1

Current Status of Veterinary Biologicals and Opportunities and Challenges for the Future

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The Animal Health market is now worth over $20Billion and continues to grow at a steady pace. Biologicals account for 25% of this business and have been increasing their share of the business by 1% per year. There are now 3 major companies who dominate the AH market with 43% of all sales. Along with the traditional biological products such as vaccines and diagnostics we are seeing an interest in genetic markers, biopharmaceuticals , stem cells and transgenic animals. New emerging diseases continue to pose a threat, such as the novel H1N1 virus and commercial companies have difficulty prioritizing these opportunities over more easily valued opportunities. With the average cost of bringing a new vaccine to the market at $10M, their appetite for high risk projects is limited. A major challenge for the AH industry is going to be to supply the increasing protein needs of our growing population and this is going to require a new revolution in technology including transgenic animals that grow more efficiently, are resistant to key diseases and have a reduced impact on our environment. The partnership between academic and industry groups will need to increase if we are to meet these challenges and gaining a better understanding of each others cultures is part of the way forward.

2

Academic discovery to commercial product â€“ a case history

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Academics often enjoy discovery but rarely development. In this case-history, where a serious clinical problem of respiratory disease was identified within a kenneled dog population, I will follow both the steps and research required of us to unravel the causes, to identify novel pathogens, attract an industrial partner and to take the development to commercial products. Working at the interface of discovery and development demands a â€œskill-setâ€ beyond purely academic expertise that both academics and industrial partners need to value. There are good opportunities for partnership between them but the cultures of academic â€˜laisser faireâ€™ and industrial â€˜secrecyâ€™ can create tension, estrangement and even divorce if not handled sympathetically! Academics must understand that timely delivery of research and Reports are critical for commercial accountability whereas industrial partners must appreciate that proper funding, often with full economic costing, and a trusting sharing of research data can develop a long-term and wonderful relationship; one almost made in heaven! Surely it can be highly cost effective for industry to cherry-pick research excellence from academia rather than in-house; the rub is often how academia funds the initial discovery?

3

The use of the canarypox virus as technology platform for veterinary vaccines

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One of the most significant contributions in the field of veterinary vaccines in the past 20 years has been the successful commercialization of several recombinant vaccines based on the canarypox vector (ALVAC) platform. With ALVAC, two innovative (and counter-intuitive) concepts were discovered and introduced in veterinary vaccinology, ie the use of a non-replicative vaccine vector and non-interfering anti-vector immunity. The registration of ALVAC-based vaccines, both in Europe and in North America reflects the excellent and robust properties of this vector combining the safety of a killed vaccine and the efficacy of a modified live vaccine. The ALVAC technology brings decisive advantages to the end-user, such as quick onset of immunity, DIVA (Differentiating Infected from Vaccinated Animals) capability and efficacy in the presence of maternally derived antibodies. The ALVAC-FeLV and ALVACâ€“rabies vaccines are still the only commercially available non-adjuvanted vaccines for cats on the market. In the past few years, research has focused on further improving the platform through optimisation of the transgene, the addition of immunomodulators and the use of adjuvant formulations. It is expected that in the foreseeable future, new vaccines based on the ALVAC platform will become available for the veterinary industry.

4

Development of a cross-protective mucosal vaccine to control porcine reproductive and respiratory syndrome in pigs

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Porcine reproductive and respiratory syndrome (PRRS) is one of the major causes of economic burden to the swine industry world-wide. Currently, we lack cross-protective killed PRRS virus (PRRSV) vaccine to effectively control the disease outbreaks. To potentiate the efficacy of killed PRRSV vaccine, we entrapped UV killed PRRSV antigens in poly(lactide-co-glycolide) (PLGA) nanoparticles and co-administered intranasally twice with a potent mucosal adjuvant, *Mycobacterium tuberculosis* whole cell lysate (*M. tb* WCL). Our results indicated that, PLGA nanoparticle-entrapped killed PRRSV vaccine adjuvanted with unentrapped *M. tb* WCL elicited better cross-protective immunity to a virulent heterologous PRRSV challenge. Clinically, immunized virus challenged pigs were free from PRRS symptoms, and immune correlates of protection at both mucosal and systemic sites comprises of: (i) significantly increased levels of PRRSV specific IgG and IgA antibody titers with enhanced avidity and virus neutralizing antibody titers; (ii) complete clearance of viremia and replicating PRRSV from the lungs; (iii) enhanced frequency of IFN-Î³ secreting cells in the lungs compared to other test groups. Results of our study are having a great promise towards development of a better cross-protective killed PRRSV vaccine to effectively control PRRS outbreaks in pigs. This project was supported by National Pork Board, USDA PRRS CAP2 and OARDC OSU to RG.

5

New approach to footrot vaccination for control and eradication of the disease in Australia

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Footrot is a contagious disease of small ruminants which is primarily caused by the bacterium Dichelobacter nodosus. The virulent form causes severe economic losses and also is a very significant animal welfare concern. Sheep and goats can be vaccinated for treatment, prevention and control of the disease. There are 10 different serogroups of D. nodosus (A,B,C,D,E,F,G,H,I,M) and immunity is serogroup-specific. The identification of the serogroup(s) of D. nodosus present in a flock is a prerequisite to specific (target) vaccination. Flocks and individual sheep can be infected with multiple serogroups. To control the disease, vaccines need to contain antigens against the serogroups that are present in the flock. When all ten serogroups are presented together in a vaccine, protection persists for only a few months due to â€œantigenic competitionâ€ . When vaccines are used as monovalents or bivalents, they induce protective antibody levels for much longer. It has been shown clearly in studies in Nepal, Bhutan and Australia that this can lead to eradication of the disease.
We evaluated the use of sequential monovalent or bivalent vaccines to control/eradicate virulent footrot on 12 commercial farms across areas of high footrot prevalence in southeast Australia. Where only one or two serogroups were present in a flock the clinical response to vaccination was very good; footrot was eradicated from a number of the trial flocks which had 3 or fewer serogroups at the start of the trial. Where there were more than 3 serogroups present, several rounds of vaccination were required for control and eradication of the disease. These results provide evidence for control and eradication of virulent footrot by sequential, flock-specific vaccination in Australia.

6

Control of bovine ringworm by vaccination in Norway

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Bovine ringworm caused by *Trichophyton verrucosum* is a notifiable disease in Norway. New infected herds are reported to the Norwegian Food Safety Authority. To limit spread of the disease, restrictions are imposed on holdings including access to common pastures and sale of live animals. Bovine ringworm has been endemic in the Norwegian dairy population for decades. Since 1980 a vaccine (Bovilis Ringvac vet, Intervet, the Netherlands) has been available. The vaccine contains an attenuated strain of *T. verrucosum* and stimulates humoral and cellular immune responses conferring protection. Efficacy and safety of the vaccine have been evaluated in experimental and field studies. Vaccination campaigns in densely populated counties have contributed to a substantial decrease in number of new infected herds. The annual incidence decreased from 1.7 % in 1980 to 0.043 % in 2004. Few herds remained with restrictions and a â€œmopping upâ€ project was established to offer assistance specifically to these holdings. In 2010, no new herds with cases of clinical ringworm caused by *T. verrucosum* were reported to the authorities. Vaccination during the last 30 years has been a key control measure in the effort to prevent disease outbreaks and eradicate bovine ringworm.

7

Vaccination with NetB toxin protects broiler chickens from necrotic enteritis

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Avian necrotic enteritis is a common bacterial disease in poultry caused by *Clostridium perfringens*. In this study we evaluated whether NetB, a major virulence factor in necrotic enteritis, could be used as a protective antigen as a subunit vaccine or supplemented in either traditional bacterin or toxoid vaccines. Immunisation with bacterin or cell free toxoid supplemented with recombinant NetB significantly protected birds against necrotic enteritis following heavy challenge with homologous and heterologous strains of *C. perfringens*, while recombinant NetB alone was only protective against a mild challenge. Birds immunised with recombinant NetB alone or supplemented in bacterin and toxoid had significantly higher levels of NetB specific IgY antibodies compared to groups immunised without additional recombinant NetB. Vaccination using toxoided culture supernatant supplemented with recombinant NetB resulted in the highest protection against necrotic enteritis. This is the first study that shows NetB can be used as a vaccine to protect chickens from necrotic enteritis.

8

Safety and efficacy studies on trivalent inactivated vaccines against infectious coryza

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The safety and efficacy of an inactivated oil-emulsion infectious coryza vaccine containing three Avibacterium paragallinarum isolates (one each of Page serovars A, B, and C) was evaluated. The safety of six batches of the vaccine was confirmed by testing with chickens vaccinated with a single large dose or vaccinated repeatedly with a normal dose. Efficacy tests were carried out on three batches of vaccine using both specific pathogen free (SPF) chickens and conventional chickens. In SPF chickens given a single vaccination at 42 days of age, the protection rate against all three serovars of Avibacterium paragallinarum was at least 80% at 30 days post vaccination. The conventional chickens, which were immunized at 42 and 110 days of age, were challenged at nine months post the second vaccination and the protection rate was at least 80% for all three serovars. The effect of storage on the vaccine was evaluated in SPF chickens using three batches of vaccine stored at 4-8 â„ƒ for one year. The protection rate against challenge from all three serovars (single vaccination at 42 days of age and challenge at 30 days post-vaccination) was at least 80%.

9

What do we look for in a vaccine opportunity: an industry perspective.

David Lowery[1]

1. Novartis Animal Health, Greensboro, NC, United States

Efficacy is certainly one of the most interesting, and critical, aspects of a new vaccine idea:Â does the vaccine work?Â However, there are many other factors to consider in evaluating a vaccine opportunity for commercialization.Â Safety, manufacturability / COG, regulatory considerations,Â intellectual propertyÂ status, potential market, strategic fit, development status, compatibility with other vaccine components, availability of appropriate models / assays....these are among the pieces of information that are considered in evaluating (and valuing) a vaccine lead.Â This talk will present one Industry perspective on the factors that might be considered in evaluating a vaccine opportunity for inclusion into a portfolio of vaccine projects.

10

Academic/Industry partnerships, how to make them work for both parties

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The partnership between academic and industry groups is critical to the commercialisation process and even more so for biological products than any other group. Many of the new pathogens or disease models are first isolated and developed by academic groups yet it is essential that industry gets involved early if there is to be a successful commercial product. Early commercial input can help to focus the research and also ensure that appropriate I.P. protection is put in place. It is also important to understand that these two groups have very different cultures and both groups need to gain a better understanding of these differences to allow them to work more effectively with each other. Academic groups are often worried about restrictions on publications, interference in their science and are focused on funding for only the next few years, while industry groups can be slow to respond to opportunities, are overly concerned about sharing their own data and determining the market value of the opportunity. If these collaborations are to be successful then they need to be genuine two way collaborations, with an open exchange of data, agreed milestones and timelines and frequent interactions between scientists.

Academic groups should look for partners who can provide more than just dollars as the input of technology such as adjuvant systems, scale up methods and Q.C. assays can help to speed up the project.

Commercial companies need to engage early, be willing to share their own data with academic partners and understand the need for academic publications . All of these topics will be discussed in this presentation.

11

Critical steps in the collaboration on the way to commercialization

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While every collaboration between academic/institutional group and industry will be different in a multitude of ways, there are usually 2 things in common; both parties are interested in supporting the producers of livestock or owners of pets, and both parties want the collaboration to fairly valueÂ their contribution in final product solution. Coming up with that fair and balanced understanding of the inputs and role requires a complete and realistic understanding of each partyâ€™s needs. This takes time and patience to understand the elements that have gone into the discovery, the exclusivity/uniqueness of the solution, and the cost and complexity of bringing and maintaining the product on the market. Several examples of successful collaborations will be presented along with suggestions on how to best plan for success in what can be a long, trying and hopefully rewarding relationship.

12

Licensing technologies to and working with big pharma: from discovery to market.

Marc Mansour[1]

1. Chief Scientific Officer, Immunovaccine Inc, Halifax, Nova Scotia, Canada

The following topics will be discussed:

-Â Pros and cons of a licensing agreement: licensorâ€™s perspective.Â Â Â

-Â Effective Licensor-licensee relationship management.Â Â Â Â Â Â Â Â Â

-Â Licensor involvement after licensing to accelerate product development.

13

Towards a hendra virus vaccine for horses

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Following its emergence in Queensland in 1994, Hendra virus (HeV) infection in horses has recurred on a regular basis with at least two disease events recognised in four of the past six years, and the 16 outbreaks to date in 2011 have already exceeded the total number identified over the past 15 years. Several outbreaks have extended to involve humans and the mortality rate in affected people is over 50%. The natural reservoir for HeV is the Australian flying-fox, with all four mainland species known to harbour the virus. However, infection of people has only been recognised following close contact with the secretions of diseased horses, especially at the time of terminal illness or during post mortem examination of horses that have died from HeV infection.

Reducing HeV exposure risk through the use of Personal Protective Equipment (PPE) during routine horse care has been heavily promoted, but there are inherent difficulties both in making reliable recommendations on this subject and relying on PPE for this purpose. However, immunisation of horses does hold promise both for providing protection against clinical illness in horses and also reducing viral shedding to a level that limits on-going transmission.

A subunit vaccine has been developed that is based on recombinantly expressed soluble versions of the HeV G glycoprotein (sG) and this is administered with adjuvant as an inactivated vaccine. Preliminary data demonstrate seroconversion of vaccinated horses and prevention of disease following exposure to an otherwise lethal HeV challenge. In addition, virus shedding was not detected in vaccinated horses and there was no evidence of virus replication in any tissue. Clearly, vaccination of horses against HeV has the potential both to protect the health of horses and to break the chain of transmission of HeV from bats to people.

14

Biosecurity considerations for the importation of veterinary vaccines/diagnostics into Australia.

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DAFF Biosecurity is responsible for ensuring Australia maintains its favourable animal health status by managing biosecurity risks through measures such as biosecurity controls. The global trade in biological products presents a continuing challenge considering the potential for contamination of these types of products with a variety of pathogens. Sourcing, processing and end use are critical elements in evaluating the risk of introduction of pathogens exotic to Australia as a result of the importation of biological products such as veterinary vaccines and diagnostics. Due to the direct pathway with the in vivo use of veterinary vaccines, the risk for the introduction and spread of pathogens is high. Veterinary vaccines are consequently, subjected to a rigorous assessment prior to the issue of an import permit. Vaccine components (master seeds, working seeds, reagents, nutrient media etc) are required to undergo testing and/or treatments to manage the risk of contamination with pathogens. To assist in this process DAFF Biosecurity has recently released a number of documents to complement and clarify existing policy on the importation of veterinary vaccines.

15

Eradication of bluetongue disease in Germany by vaccination

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Bluetongue serotype 8 occured in Germany on 21 August 2006. Vaccines were developed in 2007. Given that the vaccines that came into consideration had not been authorised they were, prior to large scale-scale use, tested for effectiveness and innocuity in a field test on cattle and sheep. A seroconversion rate ofÂ 95 to 100%, protection after experimental infection and no side effects of the norm prompted the start of obligatory vaccination against BTV-8 on 20 May 2008 that was continued in 2009. Around 80% of all bovines and 88% of all sheep were vaccinated. The success of the vaccination could also be gathered from the number of detected BT cases: 2007 20,634; 2008 5,112 and 2009 145 outbreaks. No outbreaks occured in subsequent years. Intensive monitoring was conducted in 2010 ans 2011 in order to detect BTV circulation. On the basis of the monitoring results, Germany declared itself free from BT on 15 February 2012.

The results clearly show that rigorous vaccination with corresponding monitoring is capable of eliminating animal disease agents from the susceptible population. This finding is important as conventional control measures soon reach their limits in case of vector-borne diseases.

16

Identifying patterns of Canine Parvovirus disease outbreaks in Australia using a Prospective National Disease Surveillance System

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Canine Parvovirus is a devastating disease that affects thousands of dogs in Australia every year and many more worldwide, causing severe haemorrhagic enteritis, immune suppression, and death in approximately 50% of cases. In Australia a national disease surveillance system, Disease WatchDog, has been established to record cases of disease, measure prevalence and track outbreaks. The system maps disease occurrences online in real time, allowing communication of disease outbreaks among the veterinary profession and the public.

In 2010 and 2011, there were 1,618 and 1,112 cases of Canine Parvovirus reported across Australia respectively (all states and territories). The coverage of this disease surveillance system is still expanding and the true number of cases is likely to be substantially higher. Several areas have been identified as â€œhigh riskâ€ areas for Canine Parvovirus including rural and central NSW where clustering of Canine Parvovirus case reports occurs over time. Disease trends are now able to be tracked for the first time in Australia. This provides critical information that allows veterinarians to anticipate outbreaks and thus implement strategic preventative vaccination programs in at-risk areas.

17

Rainfall and *Leptospira* infection among small animal hosts in Hawaii: assessing spatial and temporal factors

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The association between leptospirosis with a number of abiotic factors, in particular rainfall, has been well documented for humans and domestic animals; however, no study has yet examined rainfall data as a predictor of leptospiral infections in non-domestic host populations critical to the maintenance of the pathogen in the environment.Â

Using a large-scale dataset composed of 15,171 rats, mice, and mongooses collected over a period of 14 consecutive years, with 8 years of concurrent trapping across three Hawaiian islands, we performed logistic regression model analyses to estimate the association between *Leptospira* infection and rainfall at three spatial (rainfall gauge station, forecast area, island) and three temporal (trap month, season, year) levels.Â

Leptospiral infection prevalence was significantly associated with rainfall on multiple temporal-spatial scales for each of the genera studied. Specifically, associations were found between rainfall gauge station and season, and between forecast area and trap month.Â

Finding significant associations between an infectious disease and rainfall at small temporal and spatial scales is uncommon. We will discuss potential intrinsic and extrinsic factors that may be responsible for shaping the ecology of *Leptospira* in the Hawaiian islands.

18

Improving vaccine performance through understanding host-pathogen interaction in yersiniosis

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This study aimed to improve vaccine performance through understanding molecular host-pathogen interactions in yersiniosis. We investigated effects of different methods of vaccine application. Survival in an experimental challenge was significantly better after double dip and ip vaccination than control unvaccinated fish. Double dip and ip injection vaccination gave better protection than single dip or bath vaccination. The double dip vaccinated group has significantly better survival than the bath vaccinated group but there is no statistical difference between the single dip and bath. The ip vaccinated fish had a very high RPS of 95.5%. While ip vaccination maybe not practical for salmon industry, it provides a positive control for experimental research. The survival 12 weeks post vaccination further confirmed our previous results. There was a relationship between protection and antibody level above a threshold. We used cDNA microarray to characterise the differential response of host genes in the gills of naive unvaccinated and vaccinated Atlantic salmon challenged with *Y. ruckeri*. Differentially expressed genes were identified using two-way ANOVA and restricted to those with >2.5-fold change at $P<0.05$. We identified 7 genes in response to infection and 4 genes specifically associated with the protective host response to yersiniosis. These findings provide knowledge of the host-pathogen interaction in response to bacterial infection and immunisation in fish. Significantly, we identified a transcriptional biosignature consisting of predominantly immune-relevant genes (14 up and 3 down-regulated) in the gills of Atlantic salmon after immersion vaccination and before bacterial challenge. This biosignature may be used as a surrogate of protection and therefore as a predictor of vaccine success against yersiniosis.

19

Mission possible: Pan-specific vaccines to protect farmed salmonid fish against bacterial and viral pathogens in Tasmania

Richard Morrison[1] , Jeremy Carson[1]

1. DPIPWE, Prospect, TAS, Australia

Vaccination is now widely undertaken in aquaculture and is increasingly used to achieve profitable and sustainable farming of fish. However, the challenge for farming enterprises is that the inter- and intra-regional diversity of pathogens requires development of vaccines that are designed specifically for pathogen variants.

Tasmania, Australiaâ€™s southern-most state, produces in excess 30,000 tonnes of farmed salmonid fish. Since its inception in the mid-1980s, the Tasmanian salmonid aquaculture industry together with the state Government Department of Primary Industries, Parks, Water & Environment has been pro-active in developing, commercialising and producing customised vaccines specific for enzootic pathogens. The success of these vaccines has assisted in instigating an active vaccine R&D program. The broad aims of this program are to develop vaccines for emerging infectious diseases and to enhance the efficacy of existing vaccines. As production in Tasmania increases, new pathogens are emerging, of which the Tasmanian *Rickettsia*-like organism, the Tasmanian Aquabirnavirus and Tasmanian Aquareovirus are of particular importance. In this presentation, we report the successful development of a commercial-ready vaccine against the Tasmanian *Rickettsia*-like organism and how ongoing R&D is expected to deliver pan-specific vaccines for all the bacterial and viral pathogens of significance to the salmonid aquaculture industry in Tasmania.

20

Pioneering the use of aquaculture vaccines in Australia: the Tasmanian salmonid experience

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Even before marine farming of Atlantic salmon and rainbow trout commenced in Tasmania in 1985, the start up salmonid industry recognised that vaccination would be an important disease management tool. This led to the Tasmanian Government, in collaboration with the salmonid industry, developing and introducing Anguillvac-C in 1988 for the control of Vibriosis caused by *Vibrio anguillarum* and the first registered commercial aquaculture vaccine in Australia. With expansion and intensification of production, new disease threats emerged and by 1997 a second vaccine, Yersinivac-B was introduced followed in 2006 by AnguiMonas, the first bivalent vaccine for atypical *Aeromonas salmonicida* and *Vibrio anguillarum*.

While the early vaccines were killed, whole cell bacterins delivered by bath immersion, these now have largely given way to injectable formulations. The change in delivery method has opened the way for the development of formulations to protect against multiple pathogens, and current research is focussed on developing new vaccines against viral antigens that ultimately can be used in multivalent formulations.

The collaborative approach to vaccine development by industry and Government has enabled salmonid growers to be supplied with vaccines designed to meet variants of salmonid pathogens specific to Tasmania and achieve sustainable production of salmonids under challenging conditions.

21

Effect of immunization route on mucosal and systemic immune response in atlantic salmon (*Salmo Salar*)

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Amoebic gill disease (AGD) is the main disease affecting salmonids in Tasmania, Australia. The aetiological agent is *Neoparamoeba perurans* and the clinical presentation produces severe mortalities if left untreated. The current treatment (fresh water bathing) decreases the number of amoebae on the gills, but it is costly for the industry. The development of a vaccine remains a high priority.

Previous work identified a Mannose bindingâ€“ like protein (MBL) in *N. perurans*, similar to attachment factors of other amoebae, suggesting that by interfering with MBL and blocking attachment of *N. perurans*, the severity of AGD could be reduced. Before subjecting the fish to this vaccine candidate, it would be important to know the ability of Atlantic salmon in generating a mucosal immune response. The administration of an antigen with known immunogenicity would allow a proper evaluation of vaccination and sampling procedures.

Atlantic salmon were immunized with two different protein-hapten antigens: fluorescein isothiocyanate and dinitrophenol, both coupled with keyhole limpet haemocyanin. A number of exposure routes were tested, and both systemic and mucosal antibody responses were measured using ELISA for a period of 10 weeks. This will identify the best method of immunization for production of specific antibodies in the mucus.

22

Sequence diversity and cytotoxicity of the leukotoxin in isolates of *Mannheimia* species from ovine mastitis

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Leukotoxin is the best characterised virulence factor of *Mannheimia* species, and can be produced by isolates *of Mannheimia* species from ovine mastitis.Â The sequence diversity of the *lktA* gene in a panel of isolates of *Mannheimia* species obtained from cases of ovine mastitis was compared and the cross-neutralising capacities of rat antisera raised against the leukotoxin of one *M. glucosida,* one haemolytic *M. ruminalis*, and twoÂ *M. haemolytica* isolates (isolates A and B) were investigated.Â

The results indicated a higher overall nucleotide distance between the *lktA* gene sequences of *M. haemolytica* isolates compared to that between *M. glucosida isolates*.Â The *M.Â haemolytica* isolates could be divided into 2 groups based on their *lktA* gene sequences.

The neutralising capacities of polyclonal sera were tested against homologous and heterologous leukotoxins.Â Each antiserum had a neutralisation titre of 32 against its

homologous leukotoxin, while the titre differed when tested against heterologous toxins.Â The antigenic similarity coefficient calculated from the cross neutralisation data revealed that the leukotoxins from the two *M. haemolytica* isolates had the least similarity among the strains tested, while leukotoxin from *M. glucosida* had highest similarity to those from the *M. haemolytica* isolate A and the haemolytic *M. ruminalis.*

23

Improving diagnostic methods for Bovine Genital Campylobacteriosis (BGC)

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BGC is a venereal disease characterised by infertility and sporadic abortion, caused by *Campylobacter fetus* subspecies *venerealis*. Gold standard diagnosis relies upon culture isolation and differentiation from *C. fetus* subsp. *fetus* which can be difficult. Our aim is to improve diagnostic methods for BGC. Fifty-one *Campylobacter*-like (CL) isolates from bovine prepuces were screened using standard OIE and novel culture protocols, and PCR methods based on ParA and the insertion element ISCfe1. All 51 isolates were positive on one or more PCR assays and 29 were confirmed as *C. fetus* subsp. *venerealis* biochemically. Illumina genome sequencing of two Australian strains of *C. fetus* subsp. *venerealis* identified 26 conserved specific regions which did not distinguish the subspecies using PCR. Most of these regions appear to be associated with pathogenicity islands or putative virulence genes which could be present in other closely related species. Culture isolation was improved through the addition of filters to exclude contaminants, and the Thomann Transport Medium is more effective compared to current OIE protocols. Culture may remain as the gold standard and molecular tools may be useful for pathogenicity studies. Future pangenomic analyses of several *C. fetus* subspecies may assist to identify robust diagnostic targets.

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Extension of the storage time of blood in interferon gamma assays to diagnose paratuberculosis: combination of Il-7 and Il-12 stimulation

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Detection of specific interferon gamma (IFN-Î³) responses can aid in the diagnosis of paratuberculosis. IFN-Î³ detection assays offer the potential to identify more infected animals

at an earlier stage of the disease than antibody ELISA. A logistical limitation to the application of IFN-Î³ assays has been the assay needs to be performed within 8hr of blood collection. Research has shown that, for blood samples with a delayed assay setup (24hr), addition of Interleukin (IL)-12 can rescue the IFN-ï § production. For countries such as Australia, however, samples may take 2 days to reach a laboratory; therefore an improved protocol was required. IL-7 is a survival factor required to maintain resting T cells. We hypothesised that IL-7 alone or in combination with IL-12, could extend blood storage time. The combination of IL-7 and IL-12 had a synergistic effect allowing blood to be stored for up to 2 days. The same number of animals could be identified as test positive after blood was stored for 2 days using the modified IL-7 and IL-12 assay compared to the traditional assay. This practical and easily implemented potentiation protocol extends the permissible transit time of blood samples from farm to laboratory for IFN-Î³ detection.

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Novel members of the family *Pasteurellaceae* are common in kangaroos

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No specific reports exist on the presence of members of the family *Pasteurellaceae* in macropods. This initial investigation examined free wild kangaroos for the presence of *Pasteurellaceae* like bacteria and then undertook initial characterization tests. Â A total of 98 isolates of *Pasteurellaceae* were obtained from oropharyngeal swabs collected from legally harvested wild kangaroos in southern Queensland. The 98 isolates were characterized by partial *rpoB* sequence analysis and formed 3 groups (55 isolates in Group 1, 21 in Group 2 and 22 in Group 3). 16S rRNA sequencing of 18 selected strains showed a monophyletic but diverse group (>94.8% within group similarity) which appear to represent novel genera and species. Existing close relatives were as follows:- Group 1 - *Actinobacillus lignieresii* (95.6%) and *Mannheimia caviae* (95.5%); Group 2 - *A. lignieresii* (95.6%) and [*A*.] *minor* (96.6%); Group 3 - *A. arthritidis* (95.4%). Phenotypical characterization (32 tests) showed that 30 selected strains from the 3 groups were Gram negative, oxidase and nitrate positive and catalase negative. Satellitism was variable between within the groups. Separation of the 3 groups was not fully achieved. Some of the isolates demonstrated a high level V-factor requirement and special methods were required for the characterization work.

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The expression of CD2 and CD21 molecules can be used for diagnostics of involved B cell in swine infection diseases

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Anti-ovine IAH-CC51, anti-porcine BB6-11C9.6 and anti-human B-Ly4 mAbs are routinely used to detect porcine CD21 molecules on the surface of B lymphocytes. We have found that IAH-CC51 recognize only portion of B cells that are positive for BB6-11C9.6 or B-Ly4,

which indicates probably two forms of CD21 are present in the swine, and these were assigned as $CD21^{a}$ and $CD21^{b}$. While BB6-11C9.6 and B-Ly4 recognize $CD21^{a}$ that is pan-specific and identify both forms, IAH-CC51 expression discriminates $CD21^{b+}$ and $CD21^{bâ€”}$ subpopulations. We have used IAH-CC51 together with anti-CD2 to define four populations of B cells in pigs: $CD2^{â€”}CD21^{b+}$, $CD2^{+}CD21^{b+}$, $CD2^{â€”}CD21^{bâ€”}$ and $CD2^{+}CD21^{bâ€”}$. Further analysis of these four subset during ontogeny, expression profile in blood and MLN, their behavior in cell cultures and expression of other B cells related molecules revealed that they represent developmentally and functionally distinct subset of porcine peripheral B cells. The phenotypic and functional features suggest that $CD21^{b+}$ B cells are less mature than the $CD21^{bâ€”}$ subset. Although we were unable to isolate and characterize $CD21^{a}$ and $CD21^{b}$, the results of this work are significant because (1) IAH-CC51 mAb can be used to recognize functionally distinct subsets of B cells in swine, (2) previous publications needs to be revised according to which mAb was used in which study, and (3) this is the first report showing that end-stage B cells can express differential form of CD21, which can be significant for their function. This work was supported by a grant KONTAKT ME09089 from the MSMT and grants P502/10/0038 and P502/12/0110 from the Czech Science Foundation.

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Diagnosis of feline leukemia virus infection by detection of antibodies

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Up to now the diagnosis of feline leukemia virus infection is mainly based on the identification of the FeLV protein p27 present in blood. A serological test to detect FeLV antibodies for diagnosis has not been available so far, because it is difficult to differentiate between antibodies to exogenous and endogenous FeLV. In this study, we evaluated four different FeLV antigens in order to develop a diagnostic tool based on the detection of FeLV antibodies. It was the goal of this study to demonstrate byÂ indirect enzyme-linked immunosorbent assay (ELISA) wether a cat had been in contact with FeLV or not. Thus, we evaluated for use in serology a short peptide derived from the FeLV transmembrane protein, a recombinant env-gene product (p45), whole FeLV antigen, and recombinant p15E. Sera from experimentally infected and vaccinated cats as well as from field cats were examined for their reactivity to these antigens. The results of PCR detecting provirus in the blood served as reference.Â In contrast to the p45, whole virus, and the peptide, recombinant p15E displayed a diagnostic sensitivity of 95.7% and specificity of 100% using sera from experimentally infected cats. Using the sera from field cats and a different cutoff, p15E showed a sensitivity of 77.1% and a specificity of 85.6%.Â Vaccinated cats displayed only low antibody levels to p15E indicating that anti p15E antibodies are rather a sign for infection than for vaccination. The true specificity in the field may probably be even higher as many cats are PCR negative in the blood but carry the virus somewhere else in the body and thus, had contact with the virus although they are PCR negative . Â Â

We conclude that antibodies to p15E may indicate that a cat has been in contact with FeLV and together with PCR may be helpful in the control of FeLV infection.Â

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Important considerations regarding canine and feline vaccines and vaccination programs

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Canine and feline vaccines have been available for many years, but they continue to improve in quality and expand in number and types available.Â Canine and feline vaccination guidelines have been developed by species specialty groups.Â The two basic types of vaccines include 1) infectious and 2) non-infectious.Â Vaccines vary based on the specific diseases of the country or regions within the country.Â Vaccination programs are similar worldwide, in part due to the World Small Animal Veterinary Association (WSAVA) Vaccine Guidelines.Â The WSAVA Guidelines for the cat are similar to the feline guidelines of the American Association of Feline Practitioners (first in 1998, updated in 2000, 2006, and expected for 2012).Â Similarly, the WSAVA Canine Vaccine Guideilines are similar to canine guidelines developed by the American Animal Hospital Association (AAHA) (first in 2003, updated in 2006 and 2011).Â Vaccines for both species have been categorized based on need.Â Core vaccines are those that every cat or dog should receive, whereas the non-core (optional) vaccines are based on a risk/benefit analysis.Â The general vaccination recommendations for the cat and dog are to begin the vaccination program with the core vaccines at approximately 6 weeks.Â Revaccination should occur at 2 to 4 week intervals, with the last dose of vaccines at 14 to 16 weeks of age.Â Non-core vaccines can be given during this time or after the completion of the core, or not at all.Â Revaccination with core vaccines is then recommended by the first year of age, followed by revaccination every 3 years or longer.Â Most non-core vaccines must be given annually.

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Prevalence of *Bartonella* in the blood of young healthy cats relinquished to a large regional shelter in the San Francisco Bay area

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Bartonella henselae is common in the blood of otherwise healthy cats, whereas the importance of cats as reservoirs for *B. clarridgeiae* or *B. koehlerae* is uncertain because they do not grow well in routinely used medium.Â *B. henselae* is a cause of cat scratch disease, but not all isolates are zoonotic by multiple-locus variable number tandem repeat analysis (MLVA). To determine the prevalence of *B. henselae* in healthy shelter cats from the SF bay area, and to enhance isolation of other species, we tested blood from three groups of cats: A) 5-7, B) 8-10 and C) 11-12 month-olds. Blood was tested by PCR and cultured on three different media; *B. henselae* isolates were tested by MLVA. Results to date found 39% (14/36) of cats to be culture and/or PCR positive for *Bartonella.* Two of these 14 cats were PCR negative and culture positive and one PCR positive and culture negative.Â Bacteremia was 50% (10/20) in group A, 33% (3/9) in group B, and 29% (2/7) in group C.Â

Seroprevalence (titer â‰¥1:64) for *B. henselae* and *B. clarridgeiae* was 36% (44/123) and 43% (53/123), respectively. Alternative culture techniques do not appear helpful in isolation of species other than *B. henselae*.

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Canine Parvovirus-2c: an emerging virus of dogs in the USA

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Background. Canine parvovirus (CPV-2) is the number one cause of mortality in young puppies. CPV-2 infection is an emerging problem of puppies in the USA despite vaccination. CPV-2 undergoes mutations at hot spots of the viral protein-2 (VP-2). Thus, continuous monitoring of CPV-2 genotypes is required.

Methods. A PCR-based was used to genotype the CPV-2 samples submitted during the period of 2006-2012 to OADDL. The genotype of the CPV-2 was assigned based on the codon (CPV-2c= GAA; CPV-2b=GAT) at position 426 of the VP-2.

Results. Small intestine and tongue tissue samples were first screened for CPV-2 using direct fluorescent antibody test (FAT using 3BA10, anti canine parvovirus-FITC conjugate, VMRD, WA, USA). A high agreement was observed between the positive and negative FAT results on both the tissues were examined on the same puppy. Tissue and feces samples were CPV-2 genotyped using PCR followed by sequencing. CPV-2b and CPV-2c were the most common in the USA. Codon 440 of VP-2 was studied and an increase in frequency of change from ACA with GCA was also observed.

Conclusions. Both tongue and intestines are suitable for detection of CPV-2 infection. Frequency of mutations of the two major surface exposed amino-acids (426 and 440) present in the major antigenic epitopes of CPV-2 VP-2 is increasing in the USA. Thus, the efficacy of current commercial CPV-2 vaccines needs to be experimentally verified using the current emerging isolates of CPV-2c[1] .

1. Kapil, S. et al. 2007. Canine parvovirus types 2c and 2b circulating in the North American dogs in 2006-2007. J Clin Microbiol 45:4044-4047.

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An overview of The European Advisory Board on Cat Diseases (ABCD) and its achievements so far.

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The European Advisory Board on Cat Diseases (ABCD), now in its 7th year, was a joint initiative of Dr. Marian C. Horzinek, retired professor of virology at Utrecht University, The Netherlands, and Dr. Jean-Christophe Thibault, Director of Technical Services, Biologicals & NSAIDs, Merial SAS, Lyon, France. Its first aim was to issue guidelines on the prevention and management of feline infectious disease in Europe; the guidelines are based on published scientific data and follow the rules of evidence-based veterinary medicine. The

members of the Board are veterinary scientists and clinicians from the Netherlands, Belgium, Germany, Switzerland, Poland, Sweden, the United Kingdom, France, Spain, and Italy, with expertise in immunology, vaccinology and/or feline infectious diseases, opinion leaders in their respective countries. Poor vaccination rates had raised concerns about the health risks this may pose for the estimated 60 million cats in Europe. Polls conducted among >30,000 cat owners in five countries indicated that about six out of ten owners of young cats have left them unvaccinated. An essential role of the ABCD is to raise awareness about infectious disease prevention and control, through promoting vaccination as a vital part of responsible cat ownership. The Board attempts to achieve this by addressing and educating companion animal vets, emphasizing the need for evidence-based prevention and management of the major feline infectious diseases including vaccination protocols, choice of vaccines as well as the avoidance of unnecessary vaccination in cases where the duration of immunity is known. The ABCD has compiled guidelines and fact sheets (two-page abstracts highlighting the essential data contained in the guidelines, in 18 languages), has published a Special Issue on these topics in the Journal of Feline Medicine and Surgery and has participated in numerous CPD activities Europe-wide, often as satellite meetings to congresses. A recently upgraded web site (www.abcd-vets.org) is the publication platform for the most recent information, news from the research scene, upcoming events, video footage, webinars etc. Â The animal health care company Merial helped in setting up the ABCD and is committed to supporting its ongoing activities. These consist of three yearly meetings where consensus-based recommendations are formulated. The most important issue is the Board's intellectual independence from the sponsor, who may participate in these meetings as an observer.

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Novel methods for vaccine delivery

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Cells of the *innate* immune system, principally the dendritic cell (DC), are amongst the first cells to encounter antigen. Receptors of the Toll-like receptor family are particularly useful in facilitating the targeting and transport of vaccine cargos to DCs. Toll-like receptor 2 (TLR-2) is an endocytic, plasma membrane receptor which upon ligation triggers uptake of ligand into the cell. Pam2Cys is a powerful ligand and agonist for TLR-2 which initiates signal transduction pathways in the DC via MyD88 and NFkB. This leads to DC activation and maturation followed by migration of the DC to the draining lymph node. If Pam2Cys is associated with a vaccine cargo it can efficiently present processed antigen to T cells and trigger a potent immune response.

The low immunogenicity exhibited by most soluble antigens is in general due to the absence of molecular signatures that are recognized by the immune system as dangerous. We have shown that binding of a *charged* lipopeptide to an oppositely charged protein results in formation of stable complexes at physiological pH and salt concentrations. These complexes elicit very high titers of antigen-specific antibody while vaccination with similarly charged antigen and lipopeptide results in significant but lower antibody titers of antibody. Strong cell-mediated responses are also induced which are dependent on binding of lipopeptide to antigen. Induction of a $CD8^+$ T cell response correlates with the ability of lipopeptide to facilitate antigen uptake by DCs followed by trafficking of antigen-bearing cells into draining lymph nodes.

This system of adjuvanting soluble protein antigens is simple and robust and has been demonstrated to work for a variety of antigens including those from influenza, *M. tuberculosis* and the heat stable toxin of enterotoxigenic *E. coli*.

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Protection from enteric and respiratory Infections by intranasal delivery of antigens along with *E. coli* heat labile enterotoxin as an adjuvant

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We tested the efficacy in young piglets of intranasal-delivered vaccines for enteric (enterotoxigenic E. coli; ETEC) and respiratory (influenza type A) infections utilizing E. coli heat-labile enterotoxin (LT) as a mucosal adjuvant. The ETEC vaccine consisted of purified K88 (F4) antigen and LT. Inactivated swine influenza H1N1 virus was added to make the ETEC vaccine a bivalent product. Significant mucosal and systemic immune responses to the immunogens were observed. Upon challenge with K88+/LT+/STb+ ETEC, none of 20 vaccinates, but 15/19 controls became moribund or died. Vaccinates gained weight while controls lost significant weight due to dehydration. Vaccination with K88 alone afforded partial protection, while LT alone did not. Addition of influenza virus to the vaccine did not alter the efficacy of vaccine for ETEC, but provided protection against the homologous influenza H1N1 strain. Virus was isolated by nasal culture from 7/7 controls challenged with the homologous (H1N1) virus strain, and 8/8 LT-only vaccinates, but 0/12 vaccinates. Vaccinates challenged with a heterologous strain (H3N2) also shed virus. Homologous virus RNA was found in lungs of most controls, but none of the vaccinates. These studies show the efficacy of mucosal delivery with LT adjuvant in protecting piglets from diseases involving mucosal epithelium.

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Lycium barbarum polysaccharides as an adjuvant for the generation of follicular helper T cells

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Lycium barbarum polysaccharides (LBPs) have been shown playing a variety of immune-modulatory functions including activation of T cells and B cells. Follicular helper T (TFH) cells are now been recognized as a subset of helper T cells which regulate the multiple stages of B cell maturation and functions. In our current study, we found that LBPs are able to activate CD4+CXCR5+ICOS+T cells, to up-regulate the transcriptional repressor Bcl6, to induce IL-21 and IL-4 secretion. Meanwhile, LBPs could promote the formation of germinal centers (GC). Our results indicate that LBPs might enhance T cell-dependent Ab responses by acting as an adjuvant for the generation of Tfh cells.

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Early transcriptional response to ISCOM-Matrix in the pig

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The early inflammatory response to ISCOM-Matrix was evaluated in pigs.Â No side effects, determined as body temperature, appetite, activity level, reaction at the site of injection, were recorded when three doses of the adjuvant (75, 100 or 150 Î¼g) were injected in one week old piglets.Â Histological examination revealed an infiltration of leukocytes, haemorrhage and necrosis in muscle 24 hours after i.m. injection of 150 Î¼g AbISCO$^{Â®100}$ in pigs aged eleven weeks. At this time, different grades of reactive lymphoid hyperplasia was recorded in the draining lymphnode that was enlarged in three of the six pigs injected with AbISCO$^{Â®100}$.Â The global transcriptional response at the site of injection and in the draining lymph node was analysed in these pigs using Affymetrix GeneChip Porcine Genome Array. As compared to pigs injected with saline, a significant enrichment of gene signatures for the cell types â€œmyeloid cellsâ€ and â€œplasmacytoid dendritic cellsâ€ were recorded at the site of injection. In the draining lymph node, a majority of the most upregulated genes were interferon-regulated (IRGs). A number of genes encoding cytokines / chemokines or their receptors were upregulated at the injcetion site as well as in the draining lymph node.

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For the control of highly pathogenic avian Influenza

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The H5N1 highly pathogenic avian influenza virus (HPAIV) has spread to 62 countries in Eurasia and Africa and well over a billion of birds have died from infection or been killed for the control purpose. Since avian influenza vaccine induces immune response to suppress the manifestation of disease signs but does not confer complete protective immunity against infection with viruses in individual birds, misuse of vaccine consequently allows silent spread of the causative viruses.
The other serious concern is that 602 people have been infected with the H5N1 virus, 60% of whom died in 15 countries since 2004 (as of 12 April 2012). It is noted that most of the human cases (87 %) are in China, Viet Nam, Indonesia, and Egypt where bird flu vaccines are used. It is also noteworthy that in Egypt, 167 human cases have been reported since 1996 when vaccination to poultry started. In Thailand, 25 human cases had been reported until 2006, when the government decided to concentrate to stamping out policy without the use of vaccine for the control of avian influenza, no human case has been reported thereafter.
H5N1 HPAIVs isolated from dead water birds in Mongolia and Hokkaido, Japan on the way back to their nesting areas in Siberia in April to May 2005, 2006, 2008, 2009 and 2010 were genetically closely related to those isolated from domestic birds in China. It has been serious

concern that these HPAI viruses may perpetuate in the lakes where migratory water birds nest in summer. In the intensive surveillance studies on avian influenza fulfilled in autumn of 1991-2009 in Mongolia and Japan, no HPAIV had been isolated from migratory water birds that flew from Siberia, indicating that the virus had not yet dominantly perpetuated in their nesting lakes in Siberia. Whereas, on 14th October in 2010, 2 H5N1 HPAIVs, that were closely related to those isolated from dead whooper swans in spring in 2009 and 2010 in Mongolia, were isolated from fecal samples of ducks who flew from Siberia to Ohnuma Lake in Wakkanai, Hokkaido, Japan. Since then, the virus spread over Japan through wild water birds and 24 outbreaks of avian influenza occurred in chicken farms in 9 different prefectures in Japan until the end of March 2011.
Unless the H5N1 HPAIVs should be eradicated from poultry in Asia, the viruses must perpetuate in the lakes where migratory water birds nest in summer in Siberia and disastrous outbreaks of HPAI must occur in each Asian country every year. It is hereby strongly proposed to eradicate immediately the H5N1 HPAIVs from Asia by stamping-out without misuse of vaccine through international collaboration under the umbrella of One World One Health concept.

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Monitoring antigenic variation of H5N1 avian influenza viruses in Indonesia towards improved vaccine selection

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The Food and Agriculture Organization has established has established an international collaborative project for monitoring of avian influenza H5N1 virus variants in Indonesian poultry and defining an effective and sustainable vaccination strategy under the control of the OIE-FAO Network of Expertise for Animal Influenza (OFFLU). OFFLU guidance on vaccine strain selection states that all avian influenza vaccination programs should have epidemiologically relevant surveillance done to check for the emergence of genetic and antigenic variants. Since 2005 Indonesia has been the global hotspot for H5N1 infection in birds and humans. Due to the high level of H5N1 virus in poultry and the environment, and frequent contact between poultry and humans, Indonesia is widely regarded as having the highest risk of emergence of a new virus which may cause a human influenza pandemic. H5N1 has become endemic in poultry throughout much of Indonesia and vaccination can be a useful tool for short- to medium-term control if the vaccine is efficacious against the circulating field strains. Antigenic cartography was originally developed to characterize human H3N2 viruses and to facilitate selection of human vaccine candidate viruses by the WHO. This paper reports on the application of antigenic cartography to H5N1 viruses, the results of sequencing and antigenic analysis of Indonesian H5N1 virus isolates and the selection of relevant H5N1 vaccine candidates andÂ challenge viruses for evaluating efficacy of new vaccines.

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Experimental evaluation of the house flies (*Musca domestica spp.*) as a possible vector for avian influenza virus H5N1

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House flies (*Musca domestica spp*.) are one of the major pests, especially in the poultry farms resulting in not only annoyance and indirect damage to their production but also transmitting many infectious organisms mechanically and biologically. Wuryastuty *et al*. (2008) have fully succeeded in identification and isolation of highly pathogenic avian influenza virus H5N1 (AIV H5N1) from collected field house flies in Java Island, Indonesia. The AIV H5N1 isolates mentioned were further used to study the presence and persistence of the AIV H5N1 in the laboratory infected *Musca domestica spp*. One hundred house flies from a free AIV poultry farm in Yogyakarta, Indonesia were used in this study. The collected house flies were fasted for 12 hours and divided equally into the control and treated groups. The treated group was allowed to imbibe medium containing AIV H5N1 while the control group imbibed medium without virus for 1 hour. The flies from each group were collected 12 and 24 hours post exposure, respectively, immobilized at 4Â°C, immersed in absolute alcohol ethanol for a few second and dissecting under stereo microscope to collect the gastro-intestinal (GI) tract of the flies. Results showed that AIV H5N1 was first detected molecularly in dissected GI tract of the house flies and the viruses remained viable 24 hours after exposure. It is concluded that the house flies could be as AIV H5N1 vector mechanically and/or biologically.

Keywords: house flies (Musca domestica spp.), mechanical and/or biological vector, avian influenza virus H5N1

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Multiple independent recombination events between commercial attenuated ILTV vaccines caused emergence of dominant virulent field strains

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Recombination between alphaherpesviruses has been commonly seen *in vitro* and has also been seen *in vivo* under experimental conditions. While this capacity for recombination has raised concerns about the use of attenuated alphaherpesvirus vaccines in human and veterinary medicine, to date these concerns have remained theoretical and the risk of recombination has been considered to be small due to the low probability of coinfection of the same cell under natural conditions. Here we show that independent recombination events between attenuated vaccine strains of infectious laryngotracheitis virus led to the generation of two distinct virulent recombinant viruses that became the dominant strains responsible for disease in major Australian poultry production areas. Our findings highlight

the risks of using multiple attenuated herpesvirus vaccines with distinct origins in mass vaccination programs.

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Salmonella as a vaccine vector

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Oral live attenuated *Salmonella* vaccine vectors expressing recombinant foreign antigens have previously been shown to stimulate systemic, mucosal, humoral, and cell-mediated immune responses against *Salmonella* and foreign antigens; and attenuated strains of *Salmonella* have long been approved for use in both human and veterinary medicine.Â A major benefit of using *Salmonella* as a live vector is providing mucosal routes of immunization, providing the possibility of greatly enhanced protection as compared to parenteral vaccination.Â *Salmonella* vectors also have the potential advantage of being extremely inexpensive to amplify/manufacture and, as they do not have to be injected and can be administered by spray or drinking water, they are much more acceptable for widespread administration to commercial poultry.Â Recent research using attenuated *Salmonella* vaccine vectors against a variety of pathogens, including avian influenza and the food borne pathogens *Salmonella* and *Campylobacter*,has shown encouraging results.Â

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Broadly protective vaccine against enterotoxigenic *Escherichia coli*-associated porcine post-weaning diarrhea

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Enterotoxigenic *Escherichia coli* (ETEC) are the leading bacterial cause of diarrhea.Â Currently, no vaccines are available to protect against ETEC diarrhea.Â ETEC strains expressing K88ac or F18 fimbriae and heat-labile (LT) and heat-stable (ST) toxins are the predominant cause of diarrhea, particularly post-weaning diarrhea (PWD), in pigs.Â In this study, we genetically fused a K88ac major subunit FaeG peptide, a F18 minor subunit FedF peptide, and the LT_{A2} peptide and LT_B subunit peptide of LT toxoid LT_{R192G} for a tripartite fusion to develop a subunit vaccine and a live vaccine strain.Â Mice and piglets immunized with â€˜K88ac-FaeG:F18-FedF:$LT_{192A2\text{-}B}$â€™ fusion developed anti-K88ac, anti-F18 and anti-LT antibodies (IgG, seIgA) which inhibited adherence of K88ac and F18 fimbrial ETEC strains and neutralized LT toxin. Â Moreover, immunized piglets were protected when challenged with a diarrheal ETEC strain (K88ac/LT/STb).Â The live strain expressing this fusion in a holotoxin-like structure was used to orally immunize piglets.Â All immunized piglets remained healthy when challenged with the K88ac/LT/STb ETEC strain, whereas the control developed severe diarrhea and dies within 48 h.Â These studies clearly demonstrated that this tripartite fusion elicited protective antibodies, and suggest this fusion is suitable for developing broadly protective vaccines against ETEC diarrhea.

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Onset and long-term duration of immunity provided by a single vaccination with an rHVT-NDV vaccine in commercial layers

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Recombinant vaccines have been commercialized in recent years to provide more safe and efficacious vaccination than the existing ones. Negligible interference of MDA, absence of post-vaccination reactions and long-term duration of immunity are expected from vaccines using HVT as vector. We tested the onset and duration of immunity to NDV elicited by a rHVT-NDV vaccine administered at hatch as single ND vaccination or in combination with conventional live plus inactivated vaccine as part of a vaccination regime in commercial layers with MDA to MDV and NDV. The immunity to NDV was evaluated at regular intervals until 40 weeks of age by serology and challenges with a recent genotype VII velogenic NDV isolate. The effect of challenge on egg production at the peak of production and measurement of challenge virus excretion was also monitored.

Results to be presented indicated a reasonable early (74% and 95% clinical protection against challenge at 3 and 4 weeks of age, respectively) and long lasting, high level of immunity to ND, including protection against egg drop and significant reduction of challenge virus shedding.

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Novel depot based formulations for long lasting single dose vaccines

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The DepoVaxâ„¢ and VacciMaxÂ® vaccine delivery and enhancement platforms are lipid-based formulations designed to present antigen(s) and adjuvant(s) in a unique depot with a different clearance profile than water-in-oil based vaccines. Antigens and adjuvants are encapsulated in liposomes then mixed with an oil medium to allow the targeted and controlled uptake of vaccine components by antigen presenting cells (APCâ€™s) at the site of vaccination. Â In the case of DepoVaxâ„¢, a water-free formulation, Liposomes are used to bring the vaccine ingredients directly into an oil without the need for emulsification. These depot formulations are versatile and can be combined with a variety of antigens, including lysates, recombinant proteins, synthetic peptides and nucleic acids, viruses as well as a wide range of adjuvants. They have been shown to elicit potent humoral and/or cellular immunity with as little as one dose. Compared with water-in-oil emulsion-based vaccines, stronger antigen-specific IFN-Î³ responses and cytotoxic activity were generated. With a variety of model infectious disease antigens typically requiring multiple immunizations, strong and persistent antibody responses were generated rapidly with as little as one dose. DepoVaxâ„¢ and VacciMaxÂ® are versatile platforms with the potential to significantly impact the development of human and animal health vaccines.

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Effects of different adjuvant formulations on cellular migration, maturation and antigen trafficking from the site of vaccination.

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The recent recognition of innate immune activation as a critical driver of the adaptive immune response has major implications for vaccine development, and adjuvant research in particular. Using a unique and well established sheep model of pseudoafferent lymphatic cannulation, we investigated the kinetics of antigen transport and the innate inflammatory response in the afferent lymph draining the site of vaccination. Our studies demonstrated, for the first time, the *in vivo* effect of aluminium hydroxide adjuvant on soluble antigen flow and that aluminium hydroxide enhances particulate antigen uptake by dendritic cells at later time points [1]. In contrast, monophosphoryl lipidA (MPL) enhanced neutrophil and monocyte recruitment but had no effect on antigen uptake by DCs[2]. We have expanded this analysis to other classes of adjuvant including oil in water, and liposomes with/without the immunomodulators poly I:C and CpG. Both dramatic and subtle differences were observed in antigen uptake by afferent lymph cells over time and in the cell types recruited into the lymph following injection of the different adjuvant formulations. Next generation sequencing of mRNA derived from afferent lymph cells has revealed dramatic effects on gene expression by a TLR agonist over time, indicating that distinct molecular profiles can be generated for different adjuvant formulations. Further exploitation of this unique model will allow us to home in on the different cellular and molecular pathways that are activated by different adjuvants *in vivo*, and how they determine the physiological and immunological responses that impact on vaccine efficacy and safety.

1. de Veer et al. (2010). The kinetics of soluble and particulate antigen trafficking in the afferent lymph, and its modulation by aluminum-based adjuvant. Vaccine. 2010 28(40):6597-602
2. de Veer et al (2012). Modulation of soluble and particulate antigen transport in afferent lymph by Monophosphoryl lipid A. Immunology and Cell Biology, 90(4):404-10

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Efficacy of thiolated eudragit microspheres as an oral vaccine delivery system to induce mucosal immunity against enterotoxigenic Escherichia coli

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Most pathogens invade hosts via mucosal surfaces. Therefore, the induction of a mucosal immune response is necessary to prevent the invasion. Also, mucosal immunization can overcome the problems of parenteral immunization such as no induction of mucosal immune response, needle born infections, poor compliance, injection site pain, and local side effects from injections. Of the mucosal immunization methods, oral vaccinations have received attention because of their easy and acceptable properties. However, the antigens for oral vaccinations must be able to resist the harsh environment of the gastrointestinal tract, including various gastric acids, hydrolytic enzymes, and degradation of the ingested antigens. Therefore, development of an oral vaccine delivery system is needed to overcome these problems. Several delivery systems have been developed and among them, polymers exhibiting mucoadhesive or pH-sensitive properties have been reported to deliver the vaccines successfully through oral administration. Thiolated eudragit microspheres have

both mucoadhesive and pH-sensitive properties. We tried to develop an oral vaccine delivery system against enterotoxigenic Escherichia coli (ETEC) using thiolated eudragit microspheres. ETEC is one of main causes of diarrhea or edema disease in piglets. The first infection of ETEC occurs by binding fimbriae of ETEC with porcine enterocytes, so F4 or F18 fimbriae, which is well-known virulence factor of ETEC, was selected as antigen protein of the vaccine.
We selected ETEC strains with multiplex PCR for F4 and F18 and purified F4 or F18 proteins by mechanical shearing and heat shock method. After that, the fimbriae proteins were loaded into thiolated eudragit microspheres (TEMS) and the characteristic feature of the vaccine was evaluated. The average particle sizes of TEMS, F4-loaded TEMS, and F18-loaded TEMS were measured as 4.2 Â± 0.75 ãŽ›, 4.7 Â± 0.50 ãŽ›, and 4.5 Â± 0.37 ãŽ›, respectively. F4 is more efficiently encapsulated than F18 in the loading with TEMS. In the release test, F4 and F18 fimbriae were protected in acidic circumstances, whereas most were released at pH 7.4 of intestine circumstances.
For in vitro test, Raw 264.7 cells and mouse splenocytes were stimulated with unloaded TEMS, F4, F18, and F4 or F18 loaded TEMS and production levels of various cytokines were evaluated. Production of TNF-Î± and NO from Raw 264.7 cells was increased in a time-dependent manner after exposure to all groups, whereas only F4- or F18-loaded TEMS stimulated IL-6 secretion. The levels of IFN-Î³ from mouse splenocytes after exposure to F4 or F18 were increased while IL-4 was not detectable. With these results, in vitro immune stimulating activity of the vaccine was investigated.
For in vivo assay, we orally vaccinated mice with TEMS, F4, F18, F4-loaded TEMS, and F18-loaded TEMS. Mice that were orally administered with F4 or F18 loaded TEMS showed higher antigen-specific IgG antibody responses in serum and antigen-specific IgA in saliva and feces than mice that were immunized with antigens only. In addition, oral vaccination of F4 or F18 loaded TEMS resulted in higher numbers of IgG and IgA antigen-specific antibody secreting cells in the spleen, lamina propria, and Peyerâ€™s patches of immunized mice than other groups. Moreover, TEMS administration loaded with F4 or F18 induced mixed Th1 and Th2 type responses based on similarly increased levels of IgG1 and IgG2a.
These results suggest that F4 or F18 loaded TEMS may be a promising candidate for an oral vaccine delivery system to elicit systemic and mucosal immunity against ETEC.

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Novel particulate vaccines utilising polyester nanoparticles (Bio-Beads) shows efficacy against *Mycobacterium bovis* infection

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Bioengineered bacteria have been used to produce polyester nanoparticle inclusions and these have been utilised to deliver mycobacterial antigens in vaccines for the control of tuberculosis.
Polyhydroxybutyrate (PHB) biopolyester nanoparticles (biobeads) were produced in both Escherichia coli and the generally regarded as safe (GRAS) bacterium Lactococcus lactis using relatively simple fermentation and production processes. The PHB biosynthesis pathway was engineered in these bacteria to display the mycobacterial antigens Ag85A and ESAT-6 on the surface of the bio-beads. The biobeads were purified from the bacteria and characterised using MALDI-TOF MS, ELISA and flow cytometry. Mice vaccinated with these biobeads produced cell-mediated immune responses to mycobacterial antigens and had significant protection from Mycobacterium bovis infection as shown from reductions in

bacterial counts and pathology.
This unique approach to the design and production of bacterial-derived biobeads displaying antigens enables a cost-effective way to express a diverse antigen repertoire for use as vaccines to combat tuberculosis or other diseases.

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Reverse vaccinology approach to identify novel cattle tick protective antigens

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A reverse vaccinology genomic approach was developed to identify novel antigens for the protection of cattle from the tick:Â *Rhipicephalus (Boophilus) microplus*. This ectoparasite causes extensive economic losses to cattle production in tropical and sub-tropical regions of the world through tick burdens, and the transmission of tick fever (babesiosis and anaplasmosis). Ticks rapidly develop resistance to acaricides and thus the development of a vaccine with long duration of immunity is an industry priority. Such a vaccine needs to be capable of producing an anamnestic (â€œememberedâ€) response following natural tick infestations. This research commenced with the analysis of available expressed sequence tags (ESTs) as theÂ *R. microplus*Â genome is large (7.1Gb) and consists of ~80% repeat sequences. Subsequent steps included bioinformatics screening of existing (USDA database V.2 ~13,643 ESTs) and new targetedÂ *R. microplus*Â sequences (subtraction library analysis, and cDNA microarray analysis of ticks collected from resistant vs susceptible cattle~300 ESTs). Identified candidates were further scrutinised using expression analysis (localisation to stages and organs), and further informatics (membrane and secretion predictions; domain analysis and B cell epitope analysis). B cell epitopes (748 representing 240 sequences from 95 candidate protein families and singletons) were screened against pools of sera collected from tick susceptible and resistant cattle following tick challenge. Eighty peptides representing those recognised by resistant cattle were used to prepare antibodies for in vitro adult female tick feeding experiments. Simultaneously, 6 polypeptide proteins were constructed from peptides shown to be recognised by resistant cattle. In vitro feeding % effectivity for each antibody treatment was determined by monitoring adult tick death, egg output (g), and % larval emergence in comparison to naive serum/blood fed tick controls. Successful cattle vaccinations with strong protection from tick challenge were observed using a mix of 6 peptides (selected from the strongest top 9 tick feeding effectivities), a polypeptide mixture (9 antigens with peptides recognised by tick resistant cattle) and a single recombinant (selected from the top 9 feeding effectivities). Current research is focussing on vaccine product development. This approach can be easily adapted for the identification of vaccine candidates for other parasites with large genomes.

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The potential of ferritin 2-based vaccine for the control of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus*

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Ferritin2 is a newly described tick protein. It is secreted from the tick gut into the hemolymph and functions as a transporter of non-heme iron from the digested blood to the peripheral tissues. Silencing the ferritin2 by RNA interference or vaccination of experimental animals with recombinant ferritin2 have a severe impact on tick feeding and development. Sequence of ferritin2 is largely different from mammalian ferritins, which promotes it as an ideal protective antigen due to its high immunogenicity and low cross-reactivity. The main potential of ferritin2 consists in vaccinating against one-host ticks *Rhipicephalus (Boophilus) microplus*. Vaccination trials of cattle with *R. microplus* ferritin2 (Rm-Fer2) resulted in a similar protection level as was achieved with Bm86, the component of the only existing commercial anti-tick vaccine Â (Gavacâ„¢, TickGardâ„¢).Â Therefore, developing of Rm-Fer2-based vaccine or supplementing the existing vaccines with Rm-Fer2 antigen may lead to the novel, highly effective and patent-protected vaccine improving livestock production in the tropical and subtropical regions of the world.Â

Patent: (WO/2009/155886) FERRITIN 2 FOR THE HOST IMMUNIZATION AGAINST TICKS

Hajdusek et al., PNAS 106: 1033-1038, 2009

Hajdusek et al., Vaccine 28, 2993-2998, 2010

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Identifying novel vaccine candidates for schistosomiasis in water buffalo, a natural host.

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Schistosomiasis remains a major health concern in parts of China and the Philippines, where water buffaloes are a major source of human transmission. An effective livestock vaccine could simultaneously improve animal health and reduce human infection. Novel effective vaccine molecules are required, especially against the migrating larvae, which are considered an ideal target for vaccination. Here we examined the local immune response induced by the migrating larvae in the natural host, the water buffalo, in order to firstly identify novel larval-specific antigens recognised by the buffalo, and secondly to establish the type of immune response the larvae elicit. To this effect, cells were cultured from lymph nodes draining larval infection sites (skin and lung), and the secreted antibody was collected. The antibody response in skin lymph nodes from infected buffalo produced significant amounts of antibody, compared to control animals, and is being used to discover novel larval-specific vaccine targets. The type of response the larvae elicit in the buffalo was investigated by histological examination of target tissues, and cytokine analysis of cultured LN cells and PBMCs. A strong inflammatory response was observed in skin tissues shortly after infection, as well as increased IL-4 and IL-10 production in draining lymph nodes, indicative of a type-2 immune response. This study is the first to investigate the immune

response of the water buffalo against migrating larvae and is anticipated to provide new targets for generating a transmission-blocking vaccine.

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Transcriptome analysis by Illumina sequencing of full-engorged female of *Ixodes holocyclus* collected from cats and dogs with paralysis tick symptoms.

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I. holocyclus occurs along the eastern coast of Australia from far north Queensland to southern Victoria and is the most virulent tick species in terms of paralysis. Each year *I. holocyclus* affects ~100 000 domestic animals, with up to 10 000 companion animals. Also, humans are affected by tick bites provoking hypersensitivity reaction. Toxin is produced as the adult female tick engorges and paralysis is frequently induced just prior to detachment. Here we describe a transcriptome survey from salivary glands and viscera samples obtained from full-engorged *I. holocyclus* tick. Ticks were collected from Cats and Dogs with paralysis symptoms at Brisbane veterinary clinics, QLD.cDNAfrom salivary glands and viscera were sequenced using Illumina technologies producing 141 million paired reads with a total of 94 944 assembled transcripts. A total of twelve and five transcripts related with toxins were found within the samples under study. Different transcripts related with holotoxins were obtained compared with transcripts obtained from I. holocyclus fed on bandicoots. Data shows that the holotoxins are members of a multivariable protein family with highly conserved structure.

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Strangvac, an effective and safe vaccine against strangles in the horse, based on recombinant fusion proteins.

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Streptococcus equi causes strangles, which is the most severe and costly bacterial infection in the horse. It is highly contagious and a single case can lead to inhibition of activities in riding schools, cessation of racing events etc. Conventional vaccines on the market, based on attenuated strains or killed bacteria, are inefficient or have serious side effects.

A new vaccine, Strangvac, has been developed which is based on three recombinant fusion proteins derived from *S. equi*. Sixteen Welsh Mountain Ponies have been vaccinated with Strangvac, followed by experimental infection with *S. equi*. Only one of them became pyrexic, whereas 16 non-vaccinated control ponies all became ill by infection. Significant differences between the groups were found also in clinical signs of infection, levels of

inflammatory markers and post mortem analysis. Â No adverse reactions were noted. The proteins in Strangvac are surface localised on *S. equi*, required for bacterial adherence and targets for opsonic antibodies, and one is an IgG-protease of key importance for immune evasion. Other combinations of fusion proteins, than those used in Strangvac, have also been tested as vaccines, with protection levels ranging from poor to excellent (Strangvac).

Immunogenicity of Strangvac is good; high titre antibodies are formed in sera and in nasal washes against all proteins encompassed in the fusion constructs.

Shelf life stability of Strangvacis satisfactory and process development for large-scale production under GMP has begun. An on going clinical study will address the duration of protection and vaccination schedule.

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â€˜Cairns, the most venomous place on the planet; Will I die from a venomous animal at this conference!â€™

Jamie Seymour[1]

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Associate Professor Jamie Seymour or the â€œJelly Dude from Nemo landâ€ has been researching and working with venomous and dangerous animals for over 20 yrs with his present interest being â€œWhy do animals have venom?â€ Based in Cairns, in Northern Australia, an area that has an over abundance of venomous animals, he is uniquely placed to study the ecology and biology of Australiaâ€™s venomous species. He teaches at all levels at James Cook University, one of the top 5% of research universities in the world with his favourite subject being â€œVenomous Australian Animalsâ€ , a subject designed and taught by this effervescent academic. He has been successfully involved in programs designed to decrease the envenomings of humans by jellyfish, namely in Australia, Timor Leste (for the United Nations), Thailand and Hawaii. His research has been directly responsible for changes in the present treatment protocol for Australian jellyfish stings. He established and is the director of the Tropical Australian Venom Research Unit (TASRU) which is now recognised as one of the premier research groups in the world for the studies of the ecology and biology of box jellyfish and research into medical treatment of box jellyfish envenomings.

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â€œEfficacy of non-mineral oil adjuvants for intra peritoneal vaccination of olive flounder (Paralichthys olivaceus) against viral hemorrhagic septicaemiaâ€

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Adjuvants can enhance immune responseÂ to an antigen. Viral haemorrhagic septecemia (VHS) causes heavy losses in flounder aquaculture in Korea. In this study, we evaluated protective efficacy of vaccine formulations against VHS. Formalin inactivated vaccine, prepared with or without adjuvants. Groups included, inactivated virus alone (IV), IV emulsified with squalene (IV+Sq), IV emulsified with squalene and 0.5% of aluminium hydroxide (IV+Sq+Al), IV with addition of 0.5% of aluminium hydroxide (IV+Al), IV emulsified

with FIA (IV+FIA) and Sq+Al without inclusion of IV. Olive flounder (12-15 gm) were divided into 7 groups of 50 fish and 100Âµl of either of the above mentioned vaccine formulation was administered intra-peritonealy; control group was injected with same volume of MEM. Protection against VHSV was evaluated at 4th and 10th week post vaccination (wpv). Relative percent survival (RPS) obtained at 4th wpv was 37%, 31%, 58%, 19 %, 3% , 3% and at 10th wpv 48%, 65%, 83%, 53%, 71% and 6% respectively for IV, IV+Sq, IV+Sq+Al, IV+Al, IV+FIA and Sq+Al. Our results demonstrate that these vaccines formulated with squalene adjuvants can effectively protect olive flounder against VHSV and could offer an appropriate strategy to prevent this infection in olive flounder culture without serious side effects.

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CAF01 adjuvant increases the protection conferred by a commercially available influenza split vaccine in a ferret model.

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The immunogenicity and protective efficacy of current preventive vaccines against influenza are considered suboptimal, and the development of novel effective influenza vaccination strategies is urgently needed. Commercially available trivalent split vaccines are known to elicit mainly a humoral immune response, whereas the induction of cell-mediated immune responses is negligible. Recently, a cationic liposomal adjuvant (CAF01, dimethyldioctadecylammonium/trehalose 6,6â€™-dibehenate) was developed. In the current study, we compared the immune response in ferrets vaccinated with a commercially available influenza split vaccine with the same vaccine mixed with the CAF01 adjuvant and furthermore used two recently circulating H1N1 viruses for the challenge of the animals. CAF01 improved the immunogenicity of the vaccine, increasing the influenza-specific IgA and IgG levels as well as triggering cellular-mediated immunity, measured by flow cytometry as the production of interferon-gamma by lymphocytes. The adjuvant also enhanced the protection conferred by the vaccine, reducing the viral load measured in nasal washes by RT-PCR. The protection data obtained in the human relevant challenge model supports the potential of CAF01 in future influenza vaccines.

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Full-length genome sequence of class I Newcastle disease virus isolated from domestic duck

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Newcastle disease virus (NDV), avian paramyxovirus type 1 is the causative agent of Newcastle disease which is a highly contagious disease of poultry worldwide. NDV exists in two distinct classes, class I(genotypes 1-9) and class II(genotype I-XI), within a single serotype. Class I viruses are found in waterfowl, live bird markets and domestic poultry.

Â KR/duck/01/06(DK1) was isolated from domestic ducks during 2006/2007 winter season in Korea. DK1 was classified as Class I genotype 2 based on partial F gene and characterized as lentogenic NDV through pathogenicity test. The complete genome of DK1 contained 15,198 nucleotides, which conformed to the "rule of six", and consists of six genes in the following order: 3'-NP-P-M-F-HN-L-5'. The leader and trailer sequences were 55 and 17 nt in length. The cleavage site for the F protein in DK1 was 112ERQERL117. The HN protein in DK1 consisted of 585 amino acids, which is similar to DE-R49/99. The linear epitope of DK1 HN protein is 345PDDQWYQV353, which is conserved among class I strains. Â This is the first report of complete genome sequences of Class I NDV strain isolated in Korea.

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Detection of Mycobacterium avium subsp. paratuberculosis with immunomagnetic capture method from fecal samples

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Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of Johneâ€™s disease which induces chronic and fatal intestinal disorder in ruminants. MAP isolation from fecal samples is often difficult because of the slow growth bacteria that usually take long incubation time when using standard culture method. To improve MAP recovery from fecal samples, we developed an immunomagnetic separation (IMS) assay using chicken anti-MAP IgY. A diagnostic assay using immunomagnetic separation was developed to capture MAP from bovine feces by means of IgY derived from chicken eggs. The antibody was coupled directly onto the surface of magnetic nanoparticles by being mixed with magnetic nanoparticles and chicken antibodies. Analytical sensitivity and specificity were determined by extracting DNA from the captured MAP and amplifying it by IS900 PCR. The immunomagnetic separation capture followed by PCR (IMS-PCR) based on the IS900 element was evaluated in this study and showed a detection threshold corresponding to 20 cells/g in MAP-spiked fecal sample. In contrast, DNA extracted with heat treatment showed low detection efficiency of MAP-spiked fecal sample. No PCR inhibition was observed with DNA from the organisms captured with use of the nanoparticle-IgY beads. Thus, we revealed that this IMS-PCR method is particularly well adapted to the detection of MAP in fecal samples with rapid and reliable results.

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Indentification of a Novel Bunyavirus Using Next Generation Sequencing

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In 2002, serum and ectoparasite samples originating from a disease event in Tasmanian Shy Albatross were sent to Australian Animal Health Laboratory for diagnostic investigation. Antibodies against viral infections of avian species, including avian influenza, Newcastle disease, infectious bursal disease and fowlpox, were not detected and none of these viruses were isolated from the birds. Subsequently a virus was isolated in Vero cells from pooled

ectoparasite preparations from both affected and clinically normal birds. Electron microscopy examination revealed the virus isolate was morphologically similar to members of the Bunyaviridae. However there were no significant cross-reactions observed using antisera against known orthobuynaviruses and at the time this virus could not be further identified. Further studies were conducted in 2011 using next generation sequencing and identified the virus isolate as a novel phlebovirus. Of particular significance, the virus exhibited highest sequence identity with a recently characterised novel phlebovirus from China identified as the causal agent of Severe Fever with Thrombocytopenia Syndrome (SFTS) in humans. However, it should be noted, that the ectoparasites from which the virus was isolated were collected from both affected and clinical normal birds and the pathogenic and zoonotic potential of this novel virus remains to be determined.

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Application of pH-Sensitive Fusogenic Polymer-Modified Liposomes for Development of Mucosal Vaccines

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Â Â Â Â Â Â Â The purpose of the study is to evaluate the usefulness of pH-sensitive fusogenic polymer (succinylated poly(glycidol) (SucPG) and 3-methylglutarylated poly(glycidol) (MGluPG))-modified liposomes as aÂ mucosal vaccine.Â Immune response in mice immunized with OVA-containing SucPG-modified liposome(OVA-liposome) or in chickens with immunized with Salmonella Enteritidis antigen-containing MGluPG-modified liposome(liposome vaccine) was examined.Â Mice wereÂ immunized with OVA-liposomes intranasally. After immunization, significant antigen-specific antibodies were detected in the serum and intestine. When sera were analyzed for isotype distribution, antigen-specific IgG1 antibody responses wereÂ detected in mice immunized with OVA-containing unmodified liposomes, whereas immunization with OVA-liposome resulted in the induction of OVA-specific IgG1, IgG2a and IgG3 antibody responses. In spleen lymphocytes from mice immunized with OVA-liposome, both IFN-Î³ and IL-4 mRNA were detected. The same result was also obtainedÂ inÂ mice imminized with OVA-containing MGluPG-modified liposomes. Furthermore, we examined the induction of immune responses in chickens following intraocular immunization withÂ liposome vaccine, and the protective effect against the challenge with S. Enteritidis. Immunization with liposome vaccine induced significant antibody responses against S. Enteritidis in the serum and intestine. Less fecal excretion of bacteria was observed in chickens imminized with liposome vaccine after challenge. The numbers of bacteria in the caecum were also lower in immunized chickens than in unimmunized controls.

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Practical Use Of Bacucheck^{tm} Elisa For International Compliance Testing

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PCV2 infection in pigs is a worldwide problem. PCV2 vaccinated pigs can be differentiated from PVC2 infected pigs. BacuCheck™ ELISA provides evidence of PorcilisÂ® PCV vaccination by detecting antibodies against baculovirus used in the production of the ORF2 coded subunit. This study demonstrates its practical use in international trade.

From November 2011, a German finishing farm purchased batches of 10-week old PCV2-vaccinated pigs from a Danish producer which were blood sampled on arrival, and the sera tested with BacuCheck™ ELISA, and with Synbiotics SerelisaÂ® to detect and quantify PCV2 antibodies. This combination showed that PorcilisÂ® PCV had been used correctly at group level. This accords with the results in vaccinated animals of 6 to 12 weeks old in ongoing routine diagnostics in various other field trials.

Negative results need to be assessed properly, by individual interpretation, which takes account of the effect of dose volume, administration technique, concurrent use of other products, the animalsâ€™ age, health and MDA levels at time of vaccination. With the cooperation of piglet supplying- and finishers receiving farms, this trial showed that the test is a useful tool for strengthening international pig trade.

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Bacucheck Elisa To Differentiate Pcv2-Vaccinated And Unvaccinated Pigs

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PCV2 virus infection in pigs is a worldwide problem. Most pigs have PCV2 antibodies, but it has been difficult to differentiate between those to vaccinal and field antigens. Serological interpretation is complicated by MDA and field exposure.

BacuCheck ELISA provides proof of PorcilisÂ® PCV vaccination by detecting antibodies against baculovirus used in the production of the ORF2-coded subunit. It was used in this study to measure antibody levels over time in PorcilisÂ® PCV-vaccinated and unvaccinated pigs.

Vaccinated and unvaccinated pigs were blood sampled regularly between 7 and 18 weeks old, and the sera tested with BacuCheckELISA and Synbiotics SerelisaÂ®, to detect and quantify baculo virus and PCV2 antibodies.

Most vaccinated pigs tested positive for BacuCheck ELISA all ages. Whereas controls were negative at 7 weeks old but percentage of samples positive in BacuCheck ELISA increased over time.

Combining the results from these tests differentiated between PorcilisÂ® PCV-vaccinated and unvaccinated groups up to 12 weeks of age. In line with other studies, the results indicate that pigs do contract baculoviruses during fattening. The BacuCheckELISA should only be used in pigs up to 12 weeks old, with samples taken 4 weeks after vaccination.

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Prevalence and phylogenetic analysis of feline Torque teno virus in the Czech Republic

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Torque teno viruses are small, non-enveloped viruses containing a single-stranded, negative-sense circular DNA genome enclosed within icosahedral nucleocapsid. The genome of TTV has a range of 2.1â€“3.9 kb in length. Torque teno felis virus (FcTTV) belong to the genus Etatorquevirus within the family Anelloviridae International Committee on Taxonomy of Viruses (ICTV 2009). Currently only five complete feline TTV genome sequences from GenBank are available, and have been detected in serum and saliva samples. While the prevalence of feline TTV wasnÂ´t described yet.
The objective of this work was to estimate the prevalence of feline TT virus in the Czech Republic. Nested primers were situated in the highly conserved untranslated region of the virus genome. One hundred and ten blood serum samples from cats were examined. The prevalence of FcTT viruses in the Czech Republic was estimated to be 30.9%. In order to confirm specificity of PCR reaction and to perform phylogenetic analysis 300 b.p. DNA fragments was sequenced. Nucleotide sequence analysis showed that sequenced strains belong in one virus species.

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Epitope mapping of capsid protein Torque teno sus virus 1 and Torque teno sus virus 2 and serological prevalence of infection in pigs

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Torque teno sus viruses (TTSV) are small non-enveloped viruses with circular, single-stranded DNA genome. TT viruses belong to the family Anelloviridae and may infect humans and different animal species. Two different virus species have been identified to date in pigs, Torque teno sus virus 1 (TTSV1) and Torque teno virus sus 2 (TTSV2), which belong to the genus Iotatorquevirus. TTSV are widely prevalent in the pig population but TTSV infection is not directly responsible for any disease. The genome of porcine TT virus contains three open reading frames. ORF1 is coding for nucleocapsid protein which potentially induces immune response of infected animals.
The goal of this work was to identify immunodominant peptides in the structure of ORF1 protein of TTSV1 and TTSV2 which could be used as antigens to detect TTSV1 and TTSV2 antibodies following natural infection in pigs. Translated ORF1 genes of both viruses were analyzed for prediction of linear B-cell epitopes. Peptides were synthesized and used as the antigens in indirect ELISA test. Selected and synthesized peptides covered more than 40% of the length of ORF1 proteins. The total of 307 pig serum samples was collected from 16 herds and different age categories of animals were examined. TTSV1 and TTSV2 specific IgG antibodies were detected in piglets at age of 4 weeks. After vanishing of these

presumably maternally derived antibodies post-infection antibody titers started to increase significantly and peaked at week 20. Relatively high antibody titer persisted till the adulthood.

1. This work was supported by the Grant agency of Ministry of youth and education project no. ME08108, and COST CZ project no. LD12001 and IGA VFU Brno project no. IG121241.

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Assembly of *Campylobacter fetus* subspecies and biovars genomes reveal unique glycine intolerance and pathogenicity islands

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Campylobacter fetus subsp. *fetus* and *C. fetus* subsp. *venerealis* both cause disease in livestock but each subspecies generally occupy different specific host niches. *C. fetus* subsp. *fetus* is normally found in the gut while *C. fetus* subsp. *venerealis* in the genital tract of ruminants. *C. fetus* subsp. *venerealis* infections can cause abortion and infertility in cattle, while subsp. *fetus* is unlikely to result is such severe outcomes. To understand the molecular basis for such differences we sequenced the genomes of two *C. fetus* subsp. *venerealis* biovars (Venerealis and Intermedius) using Illumina technology. Our current draft assemblies suggest a similar genome size for both biovar genomes (~1.94Mbp) that are longer than the 1.77 Mbp genome of the reference *C. fetus* subsp. *fetus* ATCC 19438 genome. As compared to the *C. fetus* subsp. *fetus* genome 99kb and 94kb unique regions were found in the *C. fetus* subsp. *venerealis* Biovar Venerealis and Intermedius genomes, respectively. These sequences have been utilized to design biovar specific assays for diagnosis of field samples. The presence of a pathogenicity island (PAI) is known to be associated with the virulence of these strains, we identified unique features in the *C. fetus* subsp. *venerealis* bv. Venerealis PAI and a significant sequence divergence in the bv. Intermedius PAI. In the genome, we found a mutation in the alanine/glycine amino acid carrier protein (AACP) that correlates with the increased intolerance of *C. fetus* subsp. *venerealis* bv. Venerealis strains when grown in 1% glycine. This toxicity can be rescued with exogenous addition of Mg2+ ions or growth at pH 9.5. Finally, we found divergent flagellin genes in *C. fetus* subsp. *venerealis* bv. Venerealis that may increase robustness of the flagella and hence increase their motility fitness necessary to find a proper niche for their growth and/or enable colonization of placenta in cattle that results in abortion.

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Antigen trafficking and cellular migration into ovine afferent lymph following vaccination with liposomes containing Poly I:C and CpG

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A comprehensive understanding of the immune response is required to expand the range of vaccines available, as well as increase their efficacy and reduce side effects. Recent immunological breakthroughs in the understanding of the innate immune system and its role in shaping the immune response has lead to great interest in vaccines that selectively target innate immune pathways. Several well defined innate stimulators, such as TLR agonists, are now being developed to serve as adjuvants in the hopes of developing vaccines that induce safe, effective and sustained immune responses. Using a unique *in vivo* model of ovine pseudoafferent lymphatic cannulation, we investigated the innate response in afferent lymph following vaccination with Poly I:C and CpG. We have shown that cellular migration patterns differ between adjuvants, with Poly I:C inducing a greater neutrophil response when compared to CpG. In addition, CpG was shown to both increase the expression of MHC class II on antigen positive monocytes and induce greater antigen uptake by dendritic cells after vaccination. Further research with this cannulation model provides a unique opportunity to investigate the *in vivo* mechanisms underlying the action of adjuvants and contribute to the understanding of the complex immune response generated after vaccination.

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Identification and antimicrobial susceptibility patterns of Pasteurella multocida isolated from chickens and Japanese quails in Brazil

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A study was performed to verify the presence of *Pasteurella multocida* in eight different poultry groups of 90 birds each.Â Groups I to IV were chickens with I being >6 weeks of age with a history of respiratory illness, II > 6 weeks of age and free of respiratory illness, III < 6 weeks of age with respiratory illness and IV being < 6 weeks of age and with no respiratory illness.Â Groups V to VIII had the matching characteristics of Groups I to V but consisted of Japanese Quails. The *P. multocida* isolation rate from the groups was as follows; Group I 56/90 (62.3%) Group II 18/90 (20.0%), Group III 12/90 (13.3%), Group IV 3/90 (3.33%), Group V 8/90 (8.88%), Group VI 2/90 (2.22%) Group VII 2/90 (2.22%) and Group VIII 1/90 (1.11%). These isolation rates were not significantly different within the groups of a bird type but the overall chicken isolation rate was significantly higher than the quail isolation rate (p<0.01).Â All isolates were examined for their sensitivity to four antimicrobial agents. The results showed only low levels of resistance to the agents tested. The highest level of resistance was detected to cephalothin (5.1% of isolates) followed by amikacin (3.4%).Â

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Pathogenicity Of Current Porcine Epidemic Diarrhea Virus Infections In China

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Methods:In 2011, we received a total of 756 intestinal samples from 48 pig farms, located in the major pig-raising provinces across China. We performed PCR and viral culture assays (1) on all suitable samples. Positive PCR products corresponding to the full ORF3 and full M genes derived from Vero cell-cultured PED isolates were extracted from gels by commercial kits (Qiagen). The complete nucleotide sequence of ORF3 and M genes within these recombinant clones were then sequenced (both nucleotide strands) by a commercial service. The molecular relationships among the nucleotides of the ORF3 and M genes from our Chinese PED isolates were inferred using the Neighbour-joining method. The evolutionary distances were computed by the p-distance method involving nucleotide base (ORF3 gene) and amino acid (M gene) differences. Results: We detected PCR products consistent with PED virus in 426/756 intestinal samples (56%) derived in 2011 from 26/48 farms (54%) located across China, which had had clinical outbreaks resembling PED. We isolated and partially sequenced wild-type PED virus from nine of the PCR-positive samples. Phylogeny tree analyses based on the deduced amino acid sequences of the M gene and the nucleotide sequence of ORF3 gene of these recent isolates indicates that several isolates formed new clusters of distinct lineages, suggesting that the Chinese PED coronavirus strains show strong mutation and possible recombination potential. A total of 24 piglets were challenged orally (4 pigs per isolate) with 4 control sham-inoculation piglets. Three days after inoculation, all challenged piglets showed clinical, pathological and PCR reactions consistent with PED virus. Conclusion: The phylogeny tree analyses we conducted on both the M gene and ORF3 gene showed that several of the recent Chinese isolates appeared to form new clusters, particularly the Hebei and Guangxi origin isolates. These provinces are not connected by farming practices suggesting that the Chinese PED coronavirus strains show the strong mutation and possible recombination potential of other coronaviruses. This suggests that the recent PED isolates retain their strong pathogenicity in the field and challenge study situation, even though considerable shifts have occurred in major viral membrane protein genes.

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Duration of immunity of a live attenuated sheep pox vaccine.

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Sheep pox and goat pox are the most important kind of pox with high mortality in young animals. Inactivated and lived attenuated vaccines are used commonly in countries which sheep pox is endemic in. Sheep pox is endemic in Iran, abortion and mortality of lambs made high costs in live stock. In order to preventing and controlling these diseases mass vaccination program by a lived attenuated sheep pox vaccine (manufactured by Razi Vaccine and Serum Research Institute) has utilized since over 40 years ago. In this study, protection against wild strain of sheep pox virus and humeral immunity of the mentioned lived attenuated sheep pox vaccine was investigated. This experimental study was done in a

15 months monitoring in quality control department of RVSRI. For assaying immunity of the vaccine, efficacy test and neutralization Index was carried in 21 days, 3, 6, 9, 12 and 15 months after vaccination.

Â Â Â To sum up, as can be seen, according to result of challenging and VN tests, it seems duration of immunity of lived attenuated vaccine manufactured by RVSRI is approximately 1 year.

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Immunogenicity evaluation of *in silico* identified *Mycobacterium avium* subsp *paratuberculosis* recombinant proteins that were upregulated under stress conditions

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Mycobacterium avium subsp *paratuberculosis* (MAP)is the causative agent of Johneâ€™s disease (JD) in ruminants. MAP is known to enter a dormant phase outside the host, typically on soil. *In vitro* experiments have reported regulation of certain MAP proteins when exposed to stressors similar to what is thought to produce dormancy. It is believed that *in vivo* regulation of dormancy associated proteins by MAP may play a role in evading the host defence mechanisms and the host may induce immune responses against such proteins. Five dormancy-related proteins that were found to be upregulated under stress conditions and predicted through *in silico* analysis to possess immune epitopes were selected. Recombinant proteins were produced and evaluated by indirect enzyme-linked immunosorbent assay for immunogenicity using a panel of sera obtained from sheep unexposed and exposed to MAP. The antibody levels of the exposed group were significantly higher than the unexposed group ($P < 0.001$). All five dormancy-related proteins identified as immunogenic by *in silico* analysis were found to discriminate between sheep sera from control and MAP exposed sheep.

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A New Tetravalent Canine Leptospirosis Vaccine Provides Atleast 12 Months Immunity Against Infection

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INTRODUCTION
Most commercially available canine leptospira vaccines are effective at controlling clinical disease and preventing mortality. Only a few claim to control infection or renal excretion; important parameters in limiting the spread of this zoonotic disease. Additionally, there are concerns about whether vaccine immunity persists for a full 12 months. The following studies investigate the ability of a new European tetravalent vaccine (NobivacÂ® L4 â€“ MSD Animal Health) to control infection in dogs following challenge with strains from serogroups Canicola, Icterohaemorrhagiae, Grippotyphosa and Australis at 12 months post-vaccination.

MATERIALS AND METHODS
For each study two groups of conventional beagle dogs,were used. One group was

subcutaneously vaccinated with Nobivac DHPPi reconstituted in Nobivac L4 at 6 and 10 weeks. The second (control) group was vaccinated with just Nobivac DHPPi. In each study dogs were challenged with a pathogenic strain from one of the four serogroups, 12 months after the second vaccination. Samples of blood, serum and urine were collected at intervals during the four weeks following challenge and were evaluated for the presence of challenge organisms or leptospiral antigen by culture and PCR.

CONCLUSION
Vaccination of dogs with Nobivac L4 is effective at reducing infection post-challenge for at least 12 months.

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Development of Immunoassays to analyze the role of NetB in avian necrotic enteritis

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Clostridium perfringens is the etiological agent of avian necrotic enteritis, a disease that causes significant economic losses to the poultry industry. NetB has been identified as a major virulence factor in disease pathogenesis and could be an effective immunogen in necrotic enteritis vaccines. The objective of this work was to develop assays to evaluate specific anti-NetB immune responses in chickens and to measure NetB levels in *C. perfringens* culture supernatants. Chickens were challenged with virulent *C. perfringens* strains, EHE-NE18 or WER-NE36, and convalescent blood samples were collected. An indirect ELISA was developed to quantify the levels of anti-NetB IgY antibodies in serum and it was found that infected birds have a humoral immune response to NetB. The assay has been used to evaluate anti-NetB IgY responses to various vaccine formulations. A chemiluminescent ELISA was developed to quantify NetB levels in these samples. Although strain WER-NE36 was able to induce a higher IgY response than strain EHE-NE18, no difference in *in vitro* NetB production was observed. Further studies are being conducted on how the immune system is activated by these two strains, and on the *in vivo* production of NetB in different culture conditions.

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A new high-throughput direct faecal PCR test for Johneâ€™s disease.

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The development of a direct faecal PCR (DPCR) test for both ovine JD and bovine JD has been a goal in Johneâ€™s disease research since the mid 1990s. We have developed a new high throughput DPCR test (termed the HT-J test), suitable for faecal samples from both cattle and sheep. The HT-J test is based on a highly sensitive DPCR test that was applicable mainly to research applications because of its complexity and labour intensity. Here we report the development, optimisation and performance of the HT-J test for the detection Johneâ€™s disease in cattle and sheep. To validate the HT-J test, 1329 cattle and 596 sheep faecal samples, representing unexposed and exposed herds and flocks, were tested in two laboratories. The new test has demonstrated sensitivity and specificity equal to or greater than culture and is suitable for use in routine diagnostic laboratories. The HT-J test was developed to suite Australian conditions and during the course of the study it became apparent that local optimisation is paramount to the success of the test. Furthermore, sample storage prior to testing was identified as a critical issue.

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How does Galectin-11 mediate nematode parasite resistance?

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Gastrointestinal nematodes (GIN) cost the sheep industry $369 million annually. With anthelmintic resistance and no commercialised vaccine, economical control strategies will require a further understanding of immune resistance development in sheep. Galectin-11 is a protein secreted into the mucus during GIN infections. Our work has shown an inverse correlation between sheep galectin-11 production and Haemonchus contortus (H. contortus) infection suggesting its role as a marker of GIN resistant sheep. However, the role of galectin-11 in GIN resistance is unknown therefore in the present study we used the larval migration assay (LMA) and immunohistochemistry to determine the effect of galectin-11 on H. contortus larval stages. LMA results showed no clear effect of galectin-11 on exsheathed L3 migration. However, immunohistochemistry results showed specific binding of galectin-11 to the surface of L4. This work suggests that galectin-11 may have a potential role in modulating L4 H. contortus activity and future studies are under way. Understanding the function of galectin-11 will assist in understanding the mechanisms of GIN immune resistance in sheep.

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Administration Of A New Tetravalent Canine Leptospirosis Vaccine Has No Adverse Effect On Either The Safety Or Efficacy Of An Intranasal Kennel Cough Vaccine

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INTRODUCTION
Recently a new European tetravalent canine leptospirosis vaccine has been developed (NobivacÂ® L4). This new vaccine will commonly be given to dogs mixed with the routine injectable lyophilized viral components. Studies to support this simultaneous use have been undertaken. It is common for intranasal â€˜kennel coughâ€™ vaccines to also be given to

â€˜at riskâ€™ dogs during the same consultation. This study was designed to investigate any adverse effect of Nobivac L4 on the safety or efficacy of a live, bivalent (*Bordetella bronchiseptica* and canine parainfluenza virus), intranasal vaccine (Nobivac KC).

MATERIALS AND METHODS
Thirty-one SPF beagle puppies, 6 weeks of age, were divided into three groups. Group 1 pups were vaccinated intranasally with Nobivac KC and subcutaneously with Nobivac DHP reconstituted in Nobivac L4. Group 2 pups received just Nobivac KC and group 3 pups received Nobivac DHP reconstituted in Nobivac L4. Three weeks following vaccination all pups were challenged with pathogenic strains of Bb and CPiV. Adverse clinical signs, serum antibody titres and shedding of Bb and CPiV were recorded throughout the study.

CONCLUSION
There is no adverse effect of Nobivac L4 on either the efficacy or safety of Nobivac KC when used concurrently.

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Effects of *Lactobacillus brevis* on growth performance and immune function in swine: a feeding trial

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Lactobacillus brevis ATCC 8287 is a potential probiotic lactic acid bacterium containing an S-layer, which makes it an interesting vaccine vector candidate. The aim of this study was to investigate the effects of *L. brevis* on growth performance and immune response in newly weaned piglets. Twenty piglets were assigned into two groups (treatment and control). The treatment group (n = 10) was fed $1x10^{10}$ *L.brevis* daily for three weeks. Â An equivalent amount of probiotic free-vehicle was provided as a placebo for the control group (n=10). Â Health status and weight gain was monitored during the trial.Â Blood and stool samples were collected at the beginning and the end of the trial and samples of the intestinal mucosa and luminal contents were collected at slaughter. The presence of *L. brevis* in stool samples was determined with bacterial isolation and S-layer specific PCR, and the abundance of total bacteria and *L. brevis* in digesta was investigated with quantitative PCR. Intestinal biopsy samples were sectioned for histopathological evaluation and immunofluorescence detection of *L. brevis*.Intestinal mucosal samples were analyzed for cytokine gene expression. *S*erum IgG and IgA concentrations were measured with ELISA. The results of the feeding trial will be discussed.

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Application of a *Campylobacter fetus* subspecies *venerealis* guinea pig infection model for the assessment of strain virulence variation

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Bovine Genital Campylobacteriosis, caused by *Campylobacter fetus* subsp.v*enerealis* is associated with production losses due to herd infertility and sporadic abortions. The aim of this study was to compare abortifacient properties between *C. fetus* subsp. *venerealis* strains. Four groups of 10 pregnant guinea pigs (4 vaccinated (Vibrovax^{TM} twice, five weeks apart prior to mating) and 6 non-vaccinated) were challenged intraperitoneally at week five of gestation with four different strains at a concentration of 10^7 cfu/ml. Tissues from dams and foetuses were examined by culture. Proportions were compared using Fisherâ€™s Exact test. Strain 76223 resulted in 8/10 abortions (6/6 non-vaccinated, 2/4 vaccinated), strain 924 2/10 (2/6 non-vaccinated, 0/4 vaccinated), strain 635 0/10 and strain B6 2/10 (1/6 non-vaccinated, 1/4 vaccinated), within 12 days (p=0.007). *C. fetus* subsp. *venerealis* was reisolated from peritoneum, uterus horns, placenta, amniotic fluid and foetal stomach contents in aborting and non-aborting animals. Reisolation was unsuccessful in five of the vaccinated animals. Virulence variation was present with differences in abortion rates and culture results. Intra-peritoneal administration of *C. fetus* subsp. *venerealis* to pregnant guinea pigs is a promising small animal model for investigation of *C. fetus* subsp. *venerealis* strain variation and abortion mechanisms.

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Experimental infections with *Campylobacter fetus* subspecies *venerealis* in cattle

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Development of effective protocols to replicate the transmission of *C. fetus* subsp. *venerealis* in cattle is critical for the evaluation of the biology of the organism as well as the host immune response. Â Protocols for experimental infections with *C. fetus venerealis* in bulls and heifers were developed. A total of 6 Brahman bulls and 11 Brahman heifers were randomly allocated to four experimental groups containing exposed and unexposed animals. One group of bulls and one group of heifers were challenged by experimental exposure by intrapreputial and intracervical inoculation with a *C. fetus venerealis* suspension respectively. The remaining groups were challenged by natural mating with previously infected animals. Detection of *C. fetus venerealis* using culture and real-time PCR in smegma samples from

bulls and cervico-vaginal samples from heifers; and macro and histological examination of tissue samples from the reproductive tracts collected after slaughter were performed. *C. fetus venerealis* was detected by culture and/or real time PCR in all groups post-challenge. Experimental exposure was more effective in establishing infection in bulls and heifers and the infectious status appeared to last longer (up to 10 weeks in bulls and 7 weeks in heifers). There was minimal cellular inflammatory response after experimental and natural challenge.Â

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Mosquito Vector Competence Tests for *Bovine ephemeral fever virus*

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Bovine ephemeral fever, often referred to as three-day sickness, is a viral disease of cattle and buffalo around tropical and subtropical areas. The disease is caused by *Bovine ephemeral fever virus* (BEFV) that belongs to the genus *Ephemerovirus* of the family *Rhabdoviridae*. In Taiwan, the epizootic of bovine ephemeral fever has been recorded several times in the past twenty years. The morbidity of the disease can be up to 100% although the mortality is low (1-2%). Bovine ephemeral fever causes significant economic loses to dairy farming. Epidemiological studies implicate that a range of biting insects transmit BEFV, but their identity remains unclear. Disparity between the distribution of *Culicoides* biting midges and BEFV transmission pattern suggests there must be several vectors other than midges involved in its transmission. Moreover, BEFV has been isolated from *Culicoides* spp. and mix pools of mosquitoes. Therefore, to distinguish their role as the primary vectors of BEFV and to determine the relative importance of *Culicoides* biting midges and mosquitoes in the epidemiology are major works. The objective of our research is to study the susceptibility and transmission potential of several mosquito species including *Anopheles*, *Aedes*, and *Culex* species. The susceptibility of mosquitoes to BEFV was examined by oral infection and intrathoracic injection. The potential of virus transmission by mosquitoes was evaluated by the recovery of virus from capillary tube contents. The results show BEFV replicated inÂ *Culex tritaeniorhynchusÂ* and was detected in the saliva of the mosquito. The study will identify the potential vectors of BEFV and help understand the vector-virus interactions on BEFV transmission.

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Towards a method for antimicrobial susceptibility testing of *Haemophilus parasuis*

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Haemophilus parasuis causes GlÃ¤ssserâ€™s disease, a disease of significant economic impact in the pork industry.Â Antimicrobial treatment is an important disease control option.Â However, there are no accepted standardised methods for antimicrobial susceptibility testing of *H. parasuis*.Â We are evaluating the capacity of a medium that has a known capacity to support good growth of *H. parasuis* to be used in disc diffusion (as an

agar) and minimal inhibitory concentration (MIC) testing (as a broth).Â To date, the medium (BA/SN) has been evaluated for use in disc diffusion.Â Using the Clinical and Laboratory Standards Institute(CLSI) recommended guidelines and Quality Control (QC) strains, all five tested antimicrobials (ampicillin, ceftiofur, co-trimoxazole, florfenicol and tetracycline) have given the anticipated results across six independent repeats with *Escherichia coli* (with one exception when both the CLSI medium and the new medium gave an unacceptable result with ceftiofur).Â For the *Staphylococcus aureus* QC strain, the new medium gave the correct results for seven antimicrobials (ampicillin, ceftiofur, erythromycin, florfenicol, tetracycline, penicillin and tilmicosin) but consistently gave an unacceptable result with co-trimoxazole. The preliminary results suggest that this new medium has the potential to be an acceptable medium for antimicrobial susceptibility testing for *H. parasuis*.

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Comparative proteome and transcriptome analyses of wild-type and live vaccine strains of *Salmonella enterica* serovar Gallinarum

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Salmonella enterica serovar Gallinarum causes fowl typhoid in chickens and has been of economic importance to the chicken industry. A serovar Gallinarum live vaccine strain 9R (SG 9R) has been used to control fowl typhoid in many areas where the disease is endemic. Because the attenuation mechanism of SG 9R was not completely defined, there has been continued concern about reversion to virulence. In this study, we examined the molecular basis of attenuation of SG 9R by comparing its proteome and transcriptome with those of two wild-type strains (287/91 and 06Q110). The constantly detected spots of two wide-type strains with concomitant absence in SG 9R in two-dimensional gel electrophoresis were subjected for MALDI-TOF MS identification. Genes up- or down-regulated in SG 9R compared to wild type strains were identified using an expression array. The proteome analysis identified nine proteins absent in SG 9R. The transcriptome analysis revealed 24 up-regulated and 97 down-regulated genes in SG 9R. Approximately one-half of down-regulated genes (42 genes) were associated with virulence mechanisms. This finding suggests that attenuation of SG 9R may be associated with a large combination of impaired multiple virulence factors and reversion to virulence wonâ€™t be caused simply by any single event.

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Differentiation of marsupial herpesviruses using high resolution melt (HRM) technology.

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The recent identification of two koala gammaherpesviruses (phascolarctid herpesviruses -1 and 2 (PhaHV-1 and PhaHV-2) and an additional macropod herpesvirus (macropodid herpesvirus 3, MaHV-3) from clinical cases of disease in Australian wildlife has highlighted the need for improved diagnostic tests to detect and differentiate marsupial herpesviruses. In this study a commonly used pan-herpesvirus PCR was modified to enable differentiation of

PhaHV and MaHVs by high resolution melt (HRM) curve analysis of the amplicons. The amplicons derived from the PhaHVâ€™s showed vastly different HRM profiles. The three MaHVâ€™s showed more similar melt profiles, but were still able to be differentiated using this method. This approach has cost and time advantages over PCR and DNA sequencing, and has the potential to be extended to include the differentiation of additional marsupial herpesviruses as other novel viruses are detected in our wildlife.

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Epidemiology of fowl cholera

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One of the major economic important diseases of poultry worldwide is fowl cholera, caused by the bacterium Pasteurella multocida. Studies on free range layer and organic broiler cases have shown that these outbreaks of fowl cholera are caused by a single genotypic type of P. multocida, with the same strain causing outbreaks at different time periods. This re-occurrence could be explained by a common source, such as the cat, re-introducing the strain at intermittent periods. This project looked at genotypes and serotypes of P. multocida from poultry from seven free range farms and P. multocida from cats from two of these farms. Genotyping was done by Multi Locus Sequencing typing, High Resolution Melt typing and Enterobacterial Repetitive Intragenic Concensus PCR, while for serotyping the Heddleston serotyping scheme was used. The results show that the diversity of P. multocida is greater than previously thought with more than one isolate involved in some outbreaks. The cat is a potential source for P. multocida as the same strain was found in chicken and cats during one outbreak of fowl cholera. These results point to the importance of serotyping/genotyping when outbreaks occur and that cats need to be eliminated from chicken farms.

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Construction and evaluation of an adenovirus/alphavirus replicon chimeric vectored vaccine against classical swine fever

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Classical swine fever (CSF), which is caused by classical swine fever virus (CSFV), is one of the most devastating epizootic diseases of pigs worldwide. Efficacious and safe attenuated vaccines have played a key role in CSF control, but they have some disadvantages, for example, they are often contaminated with bovine viral diarrhea virus (BVDV) and do not allow differentiation of infected from vaccinated animals (DIVA principle).
Previously, we showed that prime-boost immunization using an adenovirus-vectored vaccine rAdV-E2 and an alphavirus replicon-vectored DNA vaccine pSFV1CS-E2, but none of the two vaccines alone, induced complete protection from virulent challenge [1-3]. In this study, we used the adenoviral vector to deliver the alphavirus replicon vector and constructed an adenovirus/alphavirus replicon chimeric virus expressing the E2 protein of CSFV. Pigs

immunized with rAdV-SFV-E2 (*n* = 5) developed robust humoral and cell-mediated responses to CSFV and were completely protected from subsequent lethal CSFV infection clinically and virologically. The level of immunity and protection induced by rAdV-SFV-E2 was comparable to that provided by the currently used live attenuated vaccine, C-strain. By contrast, both the conventional alphavirus replicon-vectored vaccine pSFV1CS-E2 and conventional adenovirus-vectored vaccine rAdV-E2 provided incomplete protection.
We further evaluated the safety and efficacy of the chimeric virus with respect to different immunization times and doses, pre-existing antibodies to the adenovirus vector, maternal antibody interference and combination immunization with PRV vaccine. The results showed that the minimal immunization dose is 6.25Ã—10^5 $TCID_{50}$ in pigs. A single immunization provided complete protection against lethal CSFV challenge, though specific neutralizing antibodies were undetectable before challenge. The chimeric virus can overcome CSF maternal antibody interference and immunization with the chimeric virus did not affect re-immunization of adenovirus-vectored vaccines. When the chimeric virus was co-administered with pseudorabies vaccine, all immunized pigs developed high-level specific antibodies against CSFV and PRV comparable to either vaccine administered alone.
The chimeric vector-based vaccine represents the first gene-based vaccine that is able to confer sterile immunity and complete protection against CSFV.

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Microbial ecological dynamics in the pathogenesis of a model polymicrobial disease: The impact on vaccine design and therapeutics

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Periodontal diseases (PD) involve inflammation and/or necrosis of the tooth and the adjoining tissues of the oral cavity and are largely due to polymicrobial aetiology associated with ecological changes in the microbial community. Most polymicrobial diseases are difficult to manage and known to re-occur after vaccination or treatment, leading to continuous ill-health, extinction and economic loss.

To understand the aetiology of periodontal diseases in macropods, culture dependent methods have largely been employed, with limitations due to the great-plate-count anomaly coupled with the inability to directly associate isolated microbes with disease or healthy condition. This brings to question, the identification of the major aetiological agents of the disease; the choice of immuno-protective antigens for vaccine design; and the choice of appropriate antimicrobial agent(s) for the effective management of the disease.
In this study, molecular ecological analysis and DNA barcoding were employed to determine the associated aetiological agents in the pathogenesis of oral necrobacillosis (ON) from an ecological perspective. ON is a chronic form of periodontal disease which has been recognized as one of the most significant diseases responsible for most ill-health and death in macropods. Using in vitro assays and statistical analysis, the microbial ecological changes/dynamics and bacteria-fungal interactions associated with periodontal health and PD in macropods were examined. The impact on vaccine design and therapeutics are discussed.

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The F17-like Fimbrial Protein GalF-A from *Gallibacterium anatis* is a Virulence Factor and Potential Vaccine Candidate

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The Gram-negative bacterium *Gallibacterium anatis* is a recently recognized, yet major, cause of salpingitis and peritonitis in egg-laying chickens, leading to decreased egg-production and animal welfare issues worldwide. Insight into the pathogenesis of disease and knowledge of important virulence factors is urgently required to combat high levels of drug resistance and antigenic diversity. Recently, varying numbers of loci encoding putative F17-like fimbriae were identified in the genomes of *G. anatis*. The objective of this study was to investigate the basic characteristics of the F17-like putative fimbrial protein precursor GalF-A and its immunogenic potential. In vitro expression and surface-exposure of GalF-A was demonstrated by flow cytometry and immunofluorescence microscopy, and the predicted function of GalF-A as a F17-like fimbrial subunit was confirmed by immunogold electron microscopy. A fimbrial knock-out mutant was generated and used to confirm the in vitro results. Importantly, the mutant was significantly attenuated, as compared to the wild-type, following intraperitoneal challenge of the natural host, the chicken. Finally, in vivo immunization with GalF-A in chickens demonstrated an 80% protection rate, thus a clear immunogenic potential. In conclusion, we describe the first fimbrial protein precursor from *G. anatis* and demonstrate its potential as a future vaccine candidate.

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Successful field vaccination with a larval-specific antigen of the gastrointestinal nematode, Haemonchus contortus

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Haemonchus contortus is the most pathogenic of the gastrointestinal nematode parasites of sheep with a worldwide distribution. Most successful vaccines currently available for viral, bacterial or protozoal infections accelerate the development of immunity naturally acquired by the host after infection. Natural immunity to gastrointestinal nematode (GIN) parasites develops in ruminants after repeated infections, and most animals are refractive to infection after 1-2 grazing seasons. This indicates that the development of GIN vaccines based on â€˜naturalâ€™ antigens, is an achievable goal. By focusing on the local antibody response generated in the lymph nodes draining the infected tissue (abomasum or true stomach), we were able to identify and isolate a surface antigen, specific to the infective larval stage (HcsL3). Antibodies generated against HcsL3were able to mediate larval killing by eosinophils in an in vitro assay and vaccination with HcsL3 significantly reduced worm burden after challenge in a small experimental pen trial.
Translation of experimental vaccine studies to the field is not always successful as it has to comply with commercial realities, including fewer vaccinations with licensed adjuvants, and using animals with varying levels of exposure in the field. In the present study, we performed a larger dose-response trial with the HcsL3antigen by vaccinating sheep in the field using a commercially acceptable adjuvant and vaccination protocol. The results suggest that sufficient protection against infection may be achievable with this vaccine regimen under acceptable management practices.

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Investigations of the antigenic differences between the EG95-G1 vaccine and related protein from the G6 genotype of *Echinococcus granulosus*

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The EG95 vaccine is based on a recombinant protein which native antigen is encoded by four genes in the G1 genotype *of E. granulosus*. The EG95 vaccine has been successfully used under experimental conditions raising protective immune response in intermediate hosts against the challenge with *E. granulosus* from the same G1 genotype. No information is available about the ability of the current EG95 vaccine to elicit a protective immune response against other genotypes of *E. granulosus*. Using genomic DNA cloning techniques, seven *eg95*â€“related genes were characterised from the G6 genotype of *E. granulosus*. Three proteins are predicted to be encoded by these genes. Investigations were undertaken to determine the ability of two of these proteins to react with specific antibodies in the sera of sheep vaccinated with EG95 and shown to be protected against a challenge infection with the G1 genotype. Proteins from the G6 genotype displayed only limited antigenic cross-reactivity with the current EG95 vaccine antigen, suggesting that the current vaccine may not protect animals against an infection with parasites of the G6 genotype. Data presented provides the information that would enable a G6 genotype-specific vaccine to be developed against *E. granulosus*.