MEETING

Ole Sereni Hotel, Kenya

26 MAR

27 MAR

International Veterinary Vaccinology Network

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Welcome

Dear delegates,

We are delighted to welcome you to Nairobi for the first annual meeting of the International Veterinary Vaccinology Network (IVVN). We hope that you are as excited about this event as we are, and that you will find the meeting rewarding, interesting and productive.

The veterinary vaccine research community embraces a large number of people with diverse expertise, skills and knowledge. We hope that the IVVN annual meetings will enable individuals from across the community with complimentary expertise to meet, and exchange ideas, that will form the basis of novel approaches to solve challenges that are preventing the development of vaccines for the many diseases that impact on the food and financial security of farmers in low-and-middle income countries. To achieve this, we encourage you to use this opportunity to engage and discuss your work with as many other delegates as possible over the next two days.

The IVVN is still very much in its infancy and we hope to see it develop over the coming years to support those that work in veterinary vaccinology, including its academic, industrial and other members. The IVVN is community led, therefore it is essential that you tell us what you want from the Network, what you would like to see the Network offer, and how it could help you in your work to develop new vaccines. Please use this opportunity to let us know what you would like to see the Network achieve in the future.

We look forward to talking to you all over the next two days, and wish you all an enjoyable and productive meeting.

Tim, Bryan, Carly and Network Management Board.

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@IntVetVaccNet

Please use the hashtag
#IVVNKenya18
when tweeting during
the meeting

Agenda

0830 - 0930	**Registration**
0930 - 0945	**Welcome & Introduction** Dr. Vish Nene (ILRI) & Dr. Bryan Charleston (The Pirbright Institute)
0945 - 1000	**The International Veterinary Vaccinology Network** Dr. Tim Connelley & Dr. Carly Hamilton (The Roslin Institute)
	Theme 1: Vaccines for Zoonotic Diseases Chair: Prof Fiona Tomley
1000 - 1045	**Unravelling the pathogenesis of visceral leishmaniasis: a one-health, interdisciplinary research agenda** Prof Paul Kaye (The University of York)
1045 - 1130	**More effective vaccination strategies for Rift Valley fever in livestock in East Africa** Dr. John Gachohi (Washington State University Global Health Program – Kenya)
1130 - 1200	Tea & Coffee
1200 - 1220	**Brucellosis and the BactiVac Network** Dr. John McGiven (BactiVac & the OIE Brucellosis Reference Laboratory, APHA, UK)
1220 - 1240	**The respiratory virus community in households of rural coastal Kenya** Dr. Patrick Munywoki (KEMRI – Wellcome Trust Research Programme, Kenya)
1240 - 1300	**Vaccine needs for zoonotic diseases in humans and animals** Dr. Baptiste Dungu (MCI Sante Animale)
1300 - 1400	Lunch
	Theme 2: Veterinary Vaccine Production in Africa Chair: Prof Brian Perry
1400 - 1445	**Economic best practice in veterinary vaccinology** Dr. Lian Thomas (University of Liverpool)
1445 - 1515	**The Production of Good Quality Veterinary Vaccines In Africa: Challenges And Prospects** Dr. Nick Nwankpa (AU PANVAC)
1515 - 1545	**The use of next generation adjuvants in animal health** Dr. Caryn Fenner (Afrigen Biologics and Vaccines)
1545 - 1615	Tea & Coffee
1615 - 1630	**Strategic Role of KEVEVAPI in disease control in Kenya and neighbouring countries** Dr. Jane Wachira (KEVEVAPI)
1630 - 1645	**Control of Foot-and-Mouth Disease in Southern Africa: Current and future prospects in FMDV- SAT vaccinology** Dr. George Matlho (Botswana Vaccine Institute)
1645 - 1700	**MCI Sante Animale** Dr Khalid Tadloui (MCI Sante Animale)
1700 - 1715	**Global Vaccine Security - why it matters – with specific reference to FMD and re-thinking on vaccine supply for emergencies and to meet surge in demands** Dr. Keith Sumption (EuFMD Secretariat, Food-and Agriculture)
1715 - 1815	**Poster Session & Networking**
1930	**Dinner**

	Theme 3: Synthetic Biology in Vaccine Development Chair: Dr. Pip Beard
0900 - 0945	**Think Globally: Vaccine development using synthetic genomics** Dr. Lauren Oldfield (J. Craig Venter Institute)
0945 - 1015	**How could the power of the emerging area of Synthetic Biology be harnessed to enable veterinary vaccine development?** Prof Susan Rosser (University of Edinburgh)
1015 - 1045	Tea & Coffee
1045 - 1115	**Plug and Display' Virus-like Particle Platform using Spycatcher/ Spytag technology** Prof Sumi Biswas (The Jenner Institute)
1115 - 1145	**New Vaccinology Approaches to Fight an Old Foe, T. Parva: New Technologies Applied On The P67 Antigen** Dr. Anna Lacasta-Marin (International Livestock Research Institute)
1145 - 1310	Lunch & Networking
	Theme 4: Livestock Vaccines Here & Now Chair: Prof Sandra Adams
1310 - 1330	**STAR IDAZ IRC - Global Coordination of Animal Health** Dr. Sadhana Sharma (BBSRC)
1330 - 1400	**Introduction to the African Livestock Productivity & Health Advancement (A.L.P.H.A.) initiative, co-funded by Zoetis and the Gates Foundation to work towards sustainable livestock production in Sub-Saharan Africa through animal health improvement** Dr. Gabriel Varga (Zoetis)
1400 - 1420	**Vaccine Production: Experiences of a Start-up** Dr. Brian Bigirwa (Brentec Vaccines)
1420 - 1440	**The Livestock Vaccine Innovation Fund at IDRC** Dr. Victor Mbao (Livestock Vaccine Innovation Fund)
1440 - 1510	**PPR Global Eradication Programme: contributing to food security, poverty alleviation and resilience** Dr. Bouna Diop (FAO)
1510 - 1540	Tea & Coffee
1540 - 1600	**The Brucellosis Vaccine Prize: an incentive for innovation in Animal Health** Prof Brian Perry (Afrique One Aspire)
1600 - 1700	**Livestock vaccination as a driver to achieve the Sustainability Development Goals** Prof Guy Palmer (Washington State University)
1700 - 1715	**Concluding comments** Dr. George Warimwe (KEMRI – Wellcome Trust Research Programme, Kenya)

Speaker Abstracts

Theme 1: Vaccines for Zoonotic Diseases

Unravelling the pathogenesis of visceral leishmaniasis: a one-health, interdisciplinary research agenda

Professor Paul Kaye, University of York

Leishmaniasis is one of the most important of the neglected diseases of poverty, affecting some 12M people in 98 countries worldwide but with a disproportionate impact in lower and middle-income countries. This parasitic disease may present as chronic skin lesions, with or without mucosal involvement (tegumentary leishmaniasis) or as a systemic multi-organ disease (visceral leishmaniasis or kala azar) that is usually fatal without treatment. Transmitted by the bite of female phlebotomine sand flies, leishmaniasis may be zoonotic, commonly with rodents and canids as reservoir hosts, or anthroponotic. Canine VL is an important veterinary problem in its own right, with CVL endemic in over 70 countries, often with prevalence rates in double figures. Drugs to treat leishmaniasis are limited, have variable efficacy and may cause serious side effects. Whilst vaccines for CVL are registered in Brazil and Europe, progress in developing vaccine for humans has been frustratingly slow.

In this talk, I will discuss recent data derived from mouse, hamster, dog and humans that is starting to provide a clearer picture of the pathogenesis of visceral leishmaniasis and how these findings are being used to develop a translational research agenda that truly encompasses a one-health approach. Drawing on recently published and unpublished data, I will summarise how exploiting interdisciplinary approaches such as computational modelling can add value to this research, by posing new questions and challenging old assumptions. I will also summarise the planned activities of the MRC/BBSRC Vaccines RD Network VALIDATE, that is devoted to enhancing research on vaccines for complex intracellular pathogens (initially TB, leishmaniasis, meliodosis and leprosy; **www.validate-network.org**).

More effective vaccination strategies for Rift Valley fever in livestock in East Africa

Dr. John Gachohi, Washington State University Global Health Program – Kenya

Rift Valley fever (RVF) is a viral zoonosis associated with febrile syndromes in humans and widespread abortions in livestock. Its outbreaks cause wide-ranging socio-economic losses mainly due to embargoes on livestock trade, abortion and neonatal mortality in sheep, goats, cattle and camels. Vaccination of livestock has been a key intervention for controlling these outbreaks, but to date, no effective RVF vaccination strategies have been developed. This work uses multiple approaches to identify reliable RVF vaccination strategies that can be used in East Africa. Through stakeholder consultations, three alternative approaches have been identified, namely (i) annual vaccination in high risk areas, (ii) reactive vaccination during periods of heightened risk, and (iii) intermittent vaccination in high risk areas at intervals of 3 years. Mathematical models have also been developed to determine benefits and costs of each strategy. In addition, field studies are being implemented in a pastoral area in Kenya to generate data for parameterizing these models. So far, the data collected demonstrate a high population turn-over of vaccinated animals which would, in a short period, erode the herd immunity that was established when animals were vaccinated at the start of the study. We discuss these observations and highlight that reliable vaccination strategies that accommodate the dynamics of the target production system should be developed to ensure that livestock vaccines generate the expected impacts.

Brucellosis & the BactiVac Network

Dr. John McGiven, BactiVac & the OIE Brucellosis Reference Laboratory, APHA, UK

The BactiVac Network was established at the same time as the IVVN and has the same funding source. Its aim is to accelerate the development of vaccines against bacterial infections relevant to low and middle-income countries. This will be delivered through the dissemination of information, catalyst projects and training awards to encourage collaboration between academic and industrial sectors and promote partnerships between developed and developing nations. The network is not exclusive to human vaccine development. Bacterial diseases that have one-health implications are within the scope of the network. This includes zoonoses as well as those that have significant indirect human health impact. Projects that develop new knowledge and techniques in bacterial vaccinology and strengthening of networks between LMICs and UK partners are of great interest to BactiVac. Joining the BactiVac Network will enable you to contribute to and benefit from a diverse network of experts in all areas of bacterial vaccine production, from R&D, licensure and implementation.

Brucellosis is an example of a disease where solutions to control may be expedited by membership of BactiVac as well as the IVVN. It is an endemic disease in many LMIC countries and vaccination is an essential component of control. However existing vaccines have considerable drawbacks including retention of significant pathogenicity and the most effective confound serological diagnosis. We received a phase 1 AgResults award to develop a novel, DIVA compatible, glycoconjugate vaccine concept by applying lessons learnt from the production of human vaccines.

See the webpage at :
www.birmingham.ac.uk/research/activity/immunology-immunotherapy/research/bactivac/index.aspx

The respiratory virus community in households of rural coastal Kenya

Dr. Patrick Munyoki, KEMRI-Wellcome Trust Research

Households provide a high intensity close-contact environment favorable for the transmission of respiratory pathogens. Few studies have characterized intra-family spread of respiratory viruses. Improved study design and methods now enable more detailed elucidation of transmission patterns.

Data are from active surveillance of 47 households in rural coastal Kenya over six months during a respiratory syncytial virus (RSV) seasonal outbreak. A total of 16,918 deep nasopharyngeal swabs (NPS) were taken from 483 household members, sampling twice-weekly irrespective of symptoms. By multiplex real time PCR assay, a sub-sample of swabs were screened for 15 respiratory virus targets, and then all samples were screened for viruses most common during the surveillance period, i.e., rhinovirus (hRV), human coronaviruses (hCoV), adenovirus (AdV) and RSV (A and B).

Of the NPS tested, 4,259 (25.2%) were positive for ≥1 of the common respiratory viruses tested, of which 599 (14.1%) had co-infections. The detected viruses in order of frequency were hRV (1780, 10.5%), hCoV (1274, 7.5%), AdV (1232, 7.3%), RSV (537,3.2%). On average, each household and individual had six and three different viral infections over the study period, respectively. HRV and hCoV were detected in all the 47 households while AdV and RSV were detected in 45 (95.7%) and 40 (85.1%) households, respectively. The individual crude attack rates were 93.4%, 80.1%, 71.6%, 61.5% and 37.1% for any virus, hRV, hCoV, AdV and RSV, respectively.

Co-circulation of and co-infections with respiratory viruses are highly prevalent suggesting a strong possibility of interaction, and emphasizing problems with identifying disease causality.

Vaccine needs for zoonotic diseases in humans and animals

Dr. Baptiste Dungu, MCI Sante Animale, Morocco & INAND, Initiative for Neglected Animal Diseases

It is estimated that over 850 infectious agents can cause zoonotic diseases or infections naturally transmitted between vertebrate animals and humans. These infectious agents could be viruses, bacteria, parasite, fungi or infectious proteins. Different criteria are used to classify zoonotic diseases. Of interest are the Neglected zoonotic diseases (NZD), which affect mainly communities and livestock in low-resource settings worldwide.

Vaccines and vaccination are among the most effective ways of controlling and preventing diseases, and for zoonotic diseases, vaccines can be potentially used in humans, animals, or both. While human vaccines are more expensive to develop and necessary for acute zoonoses with high mortality, animal vaccines can be brought to market at lower cost and faster.

The control of most NZD is hampered by several factors, including the availability and access to vaccines and other control tools. Though research toward potential new vaccines has been ongoing, the full development and commercialisation of new NZD vaccines does not often follow, due to generally limited market prospects. The access to existing vaccines by affected communities can be improved through vaccine characteristics, such as thermotolerance, one-shot vaccines, multivalence, or through public and official initiatives including supported vaccination programs, vaccination activities integrated to other human or animal health interventions, or vaccine stockpiles. Awareness activities targeting affected communities, preferably covering both public health and animal health, are always required in order to improve the adoption of control and vaccination programs. The existence of international guidelines for NZD control which include the use of vaccines, can guide governments in the implementation of NZD control and vaccination activities. The adoption of NZD vaccines in affected communities requires therefore the availability of such vaccines, their access, awareness and demand by the end user.

Theme 2: Veterinary Vaccine Production in Africa

Economic best practice in veterinary vaccinology

Dr. Lian Thomas, University of Liverpool

The World Health Organization has cost-effectiveness guidelines and there have been several excellent economic evaluations of human vaccination programmes using real-world costing and effectiveness data. The information generated from such analyses allow policy makers, implementing partners, industry and researchers to make evidence based decisions on human vaccination programmes. The paper will ask whether there is anything similar in the veterinary vaccinology system, and through this process raise what needs to be thought about to improve vaccination programmes in the future.

The paper will discuss the points within the vaccine development cycle at which economic tools are most useful. It will use the example of rotavirus vaccination in Malawi, and the ability of a prospective cohort study to fully capture the costs of disease at the individual, household and healthcare provider levels as well as the program delivery costs. We will cover the different value propositions by which vaccine programs may be evaluated and ways in which we can improve the collection of both cost and outcome data for veterinary vaccine programs.

The Production of Good Quality Veterinary Vaccines in Africa: Challenges And Prospects

Dr. Nick Nwankpa, AU PANVAC

Animal diseases continue to be major constraints to efficient production of livestock especially in Sub Saharan Africa where they threaten livelihoods and food security. While the stamping out method, vaccination and the therapeutic or prophylactic use of drugs all play important roles in animal disease control, vaccination is considered as the more sustainable choice being less costly and less likely to result in disease resistance in the field. But the production and availability of good quality Veterinary vaccines in Africa has been faced with many challenges since the early days of the rinderpest eradication campaigns. While much progress has been achieved in recent years through the activities of PANVAC in the establishment of International Independent Quality Control of vaccines and greater partner support and collaboration in the provision of equipment and facilities to enhance capacity in most vaccine producing laboratories in Africa, most of them still face problems such as inadequate resources, lack of access to modern equipment, Inadequate electric power supply, lack of access to good quality media and reagents, inadequate capacity building and competence drain; and a host of others. These problems have compelled many vaccine producing laboratories to perform far below their capacities and even the viable ones among them have been stretched beyond limits. In order to ensure sustainability, there is a need for Governments to take full ownership and responsibility by putting in place appropriate financial, legal and regulatory frameworks for the operation and management of these laboratories. There is also a need for the support of the global partners in providing funds and proffering innovative solutions that will place the laboratories on the right footing to sustainability taking into consideration the challenges and gaps in the current vaccine production environment.

The use of next generation adjuvants in animal health

Dr. Caryn Fenner, Afrigen Biologics and Vaccines

Adjuvants have proven to be key components in human and veterinary vaccines, as they are often necessary to induce an early, strong and long-lasting immune response. Advancements in the understanding of innate and adaptive immune systems led to the discovery of more powerful adjuvants. These immune-potentiating adjuvants stimulate the innate immune system by interacting with pathogen-associated molecular patterns, and are used to enhance and modify desired antigen-specific immune responses. The formulation of adjuvant ligands should also be important consideration, as different formulations of the same immunomodulatory molecules may induce substantially different immune responses. Therefore, next generation adjuvants can comprise immunostimulatory molecules, formulated with first generation adjuvants such as aluminium salts, liposomes, oil emulsions and microparticles. The use of next generation adjuvants is explored extensively in human health and are currently in different stages of clinical trials, ranging from infectious diseases to cancer. In contrast, the implementation of such adjuvant formulations in veterinary vaccines have been limited, as most veterinary vaccines are still predominantly based on live attenuated or inactivated pathogens that do not require potent adjuvants. This landscape is changing, due to the challenges and safety concerns related to the use of live attenuated or inactivated vaccines. Veterinary vaccine developers are now increasingly using recombinant technologies to produce vaccines, and therefore require more potent adjuvants. In this presentation the current and future developments of modern adjuvant formulation use in veterinary vaccines will be discussed, including other important aspects, such as costs and accessibility, which are crucial considerations for vaccine development for developing countries.

Strategic Role of KEVEVAPI in disease control in Kenya and neighbouring countries

Dr. Jane Wachira, KEVEVAPI

Kenya Veterinary Vaccines Production Institute (KEVEVAPI) was established as a parastatal on 5th March 1990 by Legal Notice No. 223 under Cap 446 of the laws of Kenya. Three vaccine production laboratories were integrated to form an institute under a Board of Management and a Managing Director.

Vision
A globally recognized institute in the production and supply of high quality and affordable veterinary vaccines and services.

Mission
To produce safe, efficacious and affordable veterinary vaccines through undertaking research, providing information, marketing and distribution for improvement of the livestock industry.

Strategic Role Of Kevevapi
KEVEVAPI produces vaccines against major livestock diseases in Kenya and the neighbouring countries. It plays a significant role in disease control by working closely with the National and County Governments. KEVEVAPI has provided vaccines during emergencies caused by climatic conditions such as Elnino and Draught Recovery Programs. Vaccines produced by KEVEVAPI are to ensure that Kenyan livestock resource is preserved. Animal resource according to 2009 census: Cattle - 17.5m (Beef & Dairy), Sheep - 17.1m, Goats - 27.7m, Chicken - 41.5m. Total: 103.8m

Vaccines produced by KEVEVAPI are:
1. Foot and Mouth Disease 2. Contagious Bovine Pleuro-pneumonia 3. Rift Valley Fever
4. Lumpy Skin Disease 5. Contagious Caprine Pleuro-pneumonia 6. Sheep and Goat Pox 7. Bluetongue 8. Orf(Contagious Ecthyma) 9. PPR 10. Newcastle 11. Fowl typhoid 12. Fowl pox 13. Turkey pox.

Control of Foot-and-Mouth Disease in Southern Africa: Current and future prospects in FMDV- SAT vaccinology

Dr Onkabetso George Matlho, Botswana Vaccine Institute

Foot-and-mouth disease (FMD) is a highly infectious transboundary animal disease of great economic importance affecting cloven-hoofed livestock (cattle, sheep, goats and pigs) as well as cloven-hoofed game animals. The disease is caused by a single stranded RNA virus in the genus Aphthovirus and family Picornaviridae. There are seven immunologically distinct serotypes of the virus, namely A, O, C, Asia 1, and the Southern African territories (SAT) – 1, 2 and 3; immune response to any of these serotypes does not confer protection to another serotype. Defined by geography, Southern Africa is the southernmost region of the African continent and includes several countries, namely; Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Zambia and Zimbabwe. FMD is endemic in Angola, Malawi, Mozambique, Zambia and Zimbabwe. The African buffalo (Syncerus caffer) is the maintenance host of the SAT viruses in Southern Africa. Namibia and South Africa have zones with FMD-free with vaccination and the virus is present in game parks. Lesotho and Swaziland which are engulfed by South Africa are FMD-free without vaccination but the virus is also present in buffaloes in the game parks. Botswana is unique in that it has multiple controls zones; FMD-free without vaccination and FMD-free with vaccination; in the latter, the viruses circulate in buffaloes in various game parks.

FMD outbreaks occur sporadically in most countries of the Southern Africa region. Over the recent past four years (2014 – 2017), samples collected from outbreaks suspected to be FMD in cattle from various countries within the region were submitted to the OIE sub-Saharan Africa Regional Reference Laboratory for FMD (OIE-SSRRLFMD) housed and administered by Botswana Vaccine Institute (BVI) Ltd for confirmatory diagnosis. Almost all the outbreaks were confirmed to be FMD and were caused by SAT serotypes; out of the confirmed cases (n=29), 65.52%, 24.14% and 10.34% were caused respectively by SAT2, SAT1 and SAT3.

Phylogenetic analysis of the FMDV sequences generated at BVI was conducted by the World Reference Laboratory for FMD (WRLFMD) Pirbright Institute and revealed the new viruses to be closely related to same type of viruses isolated previously from the region. Similarly, antigenic analysis by vaccine matching using the two-dimensional virus neutralization test (2dmVNT) gave relationship coefficients (r1 values) of >0.3 indicating that the vaccine virus was most likely going to confer protection to the field virus. Indeed, this was the situation as all the outbreaks were controlled through vaccination. On the basis of both the genotyping (phylogenetic analysis) and vaccine matching results, it is concluded that, the viruses that have been in circulation over the past four years have not significantly changed antigenically; and therefore the vaccine strains hitherto used for route production of SAT vaccines at BVI are deemed still relevant.

Both conventional and highly purified FMD (Differentiate Infected from Vaccinated animals – DIVA) vaccines are produced by BVI Ltd. The conventional FMD vaccine is marketed as aftovax while the highly purified FMD vaccine is marketed as aftovaxpur. In addition to being a DIVA vaccine, the aftovaxpur has high antigen payload (superconcetrated) and this permits a rapid onset of immunity thus arresting field outbreaks within short periods of time. The aftovaxpur is however is marketed at a premium than the atfovax and for this reason most countries have been procuring the aftovax; either as bivalent (SAT1 & SAT2) or trivalent (SAT1, SAT2 & SAT3). Nevertheless, the countries are advised to shift from the conventional to the DIVA vaccine in order to unlock the potential for international trade of meat, milk and other livestock products to lucrative markets outside the African continent, particularly trade with Europe and USA. The potential has been locked by the inability of the countries to provide evidence beyond doubt that their livestock (cattle) are free from infection with FMD virus.

Research and development has recently been launched at BVI to develop and commercialise industrial production of genomic-based vaccines using plants as bioreactors. This research is undertaken in collaboration with national and international research/academic institutions. Already, a genomic based vaccine against Lumpy skin disease (LSD) virus has been successfully developed and its immunogenicity proven under laboratory and field conditions. The candidate vaccine in is now undergoing further evaluation and patenting before commissioning it for industrial production. Based on similar technology, BVI is currently pioneering to develop a genomic based FMD (SAT) vaccine, which if successful should provide a better DIVA vaccine; probably a gold standard.

MCI Santé Animale

Dr. Khalid Tadlaoui, MCI Santé Animale

The presentation will be about "MCI Santé Animale": an African GMP vaccine factory.

Global Vaccine Security - why it matters – with specific reference to FMD and re-thinking on vaccine supply for emergencies and to meet surge in demands

Dr. Keith Sumption, EuFMD Secretariat, Food-and Agriculture Organization of the UN, Rome

Driving almost all our international work is the simple fact that we do not have vaccine security; we live with the fear that suitable and effective vaccine will not be available when needed, or if available, will not be effective when used, or achieve the outcomes desired. In FMD free regions, the "'standard model"" of vaccine banks is being challenged by the potential scale of need and the diversity of circulating FMD strains. In regions not free of FMD, the efforts being made for ""risk based vaccination"" and optimizing control measures relate to the lack of vaccine security; in that the lack of availability of quality vaccines leads to public and private stakeholders facing epidemics without secure access to vaccine. Lack of supply also leads to continued use of ineffective vaccines, with disappointing results- for the animal producers as well as nationally. Vaccine security would mean the confidence that vaccines are affordable, available and effective and accessible by stakeholders. Global vaccine security would mean security to respond with vaccines to face any threat where they may be required; a need of the ""free countries"" as well as endemic regions.

Without vaccine security, we need elaborate and well drilled preparedness for an FMD emergencies, to contain incursions before they outstrip vaccine supply. In endemic regions of Africa, mid-east and Asia, millions of animals – and their owners- cannot access effective vaccines when they need them – so lack of supply does matter for food security and livelihoods. Addressing supply issues at global and regional scale requires progress on vaccine technologies as well as production capacities. It also requires progress to understand the market forces for demand for vaccine at field level, and on financing mechanisms that reduce the risk for investors who attempt to increase the supply side.

Global security of supply of FMD vaccines therefore affects all countries – so what can be done about it? and how do we manage the risks without it?

The paper will cover one of the initiatives of the EuFMD to address the lack of supply of FMD vaccines for emergencies - the development of a "'assured emergency supply option "" (AESOP), and to enter into long term agreements with vaccine suppliers to hold increased stocks of vaccine antigens for emergency and preventive responses. Addressing the lack of supply of FMD (and other vaccines) is a critical issue for achieving global vaccine security, and will involve multiple players to co-ordinate efforts to increase investment both in vaccine supply, which includes the issue of bringing new technologies into use. These issues will be a focus for the EuFMD Open Session 2018 , to be held in Puglia, Italy, 29-31st October, and all are invited who can help co-ordinate efforts to achieve vaccine security.

Theme 3: Synthetic Biology in Vaccine Development

Think Globally: Vaccine development using synthetic genomics

Dr. Lauren M. Oldfield, J. Craig Venter Institute

New and improved vaccines are needed for many human and animal diseases worldwide. Synthetic genomics techniques offer new tools to engineer bacteria and viruses on a genome-wide scale to study their basic biology and to design rationally attenuated vaccines. As a part of the JCVI project to generate a bacterial cell controlled by a chemically synthesized genome, genetic tools were developed to manipulate Mycoplasma mycoides subspecies capri (Mmc) and other closely related Mycoplasma species. We are working to transfer these tools to Mycoplasma mycoides subspecies mycoides (Mmm), the causative agent of contagious bovine pleuropneumonia (CBPP), and to generate a rationally attenuated vaccine candidate strain. CBPP is a serious issue for smallholder farmers in developing nations and an improved vaccine is of economic importance. As a proof of principle, we used yeast-derived genetic tools to delete 68 genes hypothesized to be involved in virulence in Mmc and the deletion strain had decreased virulence when administered to goats. Also, the attenuated MmcΔ68 can be used as a vaccine vector to express Mmm antigens as an alternative strategy for vaccine development. We are also applying these synthetic genomics strategies to engineer virus genomes. Recently, we published a report of the assembly and manipulation of the herpes simplex virus type 1 genome from genomic fragments and are expanding this technique to other large double-stranded DNA viruses, including viruses of livestock animals without effective vaccines, such as African swine fever virus. Our aim is to improve the genetic tools available for these infectious agents, which may increase our understanding of their basic biology and virulence and allow for rationally designed vaccine candidates.

How could the power of the emerging area of Synthetic Biology be harnessed to enable veterinary vaccine development?

Professor Susan Rosser, University of Edinburgh

"Synthetic biology aims to design and engineer biologically based parts, novel devices and systems as well as redesigning existing, natural biological systems."

In this presentation I will give a brief overview of the emerging synthetic biology techniques and technologies that could be applied to veterinary vaccine development. I will also introduce the Edinburgh Genome Foundry (EGF), a £5.3M RCUK national synthetic biology facility focusing on automating DNA design, assembly and characterization. The EGF aims to manufacture genetic material on an unprecedented scale, using a fully automated robotic platform. It has a particular focus on high-throughput projects involving large sequences (up to 1 mega base), high numbers of genetic parts, or combinatorial libraries.

Plug and Display' Virus-like Particle Platform using Spycatcher/ Spytag technology

Professor Sumi Biswas, The Jenner Institute

The Spycatcher/Spytag biochemical superglue enables the rapid and efficient development of highly immunogenic virus-like-particles (VLPs) against target pathogens. The Spy (from Streptococcus pyogenes) protein was divided into a peptide, SpyTag, and a protein partner, SpyCatcher. Once combined, the two react to form a spontaneous irreversible isopeptide bond. VLP technologies are particularly suited for the induction of strong antibody responses. The Spycatcher/Spytag technology has been used to establish a novel chimeric VLP vaccine platform technology for rapidly and irreversibly decorating VLPs simply by mixing with protein antigen in a "Plug-and-Display" manner. This approach overcomes the well-described challenges of producing VLP carriers with complex antigens genetically-fused on their surface, or low conjugation efficiency often reported when using chemical conjugation. The application of this technology for the development of malaria vaccines and outbreak pathogens will be discussed.

New Vaccinology Approaches To Fight An Old Foe, T. Parva: New Technologies Applied On The P67 Antigen

Dr. Anna Lacasta, International Livestock Research Institute

East Coast fever (ECF) is a lethal disease of cattle caused by the parasite Theileria parva. It is economically important in eastern, central and southern Africa. The current disease control vaccine strategy is based on a live parasite infection and treatment (ITM) method. Because of its limitations, efforts are underway to develop a subunit vaccine. Among various trials, an anti-sporozoite vaccine based on the p67 antigen has given consistent immunity (~50%) to ECF. Current efforts are focused on improving the vaccine efficacy of fragments of p67, in particular p67C, a 80 amino acid C-terminal peptide of p67, as they have been easier to express, and they prime similar levels of immunity as full length p67.

Antigens in a particle format are often more immunogenic than soluble proteins. Hence, we are testing new nanotechnology approaches with p67C. The first system is through presentation of protein via mesoporous silica vesicles as an antigen carrier.

The second system is through formation of virus-like particles (VLPs) using the hepatitis core antigen (HBcAg) system. The third system is another VLP system that can be configured to express different levels of more than one antigen and incorporates B-cell stimulatory ligands, e.g., C3d or CD40L. Details will be presented on these antigen delivery systems and immunogenicity data from the first two. Briefly, HBcAg-p67C VLPs prime high antibody titers with sporozoite neutralizing capacity, while p67C- silica vesicles prime high CD4+ responses, both immune parameters that are likely to contribute to immunity.

Theme 4: Livestock Vaccines Here & Now

STAR IDAZ IRC - Global Coordination of Animal Health

Dr. Sadhana Sharma, BBSRC

STAR-IDAZ International Research Consortium on Animal Health (IRC) is a network of animal health research funders and programme managers, supported by a Secretariat funded under the European Commission Horizon 2020 programme. It aims to coordinate research at international level to address research needs, share results and together deliver new and improved animal health strategies for at least 30 priority diseases, infections or issues. Deliverables include candidate vaccines, diagnostics, therapeutics and other animal health products, procedures and/or key scientific information and tools to support risk analysis and disease control.

The IRC involves 25 partner organisations/regional consortia from 16 countries including the World Organisation for Animal Health (OIE), European Commission, Bill and Melinda Gates Foundation (BMGF) and three industry bodies with a combined 5-year research budget in excess of $US 2.5 billion. In addition, there is further engagement of 30 countries through regional networks from Europe, Asia and Australasia, the Americas and Africa and the Middle East.

IRC is bringing together working groups of global experts to develop research roadmaps for key priority diseases/ issues based on Lead model. These interactive maps, intended to support researchers and funders to understand key stages and bottlenecks, will provide a structure to gap analysis activities as each "lead" on the roadmap will be underpinned by a "lead summary". The lead model and the draft veterinary vaccinology roadmap will be discussed.

Introduction to the African Livestock Productivity & Health Advancement (A.L.P.H.A.) initiative, co-funded by Zoetis and the Gates Foundation to work towards sustainable livestock production in Sub-Saharan Africa through animal health improvement

Dr. Gabriel Varga, Zoetis Belgium SA

Livestock are an essential asset to rural communities, and the health of livestock is critical to achieving food security in regions where there is exceptionally high incidence of livestock and human disease. In Sub-Saharan Africa (SSA) which has some of the highest human population growth rates in the world, livestock productivity must be improved to meet the needs of the growing population. However there are many serious constraints to animal health infrastructure in the livestock sector in SSA, including lack of access to veterinary medicines and products, poor rural extension services, limited sustainability of diagnostics services, and low education regarding animal health.

In order to make sustainable advancements to the animal health sector in SSA, the Bill & Melinda Gates Foundation partnered with the global animal health leader, Zoetis, co-funding a $14.4M 3-year grant with the key objective to advance sustainable livestock production in SSA. In the focal countries of Nigeria, Uganda and Ethiopia, the A.L.P.H.A. initiative will set-up sustainable diagnostics laboratories and outreach services incorporated into business hubs, with a large emphasis on stakeholder training and education. In addition, the objectives of the A.L.P.H.A. initiative include: the improved availability of important veterinary products by novel product registration, adaption of product packaging to improve affordability for the smallholder market, introduction of supply-chain certification schemes, delivery of business training to local stakeholders, and demonstration of the value of animal health improvement in local communities.

Vaccine Production: Experiences of a Start-up

Dr. Brian Bigirwa, Brentec Vaccines

In 2011, BRENTEC setup a pilot facility to manufacture a thermal-tolerant strain of the Newcastle disease vaccine in poultry. The journey from concept to market has been one of excitement and discovery. The logic behind starting with a Newcastle disease vaccine was based on the fact that statistically almost every household in the country kept poultry and that 90% of these households had no access to the existing vaccines on the market. Poultry is also considered a gateway into livestock production mostly because they multiply fast and offer multiple income sources. The lack of access to a quality vaccine product that was affordable has stalled the commercialisation of this resource at household level. BRENTEC will be sharing its experiences as a start- up pharmaceutical company, dealing with regulatory agencies, market systems and consumers in getting its first product to Market.

The Livestock Vaccine Innovation Fund at IDRC

Dr. Victor Mbao, Livestock Vaccine Innovation Fund

The Livestock Vaccine Innovation Fund (LVIF) is a 5.5 year initiative supported by the Bill & Melinda Gates Foundation, Foreign Affairs, Trade and Development Canada (DFATD), and Canada's International Development Research Centre (IDRC). The CAD 57 million Fund supports the development, production, and commercialization of innovative vaccines against neglected livestock diseases in Asia and Africa.

The LVIF is currently funding innovation and research to increase the efficacy and marketability of existing vaccines, develop new vaccines against neglected livestock diseases using cutting edge technology, and build effective partnerships between vaccine researchers and private sector manufacturers and distributors to accelerate commercialization and use. The goal of the Fund is to make quality vaccines more affordable, available, and acceptable to small-scale livestock farmers, and to facilitate their use at scale. In addition, the Fund seeks to accelerate the discovery and development of new livestock vaccines by encouraging experimentation in target species and targeting the vaccine development that is aimed at a product profile. LVIF is also supporting research that will lead to a better understanding of disease dynamics and their impact, as well as constraints to vaccine use by poor farmers. And by bringing together all stakeholders, from farmers to researchers, pharmaceutical companies, and regulators, the Fund is fostering a vibrant and sustainable livestock sector in sub-Saharan Africa and South Asia.

PPR Global Eradication Programme: contributing to food security, poverty alleviation and resilience

Dr. Bouna Diop, FAO

Small ruminants (sheep and goats) -2.1 billion head worldwide - are the primary livestock of many low-income food-deficit households. For livestock keeping households, sheep and goats are: a source of regular income; a means to capitalize savings; and a safety net during times of hardship. Selling animals or their products provides the necessary resources to access food, as well as educational and social services. Food products derived from sheep and goats are an essential part of the diet for many people around the world and contribute to overcoming malnutrition.

Sheep and goat milk and meat are of high nutritional value and provide high-quality protein, vitamins and minerals critical for cognitive development and physical strength. Furthermore, small ruminants are moveable assets that can be easily relocated in times of climatic stress or volatile security situations.

Peste des petits ruminants (PPR) is an acute, highly contagious disease caused by PPR virus, a member of genus morbillivirus of the family Paramyxoviridae affecting domestic and wild small ruminants. The disease is present in more than 70 countries throughout Africa, the Middle East, Asia and Europe. PPR causes annual economic losses of up to USD 2.1 billion, but the costs extend beyond monetary considerations. PPR adversely affects livelihoods, food security, and employment, including for women and youth and exacerbates poverty and malnutrition. In vulnerable countries and regions with high small ruminant smallholder populations, the loss of livestock can induce social and economic instability, contribute to the breakdown of civil society, provoke conflict and in the worst cases, even fuel terrorism.

Following the endorsement of the PPR Global Control and Eradication Strategy (Abidjan, Cote d'Ivoire, April 2015), FAO and OIE established in March 2016 a Joint PPR Secretariat to design the PPR Global Eradication Programme (PPR-GEP) and coordinate its implementation. The PPR GEP effort is framed as a 15 year process running through 2030. The plan for the first five-year phase of that effort is focused on countries where PPR is known to exist or where its status has never been assessed. It will involve activities to raise awareness among farmers, build their capacity to prevent and contain the disease, strengthen national veterinary health services and systems for control of PPR and other diseases, and implement targeted vaccination campaigns (number of animals to be vaccinated estimated to 1.5 billion). The programme approach encompasses a multi-country, multi-stage sequential process comprised epidemiological and socio-economic assessments, control, eradication and maintenance of PPRV freedom. The four stages correspond to a combination of decreasing levels of epidemiological risk and increasing levels of prevention and control. Regardless of the stage in which a country initially places itself, the country will be guided and supported to achieve the capacity needed for the implementation of five key elements of PPR prevention, control and eradication: the diagnostic system; surveillance system; prevention and control system; legal framework; and stakeholders' involvement. The PPR GEP will require more effective national Veterinary Services and will therefore support building their capacity using proven frameworks such as the PVS Pathway, as well as promoting activities geared towards reducing the prevalence of other prioritized small ruminant diseases. Furthermore, the PPR GEP will provide required technical assistance and coordination at regional and global levels. The PPR GEP goes beyond disease eradication alone– it also aims to improve national production models and help herders build the strongest, most resilient livelihoods with their animal resources.

The Brucellosis Vaccine Prize: an incentive for innovation in Animal Health

Professor Brian Perry, Afrique One Aspire

AgResults, a $122m multi-donor, multi-lateral initiative is creating incentives for / rewarding high-impact agricultural innovations that promote global food security, health and nutrition. These are pull mechanism projects rather than grant funding or aid, and were introduced at the 2010 G20 Summit as a commitment to exploring innovative, results-based methods of leveraging private sector innovation in developing countries.

The largest of these initiatives is the Prize for the development of a new vaccine for Brucella melitensis infection in small ruminants, and US$ 30 million has been allocated to this initiative.This presentation will present the results to date after the first year of opening the competition, and review the structure of the competition and the three milestones.

Livestock vaccination as a driver to achieve the Sustainability Development Goals

Professor Guy H. Palmer, Washington State University (USA) and the Nelson Mandela African Institution of Science and Technology (Tanzania)

On September 15, 2015 the General Assembly of the United Nations passed the resolution "Transforming our world: the 2030 Agenda for Sustainable Development". Better known as the Sustainability Development Goals (SDGs), these include 17 goals and over 160 targets. Although laudatory in their ambition and breadth, the SDGs lack a clear roadmap to achieve them. Improved vaccination—from development of new vaccines against both established and recently emergent pathogens through broad and equitable delivery—is not only essential to meet specific SDGs but can serve as a driver to generate additive and synergistic benefits across multiple SDGs. The benefits from vaccination provide the opportunity to improve health at its broadest and most lasting definition, not merely the absence of disease but a complete state of physical, mental, and social wellbeing. From this perspective, vaccination is essential to meeting a minimum of 13 SDGs, those addressing poverty, food, health, women, water, equality, economy, inequality, consumption, climate, marine and terrestrial ecosystems, and sustainability. Critically, to meet these goals the field of vaccinology must continue to innovate technologically but also to measure the impact of improved vaccines and immunization in its broadest contexts. This presentation will focus on the technological, economic, and social challenges, connections, and metrics needed for veterinary vaccines to drive progress in achieving the SDGs.

Poster Abstracts

1

Human brucellosis prevalence in occupationally exposed population in Punjab, India and its associated risk factors: Need urgent attention

JS Bedi[1]* and JPS Gill[2]

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Brucellosis undoubtedly is a public health concern primarily for population exposed to various livestock species. Not only the direct exposure of humans to the diseased animals is a matter of concern but people living in urban and sub-urban areas may also get exposed to brucellosis by drinking of raw milk and consumption of milk products made from unpasteurized milk. In present study, we targeted occupationally exposed high risk human population including veterinarians, veterinary pharmacists/inspector, animal attendants/artificial insemination workers, and dairy farmers/farm labor for blood sample collection to get the prevalence of brucellosis. To identify the risk factors the data was collected on age, sex, address, level of education, occupation, type of animal handled, raw milk consumption, presence of symptoms etc. in a structured questionnaire. During one year study from April 2015 to March 2016, we analysed 918 human serum samples by serological tests (RBT, SAT, ELISA IgG and ELISA IgM). The results revealed the human brucellosis prevalence of 13.8% in the occupationally exposed population. The RBT positive human samples (126), were further subjected to genus specific PCR using BCSP 31 gene targeting 223 bp, and 32 human samples were found positive by PCR indicating active brucellosis infection. The risk factors analysis using logistic regression model indicated occupation (Veterinarians; p=0.003 and Veterinary pharmacist; p=0.001), handling of dystocia (p=0.02) and aborted material (p=0.01) were appeared to be the most significant risk factors for human brucellosis.

High sero-prevalance of brucellosis in human indicating urgent need to formulate policies (mass vaccination and biosecurity practices) for prevention and control of this disease in country. The non-existence of human vaccine against brucellosis in human implies that controlling this disease in animals will directly lead to prevention in human.

2

Immunomodulatory activity of curcumin entrapped poly D,L-lactic-co-glycolic acid nanoparticles in mice

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Background: Studies have shown that curcumin from Curcuma longa has both medicinal and immunomodulatory properties. These activities have however been hindered by its low bioavailability. Meanwhile, nanoparticle has been shown to increase bioavailability of certain drugs. This study was therefore conducted to comparatively evaluate the immunomodulatory activity of free and nanoparticulate curcumin in mice.

Methodology: Animals were sensitized with 0.5 × 108 cells/ml Sheep Red Blood Cell (SRBC) and thereafter free and nanoparticulate curcumin were administered orally at doses of 5 and 10mg/kg/day for 10 days to healthy albino mice. The assessment of the immunomodulatory activity was carried out by determining the humoral and cell-mediated immune responses using haemagglutination and delayed type hypersensitivity assays respectively. Haematological components and some lymphoid organs of treated animals were further evaluated.

Results: The study showed that nanoparticulate curcumin stimulated higher early cell- mediated immune response at 5mg/kg and 10mg/kg when compared to the free drug and control. While nanoparticulate curcumin significantly stimulated primary humoral immune response with 9.00±1.00 antibody titre ($p<0.05$), the free curcumin suppressed the immunity with 3.33±0.67 antibody titre when compared to control. Similar result was observed with secondary humoral antibody titres. White blood cells production and weights of the lymphoid organs were also enhanced in the groups that received nanocurcumin.

Conclusion: This work shows that PLGA entrapped curcumin nanoparticle could increase bioavailability of curcumin for improved immunity.

Targeting East Coast fever: The approach of a subunit vaccine

Jerome Nyhalah Dinga
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East Coast fever (ECF) results in severe economic loss to livestock production, and kills 1 million animals each year. A well established, safe and affordable vaccine that can confer full and long lasting protection is still awaited. A live parasite-based vaccine, which is used to immunize the animals by an infection and treatment method (ITM), is available but its setbacks include laborious production, cold chain for delivery, etc. However, the strong immune response engendered by using the ITM vaccine indicates the feasibility of a successful recombinant vaccine against ECF and such antigens can be identified. Recent advances made in the development of a subunit vaccine against T. parva based on the p67 sporozoite surface antigen, induces protective immunity to ECF in 50% of vaccinated cattle, suggesting that more protective antigens need to be identified as it has been suggested for malaria.

A study on the apicomplexan parasite that causes malaria in humans, Plasmodium falciparum, identified an immunodominant antigen, UB05 (GenBank Accession Number DQ235690) (Titanji et al., 2009), which was shown to be a potential marker of protective immunity to malaria. The principle of the conservation of gene function suggests that most orthologous gene products play similar roles in closely related organisms. Conceivably, T. parva orthologue of UB05 could play a similar role in protective immunity to ECF. This hypothesis was tested and let to the identification of a previously T. parva hypothetical protein now termed TpUB05 (GenBank Accession Number KF875450) and proposed as a marker of protective immunity in ECF (Dinga et al., 2015). Covalently linking two antigens can create an adjuvant effect leading to a more potent bivalent vaccine candidate using immuno-epidemiology (Dinga et al., 2017) and pre-clinical studies (Dinga et al., unpublished). Hence, TpUB05 could be linked to p76c and tested in an attempt to develop a potent subunit vaccine candidate against East Coast fever.

4

Porcine Circovirus Virus Like Particle Production in Plants

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Plant expression systems are a viable technology to produce recombinant proteins as pharmaceutical agents and vaccine development. These systems are free of mammalian pathogens as well as cost-effective with rapid and scalable production potential. Plant expressed, self-assembled virus like particles (VLPs) may elicit strong antibody and cellular immune responses. Our objective in this study was to express the porcine circovirus type 2 (PCV-2) capsid protein (CP) in Nicotiana benthamiana plants so that expression would produce VLP's. Recombinant Agrobacterium tumefaciens delivered the modified pEAQ-HT vector which ensured high levels of transiently expressed foreign proteins in N. benthamiana. Immunoblotting confirmed the expression of PCV-2 CP which was optimised for days' post infiltration, and the OD600 of infiltrated recombinant A. tumefaciens. The self-assembled VLPs were purified by CsCl density purification and visualised by transmission electron microscopy. Optimum protein expression of the 27-kDa PCV-2 CP was determined and PCV-2 CP self assembling into 17 nm diameter VLPs confirmed with transmission electron microscopy. This work ads to our understanding of foreign protein and DNA production in plants, and may well serve as viable alternative for commercial vaccine development.

Molecular detection of avian leukosis virus subgroup – J as a contaminant in commercial poultry vaccines distributed in Nigeria

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Vaccines are used to combat and control the menace of infectious diseases in livestock all over the world. However, they have been source of introduction and spread of some pathogens to some disease free environments. Equally, some vaccines have been reported to be inadvertently contaminated with extraneous viruses acquired probably through vertical or horizontal transmission. With the aim of screening for avian leukosis virus (ALV) and chicken infectious anemia virus (CIAV), forty – three live vaccines {(Newcastle disease virus (NDV), Infectious Bursal disease virus (IBDV), Fowl pox virus (FPV) and Mareks disease virus (MDV)} were procured. The poultry vaccines from fourteen manufacturers in 10 different countries including Nigeria were screened by Polymerase Chain Reaction (PCR) using standard protocols. Our result showed contamination with only ALV-J subtype in 9% (4/43) of the vaccines from three foreign manufacturers while other exogenous subgroups (ALV-A, B, C, D) were negative. The contaminating virus was found in IBDV (n=1), FPV (n=2) and MDV (n=1) vaccines.

Interestingly, CIAV was not detected in all the vaccines. The result of this study underscores the importance of screening vaccines to avoid the introduction and spread of extraneous viruses that could pose a threat to poultry production in Nigeria. This is the first report of avian leukosis virus subgroup J in Nigeria.

6

Murine Immune Response to Intranasal Immunization With VIRB7 Protein from Brucella Abortus

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Brucellosis is usually transmitted to humans through mucosae. Currently, there are no human vaccine against this disease. We decided to evaluate a virulence factor of Brucella (VirB7) as a potential component of an acellular vaccine against brucellosis, in a murine model.

Six week-old Balb/c mice were weekly immunized for 3 weeks with VirB7 (10ug) plus cholera toxin (CT, 2ug), CT alone or saline. One week after last immunization serum, saliva, feces, bronchoalveolar (BAL) and vaginal lavage fluids (LV), lung homogenates and spleens were obtained. Levels of VirB7-specific antibodies were measured by indirect ELISA in all samples. In vitro production of gamma interferon (IFN-γ), interleukin 2 (IL-2) and IL-5 by spleen cells stimulated with VirB7 (1 or 10 µg), ConA or RPMI was determined. Other mice (VirB7 plus CT) were injected intradermally in opposite footpads with VirB7 or saline to evaluate DTH.

Immunized mice showed increased serum levels of total antibodies against VirB7 ($p<0.0001$), and of specific IgA in saliva, feces, BAL and LV ($p<0.01$; $p<0.05$; $p<0.001$; $p<0.1$) compared with controls. IgA titers in lungs and serum were 80 and 9600, respectively. Serum titers of IgG1 were higher than those of IgG2a (16000 vs. 3840). The IgG1/IgG2a ratio was 6. A specific DTH response was detected at all times evaluated. Splenocytes from immunized mice secreted high levels of IFN-γ (mean 34677 pg/ml and 50270 pg/ml for 1 ug and 10 µg VirB7, $p<0.01$ and $p<0.001$ vs RPMI), IL-2 (187 pg/ ml and 395 pg/ml, $p<0.05$ and $p<0.001$) and IL-5 (504 pg/ml and 700 pg/ml, $p<0.05$ and $p<0.01$).

Intranasal administration of VirB7+ CT induced a systemic and mucosal specific immune response, which may contribute to prevent the mucosal entry of Brucella and its dissemination. The activated cellular immune response, shown by DTH reactions and high levels of IFN-γ, may be crucial for this protective effect.

Insights from West Nile Virus lineages 1 and 7 virulent strains: sub-lethal infections followed by homologous or heterologous challenges to guide vaccine design

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West Nile Virus (WNV) is an emerging mosquito-transmitted flavivirus that infects different vertebrates, including humans and horses that are dead-end hosts in which it causes mild disease to severe fatal encephalitis. WNV was first isolated in Africa in 1937 and has since spread worldwide. There are at least 8 lineages of WNV classified according to their genetic diversities. Lineages I and II are the first being recognized and have been responsible for most of the disease outbreaks in humans and horses worldwide. The other WNV lineages, which were identified more recently, have not been as well characterized and their potential to cause disease outbreaks need to be evaluated. Among these is Lineage 7, called Koutango, which circulates in Africa.

Koutango has not been formally associated with disease outbreak. However, there is a unique documented case of human Koutango from a laboratory worker. Interestingly, in mouse models of infection, Koutango appears to be the most virulent WNV lineage. In these studies, the virulence of Koutango was attributed to immune escape strategies specific to this lineage. Koutango is therefore a potential global threat that deserves much attention. With the ultimate goal of developing vaccines against WNV, we are conducting researches aiming to understand the mechanisms behind the virulence of Koutango. We performed a series of mouse infection studies comparing Koutango to a Lineage 1 virulent strain. In these studies, we infected Swiss mice with sub-lethal doses of virus followed by a lethal challenge with the same (homologous challenge) or the other WNV lineage (heterologous challenge). Longitudinal analyses of the survival, viral loads in different organs, and immune responses showed important differences between the two viruses. In particular, sub-lethal administration of WNV Lineage I conferred a protection against a subsequent lethal challenge of either virus while Koutango failed to provide a similar protection. These observations will help design efficient vaccines against WNV and prevent emergence of human infection by Koutango.

8

Induction of protection against East Coast Fever in Tanzania using targeted stimulation with toll-like receptor (TLR) agonist

Paul Simon Gwakisa
Sokoine University of Agriculture, Morogoro, Tanzania

ECF continues to be the number one killer disease for cattle in terms of endemicity in 11 countries in eastern, central and southern Africa. Vaccination against ECF is based on concurrent infection of cattle with live T. parva sporozoites and treatment with long-acting oxytetracycline, a procedure known as the infection and treatment method (ITM). Due to the rise in antimicrobial resistance and global initiatives to reduce antibiotic use, particularly prophylactic use in livestock, a method to replace the antibiotic in the vaccine protocol for ECF is necessary. We have investigated the possibility of stimulation of the innate immune system of cattle with a Toll-like receptor (TLR) agonist to replace the antibiotic in ECF vaccination protocol. We have demonstrated that the TLR7 agonist protects B. taurus X B. indicus crossbred calves against challenge when administered 24 hours prior to ECF vaccination. Our preliminary study has shown that TLR7 agonist is a potent stimulator of innate immunity and can be used to induce immunity of calves prior to the infection and treatment method for ECF vaccination, without simultaneous use of long acting oxytetracycline. We report that the innate stimulation induced significant rise of several early response indicators (body temperature, skin thickness, Th1, IFNg). This work is now undergoing to optimise the dosage and optimal regimes for innate stimulation prior to parasite challenge under field conditions.

African Vaccinology Network (AfVANET): An African Organization to help solve African Problems

Mustapha Oumouna (Algeria), Yakhya Dieye (Senegal), David Lazarus (Nigeria), Kwabena Duedu (Ghana), Tesfaye Kassa (Ethiopia), Gezahegne Mamo (Ethiopia), Funmilayo Afolayan (Nigeria), Dinga Gerome (Cameroun)

African Vaccinology Network meeting was mainly motivated by the observation that while African countries suffer the most from the infectious diseases discussed during the conference; the researches and innovations used to tackle these problems mostly come from outside the continent. Indeed the knowledge of the field by local scientists constitutes an invaluable asset that can be mobilized in the fight against human and animal infectious diseases. Through this initiative, African researchers will be able to take the necessary initiatives to solve the problems of their continent and provide appropriate solutions that are in most cases different from region to region. Indeed, whatever technology provided by the Western countries, which could provide solutions to some timely issues, the implication and participation of African researchers, who are aware of the different factors that influence the emergence and re-emergence of diseases, constitute an appropriate and a critical effort to solve these problems. The African continent has been exposed for decades to different diseases that constitute a significant threat to the health of humans and animals. In order to reach this goal, an initiative was taken to establish the African Vaccinology Network (AfVANET).

The goal is to use AfVANET as a platform to:

- Bring together all stakeholders in vaccinology and related sciences in Africa;
- Identify and prioritize vaccine gaps in Africa;
- Promote vaccine research and development in Africa;
- Help the mobility of student and young researcher between research labs and universities in Africa, through regional and sub-regional African networks.
- Applying for grants and funding opportunities to pay for travel expenses for student and young researchers within African continent.

10

Vaccination of calves with Bovine Respiratory Syncytial Virus (BRSV) as a model to understand the complications with HRSV in humans

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Respiratory syncytial virus (RSV) is an important infectious agent and the leading cause of viral lower respiratory tract infection in young children worldwide. Clinical manifestations range from asymptomatic infection to bronchopneumonia, bronchiolitis, and pneumonia. Like human RSV (HRSV), bovine RSV (BRSV) is a negative-stranded RNA. It has been recognized as an important pathogen for cattle for 30 years, and it affects primarily young calves. Infection is characterized by pyrexia, coughing, and dyspnea, pneumonia, and sometimes death. The pathological lesions caused by HRSV and BRSV are very similar.

Vaccination of calves with formalin-inactivated bovine respiratory syncytial virus (FI-BRSV) induces low levels of cellular immunity that may not be protective. Since inactivated and subunit vaccines formulated with CpG oligodeoxynucleotides (ODNs) have been shown to induce cellular immune responses, we studied the ability of a FI-BRSV vaccine formulated with CpG ODN to elicit cellular immunity against BRSV. Neonatal calves were immunized with FI-BRSV, FI-BRSV formulated with CpG ODN or medium and challenged with BRSV after two immunizations. Exacerbation of disease, characterized by increases in clinical signs of infection and BRSV-specific serum IgE and decreases in IFN-γ production, has been demonstrated in calves infected with BRSV following vaccination with FI-BRSV. It is thus hypothesized that BRSV-specific cellular immune responses, characterized by interferon-γ (IFN-γ) production and BRSV-specific IgG2, could be protective against subsequent infections.

Calves vaccinated with FI-BRSV formulated with CpG ODN developed increased numbers of IFN-γ secreting cells in the peripheral blood and broncho-tracheal lymph nodes and enhanced BRSV-specific serum IgG2 in comparison to FI-BRSV immunized animals. Calves that received the FI-BRSV vaccine formulated with CpG ODN also experienced a reduction in the amount of BRSV in the lung tissue. Based on these observations, CpG ODN appears to be a suitable candidate adjuvant for inactivated BRSV vaccines.

Anti- tick vaccines; one step control of both ticks and tick borne diseases

Irene Kiio
International Livestock Research Institute

Ticks are the second worldwide vectors of human diseases after mosquitoes. They also transmit pathogens which cause livestock farmers huge economic losses in terms of mortality and morbidity of animals and disease control costs. Thus, in the control of tick borne pathogens affecting both livestock and humans, tick control is critical. The chief method of tick control is acaricide application, a method associated with selection of acaricide resistant ticks, pollution and contamination of animal products. This creates a need for alternative tick control methods.

Anti-tick vaccines control vector infestation while simultaneously reducing pathogen infection and transmission. They are based on proteins vital for vector survival such as feeding, homeostasis, reproduction and immune response. Progress in characterization of tick genomes, ribonucleic acid interference (RNAi), expression library immunization (ELI) and other technologies have been instrumental in the discovery of potential antigens for anti-vector vaccines.

The challenge so far has been identification of efficacious antigens and their expression as effective recombinants. Anti-tick vaccines based on a mid-gut protein (Bm86) from Rhipicephalus microplus were released as GAVAC in Latin America and Tick GARD in Australia in 1993 and 1994 respectively. Despite the success, these products were found not be effective against afro tropical tick species.

Numerous antigens have been evaluated for their anti-tick effects since then. The biggest challenge has been identification of efficacious antigens and their expression as effective recombinants. Consequently, the search for an ideal vaccine antigen which is protective against multiple tick species and that reduces incidences of tick borne disease continues.

12

Isolation and Characterization of Clostridium Tetani from Equine and Their Environment in Selected Sites of Central Ethiopia

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A cross sectional study was carried out to isolate and identify Clostridium tetani from tetanus suspected 46 equines and their environment in selected sites of central Ethiopia. A total 71 samples (equine deep wound swabs, feces, soil from the contaminated environment) were collected and culture based isolation of C. tetani was carried out using anaerobic Viande et Foie (VF) medium and further identification by biochemical tests. Out of the 71 samples cultured on VF medium, 27 (38 %) of them were grown on anaerobic VF medium and all confirmed to be C. tetani using spore staining and biochemical test. Experimental injection of the C tetani positive culture supernatant to mices with or without tetanus antitoxin (TAT) injection showed that those mice injected with C. tetani positive culture alone were found dead within 72hrs after showing typical tetanus clinical signs while those control groups injected with C. tetani positive culture and TAT showed no clinical sign of tetanus and survived after 72hours. The occurrence of tetanus based on culture result was 38% (CI 95%: 0.27-0.50). The association of risk factors with occurrence showed that selected sites of Bishoftu and Addis Ababa and samples type of soil had a statistically significant difference among the group ($p<0.05$) in which higher occurrence were from Bishoftu area, environmental and feces samples, while sex and age of equine were not associated with occurrence of C. tetani ($p>0.05$). In conclusion, the present study showed the occurrence of tetanus in equine population of the study sites and confirmed widespread occurrence of the causative agents, C. tetani, in deep wounds, feces and in the environments of selected sites of central Ethiopia which warrant the need for designing feasible control strategies of tetanus in livestock and reduction of contamination of the environment in central Ethiopia

Estimation of Rift Valley Fever transmission in Kilifi, Kenya

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Background
Rift valley fever (RVF) is a zoonotic disease. The disease results in great economic loses in form of loss of human lives, livestock as well as the money and time spent in seeking medical care. Despite this, there is no any licenced RVF vaccine to date.

Objectives
This project aims at establishing RVF transmission dynamics with an interest in transmission that occurs outside the known outbreak periods.

Methods
Blood samples were collected from children aged between 5- 15 years, in the period 2002/2003 (non-outbreak period) and 2006/2007 (outbreak period). Adult samples were collected from 2002 all through to 2013. The temporal patterns were plotted and presented to show RVF IgG ELISA response magnitude in both outbreak and non- outbreak periods. Age stratified RVF IgG ELISA response was also presented. Climatic data has been plotted against magnitude of response in the two periods. Risk factors for transmission were obtained by literature review.

Results
There was increased serological response in outbreak period than in non-outbreak period. The overall response in 2002/2003 period was 41.08% while the overall response in 2006/2007 period was 59.9%. RVF response increases with increase in age, being lowest in children with less than aged 5-10 years and being highest in adults.
RVF response seems to peak during periods of unusually heavy rainfall in 2006/2007 period when there was a 58.1% increase in rainfall more than in 2002/2003. There was insignificant temperature variation across the two periods.

Conclusion
These results showing circulation of RVFV outside a known epidemic period, and the risk factors for transmission will be critical in developing and validating an assay to quantify RVF seroprevalence. Ultimately this falls in the greater goal of developing a licenced, stable and effective RVFV vaccine for use in both animals and in human.

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Annual beef measles (Cysticercus bovis) incidence in the catchment areas of Molepolole slaughterhouses, Botswana.

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A retrospective study was carried out to determine the annual incidence of beef measles (Cysticercus bovis) in cattle slaughtered at Molepolole slaughterhouses for the year 2012. Meat inspection records were used to determine the cattle origin and the proportion that was positive for C. bovis. The annual incidence was found to be 6% (348/5837). The cattle came from six extension areas and the annual C. bovis incidence in the areas ranged from 7% to 19%. A Z test demonstrated a significant difference (p= 0) in the incidence in Molepolole village that is in an urban area and letlhakeng that is in a rural area. We conclude that beef measles presents a major challenge to cattle farming in Botswana and that urban/peri-urban farming increases the risk of exposure to C. bovis. Our recommendation is that where sanitary measures fail to reduce C. bovis prevalence development of an effective vaccine should be the target.

Development of a Plant-Made Recombinant Virus-like Particle (VLP) Vaccine Against African Horse Sickness

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African horse sickness is a devastating, infectious, but non-contagious disease that causes great suffering and many deaths among horses in sub-Saharan Africa. The aetiological agent is a dsRNA virus of the same name, African horse sickness virus (AHSV), an orbivirus of the family Reoviridae. The disease has significant economic consequences for the equine industry both in southern Africa, and increasingly further afield as its midge vector spreads with global warming. Live attenuated vaccines have been in use with relative success for more than 5 decades, but there is a risk of reversion to virulence as well as re-assortment of the segmented genome between outbreak and vaccine strains. Furthermore, the vaccines lack DIVA capacity, the ability to distinguish between vaccine-induced immunity and that induced by natural infection. Several studies have demonstrated the potential for the use of plant expression systems for the production of VLPs, which are excellent vaccine candidates as they do not contain the virus genetic material and there is no risk of reversion to virulence or re-assortment with wild virus strains.

In this study, we investigated the formation of VLPs of AHSV serotype 5 in N. benthamiana by expressing the four structural proteins VP2, VP3, VP5 and VP7 of AHSV 5. We successfully cloned Nicotiana spp. codon-optimised AHSV capsid protein genes into the pEAQ-HT plant transient expression vector. Recombinant Agrobacterium tumefaciens harbouring the VP genes were co-infiltrated into N. benthamiana and shown to successfully express in plants by SDS-PAGE and western blot analyses. The formation of AHSV VLPs was observed by transmission electron microscopy and large scale purification of these VLPs was carried out by density gradient ultra-centrifugation. The immunogenicity of these AHSV 5 particles was demonstrated in guinea pigs and is currently being investigated in horses which are the main target animals. Our studies provide evidence that this system holds excellent potential for the production of a novel AHSV VLP vaccine.

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Comparison of heterosubtypic protection in ferrets and pigs induced by a single cycle influenza vaccine

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Influenza is a major health threat and a broadly protective influenza vaccine (BPIV) would be a significant advance. S-FLU is a candidate BPIV, limited to a single cycle of replication which induces a strong cross-reactive T cell response, but a minimal antibody response to haemagglutinin. We tested whether an H3N2 S-FLU can protect pigs and ferrets from heterosubtypic H1N1 influenza challenge. Aerosol administration of S-FLU to pigs induced lung tissue resident memory T cells and reduced lung pathology, but not the viral load. In contrast, in ferrets S-FLU reduced viral replication and aerosol transmission. Our data show that S-FLU has different protective efficacy in pigs and ferrets, and that in the absence of antibody, lung T cell immunity can reduce disease severity without reducing challenge viral replication.

Proteomic analysis of local disease-sparing responses to bovine respiratory syncytial virus in intranasally vaccinated and challenged calves

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Bovine and human respiratory syncytial viruses (BRSV, HRSV) are primary causes of pneumonia in calves and children, respectively. Parenteral and intranasal (IN) vaccines confer protection against BRSV infection, which is associated with systemic and local antibody and cellular immune responses. To better understand the response engendered by IN vaccination, 3-8 day old calves received a single component BRSV vaccine, a "3-way" vaccine (BRSV, BHV-1, BPIV-3), or placebo, IN, and were challenged via aerosolization of BRSV 42 days later. Both groups of BRSV-vaccinated calves had significantly less pulmonary lesions compared to controls. 2D-DIGE (24cm, pH 3-10NL) proteomic analysis of pharyngeal tonsil lysates (n=8/gp) indicated a differential proteome response among the groups. Principal component analysis (PCA) of 864 detected protein spots revealed clustering of treatment groups (20.41% R2X variation – PC1 and PC2). Three placebo and 2 vaccinated calves that required euthanasia on days 6 and 7 post challenge were separated from other calves within the PCA scores plot. Vaccinated calves that overlapped on the PCA plot with the placebo group had lower serum IgG post-challenge, suggesting that alterations to the pharyngeal tonsil proteome indicate protective immunity. Seventy-six protein spots were significantly different (ANOVA, $p < 0.05$) between vaccinated and placebo groups, and 105 between animals euthanized early versus on day 8. Of these, 67 protein spots were selected based on FDR testing ($q < 0.2$) for MALDI identification. These data indicate differential responses in the pharyngeal tonsil proteome which correlates with vaccination and disease-sparing.

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Development of a Polyvalent Vaccine Against Flavobacterium Psychrophilum in Rainbow Trout (Onchorynchus Mykiss L.)

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Rainbow trout fry syndrome (RTFS) is a disease caused by the Gram-negative bacterium Flavobacterium psychrophilum, responsible for economic losses in the trout industry worldwide. The diversity of F. psychrophilum strains, the inherent difficulties in vaccinating juvenile fish and lack of a reproducible immersion challenge model has hampered the development of a vaccine for this disease. At present no commercial vaccines are available, leaving antibiotics as the only course of action to contain disease outbreaks. The development of a vaccine is required, because of the potential risk of resistance developing in the antibiotics presently licenced for use in aquaculture.

We report on the development and efficacy of a polyvalent, whole cell vaccine containing formalin-inactivated F. psychrophilum. Significant strain variability among our collection (>300 isolates of F. psychrophilum) was confirmed by PFGE, rep-PCR profiles, 16S rRNA allele groups, plasmid profiles and serotypes and these data were used to choose the isolates to produce a potentially cross-protective vaccine. The vaccine was applied as a 30s dip with a booster 315 degrees days post-vaccination to trout fry of 5 g. Vaccine combined with an immersion adjuvant (IMS1312Vg, Seppic) was tested using the same vaccination regime. Challenge was by immersion with a virulent heterologous isolate of F. psychrophilum (630 degree days post-initial vaccination).

Significant protection was achieved with an RPS of 84% achieved with the application of the immersion vaccine without adjuvant; RPS of the immersion vaccine with adjuvant was 60%. Long term efficacy of the vaccine applied via the intraperitoneal route in combination with two adjuvants- Montanide and a novel adjuvant squalene/aluminium hydroxide - was tested in 80g Rainbow trout. The fish were challenged by intramuscular injection 1155 degree days post-vaccination with a virulent heterologous isolate of F. psychrophilum. The vaccine emulsified with Montanide provided 100% protection, whereas survival in the groups given vaccine alone and vaccine combined with squalene/alum adjuvant was not significantly different from controls.

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The application of NHEJ-CRISPR/Cas9 and Cre-Lox system in the generation of bivalent duck enteritis virus based vaccine against avian influenza virus

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Duck-targeted vaccines to protect against avian influenza are critically needed to aid in influenza disease control efforts in regions where ducks are endemic for highly pathogenic avian influenza (HPAI). Duck enteritis virus (DEV) is a promising candidate viral vector for development of vaccines targeting ducks, owing to its large genome and narrow host range. The clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 system is a versatile gene editing tool that has proven beneficial for gene modification and construction of recombinant DNA viral vectored vaccines. Currently, there are two commonly used methods for gene insertion: non-homologous end joining (NHEJ) and homology-directed repair (HDR). Owing to its advantages in efficiency and independence from molecular requirements of the homologous arms, we utilized NHEJ-dependent CRISPR/Cas9 to insert influenza HA antigen expression cassette into the DEV genome. The insert was initially tagged with reporter green fluorescence protein (GFP), and a Cre-Lox system was later used to remove the GFP gene insert.

Furthermore, a universal donor plasmid system was established by introducing double bait sequences that were independent of the viral genome. In summary, we provide proof of principle for generating recombinant DEV viral vectored vaccines against influenza virus using an integrated NHEJ-CRISPR/Cas9 and Cre-Lox system.

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ChAdOx1 Rift Valley Fever (RVF)–Thermostabilisation of the One Health Vaccine

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The need for a cold-chain for storage and distribution is a massive limiting factor on the economic viability for a vaccine, especially in warmer climates. We will describe the development of a cost effective veterinary vaccine. ChAdOx1 RVF is a replication- deficient chimpanzee adenovirus expressing the RVFV surface glycoproteins; Gn and Gc. A single dose elicits high-titre neutralising antibodies and provides protection against RVFV challenge in sheep, goats and cattle: the most naturally susceptible species. 1 ChAdOx1 RVF is currently being evaluated in human and veterinary clinical trials.

We will demonstrate that a lower cost cell lysate, that has undergone fewer purification steps during manufacture than for the equivalent GMP human production, can be thermostabilised with minimal loss in potency and immunogenicity. Lyophilisation will allow for the distribution of the vaccine at not only ambient temperature but also with the capacity for a short term distribution at extended temperatures. This is especially important as RVFV mainly affects those areas that are low and middle income countries and for which the end of the chain distribution is in areas with limited resources and poor infrastructure. The ability to increase the vaccine coverage for those areas would massively benefit our One Health approach.

Previously Chen et al., 2011 evaluated the effects of different formulations on a recombinant adenovirus during lyophilisation.2 Using the excipient formulation I1 we have further improved this process by optimising the cycling conditions with a reduction in total time, from 75 hours to 55 hours, in a bid to reduce the associated costs with lengthy cycling conditions. We will describe the processes involved with; the selection of cycling parameters, capturing the end of sublimation by capacitive pressure sensor/Pirani pressure sensor difference, aseptic process operation, sample analysis & structure, stability and finally the confirmation of the in vivo viability of the vaccine and the optimal dose.

The data generated here will not only inform other low cost veterinary production of vaccines but will be applicable for the One Health approach through the deployment of human adenovirus vaccines, for which stability is a major limiting factor.

1George M. et al. Chimpanzee Adenovirus Vaccine Provides Multispecies Protection against Rift Valley Fever. Scientific Reports 6 (2016): 20617. PMC. Web. 23 Nov. 2017. 2Chen, S. et al. Investigation on formulation and preparation of adenovirus encoding human endostatin lyophilized powders Int J Pharm, 427 (2012), pp. 145-152.

An Infection for a Vaccine – Theileria-annulata infected cells as an antigen delivery model

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Bovine theileriosis caused by Theileria parva (T.parva) continues to be a major economic problem in many parts of Eastern, Southern, and Central Africa. Live vaccines against the parasite have been available for over 40 years, but although they provide strong protection, practical disadvantages have limited their widespread application. There is therefore a huge interest in developing alternative vaccines and there has been an increased focus on using defined parasite antigens. Detailed studies of the immune responses of cattle immunised with T. parva have provided clear evidence that parasite-specific CD8 T cells reactive with parasitised cells play a key role in immunity and a series of antigens recognised by CD8 T cells from immune cattle have been identified. Although cattle immunised with recombinant pox and adenoviruses expressing these antigens generate strong specific T cell responses, they show poor protection against parasite challenge. A potential reason for the failure of viral vectors to reproducibly induce protective immune responses is that they fail to promote appropriate differentiation of T cell function. To address this hypothesis, we aim utilise the related parasite Theileria annulata as a novel antigen-delivery system, by generating Theileria annulata-infected cell lines expressing defined T. parva antigens. As a first step, we have demonstrated successful transduction of T. annulata-infected cells with a red fluorescent protein (RFP)-expressing lentivirus expressing the Tp9 T. parva antigen. Cell lines from two animals expressing the A14 MHC haplotype were chosen because animals of this MHC type generate dominant CD8 T cell responses to the Tp9 antigen. The Tp9-transduced cells have been shown to express the antigen and are recognised by Tp9-specific CD8 T cells. Following further validation of Tp9 expression in vitro the Tp9-transduced T. annulata infected cells will be used to immunise animals, which will be monitored for induction of Tp9-specific T-cell responses and challenged with.

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Rapid identification of bovine MHCI haplotypes in genetically divergent cattle populations using Next-Generation Sequencing (NGS)

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The MHC region contains many genes that are key regulators of both innate and adaptive immunity and is the most polymorphic in the mammalian genome. Consequently, the characterisation of the repertoire of MHC genes is critical to understanding the variation pivotal in determining the nature of immune responses. Currently our knowledge of the bovine MHCI repertoire is limited with only the Holstein-Friesian breed having been studied intensively. Traditional methods of MHCI genotyping are of low resolution and laborious, however next-generation sequencing (NGS) technologies have been used to enable high throughput and much higher resolution MHCI typing in a number of species. We have developed a MiSeq platform approach and requisite bioinformatics pipeline to facilitate typing of bovine MHCI repertoires. The method was validated initially on a cohort of Holstein-Friesian animals and then demonstrated to enable characterisation of MHCI repertoires in African cattle breeds, for which there was limited available data. During the course of these studies we identified >140 novel classical MHCI genes and defined 62 novel MHCI haplotypes.

Hydrophobic mycobacterial antigens induce polyfunctional T-cell responses in BCG-vaccinated cattle that are associated with protection against subsequent Mycobacterium bovis challenge

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Bovine tuberculosis (bTB), caused by Mycobacterium bovis, is a chronic disease of cattle with a detrimental impact on food quality and production in many low- and middle-income countries, as well as the UK. Research on bTB vaccines has predominantly focussed on proteinaceous candidate antigens. However, Mycobacteria have a thick and intricate lipid outer layer and lipids are important for immune-evasion and virulence. In humans, lipid extracts of M. tuberculosis have been shown to elicit immune responses effective against M. tuberculosis in vitro.

We isolated hydrophic antigens from M. bovis BCG by chloroform-methanol extraction (CMEbcg). CMEbcg contains lipids and lipopeptides and has a low content of high molecular weight protein. CMEbcg stimulates polyfunctional T-cells both in BCG vaccinated and M. bovis challenged calves. The CMEbcg specific CD3+ T-cell proliferative response following BCG vaccination was the best predictor of protection against subsequent M. bovis challenge. CMEbcg stimulates CD4+T-cells via MHC class II and the immodominant antigens in CMEbcg are lipopeptides. The immune responses against CMEbcg were specific for M. bovis infection, this is in contrast to the immune response against purified protein derivative of M. bovis (PPDB) that is currently used for diagnostic testing of bTB, indicating that CMEbcg could be used a single diagnostic antigen for bTB.

We conclude that lipopeptides of M. bovis are novel vaccine candidates and have potential as a highly specific diagnostic antigen for bovine tuberculosis.

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A multicomponent vaccine composed by recombinant tick secreted proteins protect cattle against R. microplus infestation

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Ticks are important vectors for disease transmission to humans and animals, promoting significant losses in bovine production, and being a concern to veterinary and public health. Due to the importance of tick saliva during infestation, the development of multi-target vaccines that neutralize salivary activities could be useful to avoid tick infestations. Nine protein identified in R. microplus salivary transcriptome were selected and produced as recombinant proteins and used as antigens to immunize Holstein calves (n=6/group). Animals received three doses of 50 µg of each recombinant antigen adjuvanted with aluminum salt, or PBS + aluminum salt (control), applied individually by SC route, in a 28-days interval between doses. Four weeks after the 3rd immunization, both groups were challenged with 15,000 R. microplus larvae. All spontaneously detached engorged females were collected and counted, and 25% of the females collected per animal each day were kept for weighing and incubated for further evaluation of oviposition and egg fertility. Blood was collected from calves in pre-immunization period, after the 3rd dose and during infestation and the antigen specific IgG levels were assessed by ELISA. Animals from the immunized group presented positive IgG seroconversion 14 days after the 3rd immunization for all recombinant antigens ($p<0,05$) and a decay of antibody levels were observed during infestation. Compared to the control group, the vaccinated group presented a reduction of 62% in the number of engorged female recovered after infestation, 7% reduction in the eggs weight and 12% reduction in eggs fertility. The efficacy of the vaccine was 69%. The results show that multicomponent vaccines using tick secreted proteins as targets could be useful as anti-tick vaccines. New antigens and adjuvants could be associated in order to improve the results obtained and avoid R. microplus infestations in cattle.

Pre-clinical assessment of molecular adjuvants for viral-vectored vaccines for the global infectious diseases MERS coronavirus and malaria

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MERS coronavirus and malaria are two infectious diseases with important health repercussions worldwide. Despite the fact that these diseases combined have caused millions of deaths, an effective and safe vaccine is yet to come. In this regard, replication-deficient viral vectors (such as adenovirus and poxviruses) represent a promising tool due to their high levels of antigen-specific T cell responses and relative innocuity. Nevertheless, these high levels of T cell responses might not be sufficient to provide protection. My work has the aim to see how newly discovered adjuvants, such as spermidine and 2'3'-cGAMP, can be used to obtain such levels of protection in a pre-clinical setting.

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Sensitivity of the bovine BCG challenge model

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Bovine tuberculosis is a zoonotic disease affecting cattle and causing economic loss to farmers worldwide. A highly efficacious vaccine could reduce the burden of bovine tuberculosis around the world. The vaccine currently used in experimental studies in cows is the attenuated M. bovis strain Bacillus Calmette-Guérin (BCG).

However, protection induced by BCG varies between 0 to 80%. The development of new vaccines is hindered by the lack of a correlate of protection. As a result every new vaccine candidate has to be tested in lengthy and expensive M. bovis challenge experiments. Methods that would allow for a faster and cheaper pre-selection of vaccine candidates and additionally improve welfare of experimental animals are needed. We have previously developed a bovine BCG challenge model in which animals are challenged intranodally with a non-pathogenic BCG strain. Improved protection results in reduced bacterial burden in the injected lymph nodes of protected cows compared to unprotected animals three weeks after challenge.

The aim of this work was to analyse the sensitivity of the BCG challenge model and thus its ability to differentiate between different vaccine regimens resulting in varying degrees of protection. In order to do so we compared vaccination with BCG only to a heterologous prime boost vaccine regimen with BCG as the prime vaccine and a recombinant adenovirus expressing antigen 85A as a booster. The prime boost vaccine regimen has previously been demonstrated to improve protection over BCG alone in pathogenic M. bovis challenge experiments. Here we show, that the BCG challenge model is able to measure improved protection of both vaccine regimens over unvaccinated control animals. However, the model is currently not sensitive enough to distinguish between the two different vaccine regimens. Nevertheless, it allows us to determine the ability of new vaccine candidates to induce protection at least as good as BCG.

To Evaluate the Effects of Immunostimulant Drugs on the Course of Trypanosomiasis Following Antigen Injection and Challenge

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Introduction

The development of trypanosomiasis vaccine has not been successful, mainly due to bloodstream forms of trypanosomes circumventing the host immune response by continuous changes of surface glycoprotein and antigenicity. Recent studies also suggest that immune suppression prominent in trypanosomiasis, is responsible for vaccine failure. Hence, any attempt to produce an effective vaccine must address this problem of immunodepression. Continued efforts to develop vaccine resulted in the identification of non-variable molecules particularly, trypanosomal microtubulin.

Materials and Methods

Strains of T. b. brucei, T. congolense and T. vivax obtained from cryopreserved stabilates at (NITR) Vom, Nigeria and wild strains of the same species stabilized by serial passage in rats will be used. Two large rats will be inoculated intraperitoneally with 105 of each strain of trypanosome. The blood of these rats will be monitored daily. On day 4/5 the parasitaemia levels are expected to be maximal and tail blood will be obtained and diluted with physiological saline to obtain 105 trypanosomes / 0.1ml. Each trypanosome strain will then be injected intraperitoneally into twenty rats. Purified microtubulin antigen from the trypanosome strains will also be injected into separate groups of rats and infected after 21 days.

The influence of immunostimulants on the course of the disease in antigen sensitized and non-sensitized animals will be investigated.

Statistical Analysis

The means and standard deviations of means of measured parameters will be calculated. The differences in the means will be analyzed statistically with Instat(R) software (Graphpad Inc. USA) or other statistical software (SPSS) using analysis of variance (ANOVA) Probability values less or equal to 0.05 ($p < 0.05$) will be considered significant.

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Multivalent Eimeria-vectored oral vaccines for poultry

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Protocols supporting genetic complementation of Eimeria raise the prospect of generating transgenic parasite lines that function as vaccine vectors to express and deliver heterologous antigens. For example, complementation with sequences encoding immunoprotective antigens from other species of Eimeria could reduce the complexity of species/strains that are currently incorporated into live oral vaccines for poultry coccidiosis, making them more cost-effective.

EtAMA1 and EtAMA2 are members of the apical membrane antigen (AMA) family of parasite surface proteins from Eimeria tenella. In this poster we show that EtAMA1, but not EtAMA2, induces partial protection against homologous challenge with E. tenella parasites when used as a recombinant protein vaccine. To test whether transgenic parasites can induce protection against heterologous challenge, wild type E. tenella parasites were complemented with EmAMA1, the specific orthologue of EtAMA1 from E. maxima. Sequences encoding EmAMA1 were fused to delivery signals to modify intracellular trafficking and exposure of the antigen to the host immune system. Vaccination of chickens with the transgenic E. tenella parasites conferred partial protection against E. maxima challenge, at levels comparable to those obtained using EmAMA1 recombinant protein vaccination. This provides evidence that vaccination with transgenic Eimeria can induce cross protection against challenge with a heterologous coccidial species. Taken further, this technology has the potential to streamline the production of coccidiosis live vaccines through the generation of parasite lines that co-express immunoprotective antigens derived from multiple species of Eimeria.

Knowledge and Awareness of Animal Owners And Veterinarians on the Relative Efficacy and Quality of Veterinary Vaccines in Federal Capital Territory, Nigeria

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In Nigeria, veterinary vaccines are sold over-the-counter to any prospective buyer, and only few buyers are aware of the relative efficacy and quality of the vaccines purchased. The aim of the study was to determine the knowledge and awareness of the relative efficacy and quality of available vaccines in the market. A cross sectional study using standardized questionnaires was conducted. Respondents were 180 animal owners and 108 veterinarians from the six area councils of the Federal Capital Territory.

Result showed that 56.7% (102) of animal owners had no knowledge on the vaccines used on their animals while 34.4% (62) were concerned about the brand of vaccines. However, only 16.1% (10) are aware of the relative efficacy of the brands of vaccine available in Nigeria. Among the veterinarian-respondents, only 25.9% (28) updated their knowledge on vaccines relevant to their area of specialization since they began practice. 30.6% (33) of veterinarians are aware about the relative efficacy of the vaccine brands available in market while only 25% (27) were concerned about vaccine brand, and they were found to be veterinarians that had vaccinated animals for more than 15 years.

It is recommended that vaccine regulatory agency allow importation of only quality veterinary vaccines and enforce strict rules and regulations guiding sale of vaccines in Nigeria. In addition, veterinarians should update their knowledge on the efficacy and immunogenicity of available vaccines in Nigeria.

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Next-generation adjuvants: transferring the learnings of its use in humans to veterinary health

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Recent advances in the understanding of innate and adaptive immune systems led to the discovery of adjuvants more powerful than previously used adjuvants, such as Freund's adjuvants and aluminium hydroxide-based formulations. Next generation adjuvants include molecules that stimulate the innate immune system by interacting with pathogen-associated molecular patterns, for increased immunogenicity. Most modern human vaccines are subunit-based and often require at least one immunopotentiator to increase the vaccine's immunogenicity. The inclusion of immunopotentiating adjuvants in human vaccines have shown clear benefits in numerous clinical trials against diseases, ranging from infectious, chronic, and autoimmune diseases. Next-generation adjuvant formulations that are included in licenced human vaccines include AS03 and AS04 (GlaxoSmithKline) that are in currently in vaccines against influenza, human papillomavirus and hepatitis B, as well as MF59 (Novartis) that is also used in influenza vaccines.

Veterinary vaccines still consist of whole inactivated or killed organisms, or even relatively crude microbial extracts, resulting in limited uptake of next-generation adjuvants in commercial animal vaccines. This landscape is changing, as the veterinary industry, recognizing the safety profiles associated with subunit vaccines, are now adopting recombinant technologies for veterinary vaccine development. These vaccines will more than likely need strong adjuvants, as required with modern human vaccines. The use of next-generation adjuvants could also address previous shortcomings of whole inactivated or killed organism vaccines, which tended towards a Th2 response. For some animal pathogens a clear Th1 response is needed for protection and clearance, and the addition of immunopotentiators to the vaccines could make this possible. Here we draw on some of the similarities between human and animal diseases and speculate how rationally designed vaccines that include next-generation adjuvants can benefit the development of safer veterinary vaccines.

A successful vaccine trial reveals an opportunity to control Malignant Catarrhal Fever in cattle

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Background: Wildebeest associated malignant catarrhal fever (MCF) is a fatal disease of cattle caused by alcelaphine herpesvirus (AlHV). Pastoralists in wildebeest endemic areas report MCF to be one of the most important diseases of cattle. The incidence can reach 10% in affected herds.

Objectives: The aim of this study was to assess the efficacy of an attenuated AlHV C500 vaccine for preventing mortality from MCF in cattle.

Methods: The study was conducted at Kapiti Plains Ranch, east of Nairobi. In 2016, 146 cattle were selected for a randomised placebo controlled trial. Animals were stratified according to breed and age and randomly assigned vaccine or placebo treatments. Animals received prime and boost treatments one month apart. The study herd was grazed with wildebeest from one month after booster vaccination.

Results: All vaccinated animals were shown by indirect ELISA to have a serological response to vaccination. Three animals (two vaccinated and one placebo) died of unrelated causes before the study ended. Twenty-five animals developed clinical MCF; four of the 71 vaccinated animals (5.6%; 95% 2.3-13.6%) and 21 of the 72 unvaccinated animals (29.2%; 95% 19.9-40.7%). All affected animals died and the cause of death was confirmed to be AlHV by PCR and histopathology. The vaccine efficacy was determined to be 80.8% (95% CI 78.0-83.6%).

Conclusions: The AlHV C500 vaccine appears to be a safe and effective method for controlling wildebeest associated MCF in cattle in Kenya.

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Global Vaccine Security - why it matters – with specific reference to FMD and re-thinking on vaccine supply for emergencies and to meet surge in demands

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Driving almost all our international work is the simple fact that we do not have vaccine security; we live with the fear that suitable and effective vaccine will not be available when needed, or if available, will not be effective when used, or achieve the outcomes desired. In FMD free regions, the "'standard model"" of vaccine banks is being challenged by the potential scale of need and the diversity of circulating FMD strains. In regions not free of FMD, the efforts being made for ""risk based vaccination"" and optimizing control measures relate to the lack of vaccine security; in that the lack of availability of quality vaccines leads to public and private stakeholders facing epidemics without secure access to vaccine. Lack of supply also leads to continued use of ineffective vaccines, with disappointing results- for the animal producers as well as nationally. Vaccine security would mean the confidence that vaccines are affordable, available and effective and accessible by stakeholders. Global vaccine security would mean security to respond with vaccines to face any threat where they may be required; a need of the ""free countries"" as well as endemic regions.

Without vaccine security, we need elaborate and well drilled preparedness for an FMD emergencies, to contain incursions before they outstrip vaccine supply. In endemic regions of Africa, mid-east and Asia, millions of animals – and their owners- cannot access effective vaccines when they need them – so lack of supply does matter for food security and livelihoods. Addressing supply issues at global and regional scale requires progress on vaccine technologies as well as production capacities. It also requires progress to understand the market forces for demand for vaccine at field level, and on financing mechanisms that reduce the risk for investors who attempt to increase the supply side.

Global security of supply of FMD vaccines therefore affects all countries – so what can be done about it? and how do we manage the risks without it?

The paper will cover one of the initiatives of the EuFMD to address the lack of supply of FMD vaccines for emergencies - the development of a "'assured emergency supply option "" (AESOP), and to enter into long term agreements with vaccine suppliers to hold increased stocks of vaccine antigens for emergency and preventive responses. Addressing the lack of supply of FMD (and other vaccines) is a critical issue for achieving global vaccine security, and will involve multiple players to co-ordinate efforts to increase investment both in vaccine supply, which includes the issue of bringing new technologies into use. These issues will be a focus for the EuFMD Open Session 2018 , to be held in Puglia, Italy, 29-31st October (for more details see the poster), and all are invited who can help co-ordinate efforts to achieve vaccine security.

Vaccines projects at Moredun Research Institute

George Russell, W. David Smith, George Newlands, Tom McNeilly, Jacqui Matthews and Alasdair Nisbet

Moredun Research Institute is recognised worldwide for its research into infectious diseases of livestock, with outputs focused in three areas: development of novel and effective vaccines, diagnostics and disease control measures.

To date, Moredun is responsible for development of vaccines for 12 livestock diseases, protecting against bacterial, viral and parasitic infections. These live-attenuated or inactivated whole-organism vaccines include those which protect against clostridial diseases, toxoplasmosis, Pasteurella, Louping ill etc. Development of novel vaccines increasingly uses genomic and proteomic resources to identify relevant antigens and recombinant expression systems to produce the antigens in a form suitable for vaccination. In this presentation we will focus on three areas of successful recent vaccine development by Moredun scientists.

Haemonchus contortus: a blood feeding nematode parasite of small ruminants that is prevalent in tropical and sub-tropical regions has a significant impact on production. Work over many years identified enzymes on the surface of the parasite gut which were protective antigens. However, recombinant versions of these were not protective. Methods were devised for collecting large quantities of adult Haemonchus and a cost-effectively native antigen vaccine has been commercialised. This, the first vaccine for a gut dwelling worm of any host, has been on sale in Australia as Barbervax since 2014 and as Wirevax in S. Africa since 2017.

Malignant catarrhal fever (MCF): a fatal viral disease of cattle prevalent across the range of the wildebeest in east and south Africa. An attenuated form of the MCF virus alcelaphine herpesvirus 1 was found to protect against fatal challenge in experimental trials. This vaccine has now been tested in field trials in Tanzania, Kenya and South Africa with efficacy of around 90%.

Teladorsagia circumcincta: a roundworm parasite of sheep, which develops and lives in the abomasum, shedding eggs into faeces, causing significant production losses. Identification of potential antigens by immunological, proteomic and bioinformatic methods has led to the recombinant expression and testing of a panel of antigens, a combination of which was found to reduce the shedding of nematode eggs in vaccinated animals by up to 70%.

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Harnessing Superantigen Activity to Generate an Enhanced Vaccine Response

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Strangles, caused by Streptococcus equi (S. equi), is the most common infectious disease in horses worldwide. In Europe, the only available Strangles vaccine is Equilis StrepE, a live vaccine, which provides protection to horses for only 3 months. An alternative vaccine, Strangvac, currently in development comprises 8 S. equi proteins and is 95% effective 2 weeks after third vaccination, but this protection only lasts for 2 months.

Superantigens activate the immune response by cross-linking major histocompatibility complex (MHC) class II to the T cell receptor (TCR), resulting in polyclonal T cell activation. Here we show that by mutating the TCR-binding site of SzeQ, a novel superantigen, MHC class II binding properties are maintained while TCR-binding is lost. We hypothesise that fusion of the modified SzeQ to surface proteins from S. equi will direct vaccine components towards antigen presenting cells resulting in enhanced presentation, stronger immune responses and increased duration of protection. If successful, similar fusion proteins could be exploited to enhance the effectiveness of vaccines against other infectious diseases in different animal species, including humans.

Serological responses of cattle inoculated with inactivated trivalent Foot-and-Mouth Disease vaccine

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The routine prophylactic vaccination of cattle is a vital control strategy in foot-and-mouth disease (FMD) endemic settings. FMD vaccines are used on enormous scale across the globe, with over 2 billion doses used annually. Cattle within the FMD Protection Zone with Vaccination bordering the GFcorreater Kruger National Park (KNP) Area are routinely vaccinated for FMD using a trivalent (SAT 1-3) inactivated vaccine. In this study, four dip tanks within a communal area bordering KNP were purposely selected based on the scheduling of weekly animal inspections. Cattle aged >6 months were selected from available herds after obtaining informed consent from owners. Cattle were vaccinated using a trivalent inactivated FMD vaccine (SAT 1-3) and longitudinally followed for a period of 112 days. Blood samples were collected on a fortnightly basis and analysed for FMD-specific antibodies using a liquid-phase blocking ELISA. A total of 293 cattle were sampled and <50% of the enrolled cattle had evidence of pre-existing antibody responses to the three SAT viruses at the beginning of the study. However, 14 days post-vaccination, the proportion of seropositive cattle (≥1.6log10 titre) to the three SAT serotypes varied between 66% - 93% with SAT 2 having the highest proportion. Antibody responses peaked at 42 days post-vaccination. Measured antibody titres varied by FMD serotype ($P<0.001$), sex ($P=0.018$) and age ($P<0.001$). Cattle 6-12 months of age had lower serological responses compared to older cattle. The vaccine elicited high serological responses by 14 days post-vaccination. However, the duration of antibody response was short-lived as the antibody level began to decline below the cut-off threshold by 56 days post-vaccination. More research is necessary to determine the reasons for the limited duration of vaccinal antibodies in efforts to ensure sufficient herd immunity that will limit disease occurrence in vaccinated populations.

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Comparative pathological association of slc11a1 (NRAMP1) gene with susceptibility and resistance to brucellosis in cattle and buffaloes of various breeds

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As the NRAMP1 genotype may be linked with Brucella resistance and susceptibility therefore it is important for us to know the status of our local breeds of bovines. Two breeds of buffaloes and three breeds of cattle were screened for brucellosis and comparative genomics for NRAMP1 genotype. The target breeds were classified into infected and non-infected groups after PCR confirmation of Brucella abortus. Both the PCR positive and negative groups were analyzed for NRAMP1 genotype. It was interesting to note that Nili-Ravi buffaloes were more resistant as compare to Kundi breeds of buffaloes. Similarly Sahiwal breed was more resistant for brucellosis as compare to cross breeds and Friesian. This genetic resistant can be used for genomic selection of animals to reduce disease cost and economic losses due to brucellosis.

Assessing the feasibility of using Bacillus subtilis as a vaccine display system for Betanodavirus in European sea bass

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Over the past three decades, Betanodavirus, the causative agent of viral encephalopathy and retinopathy (VER) disease, has become a serious problem in marine finfish aquaculture, particularly European sea bass (Dicentrarchus labrax) in the Mediterranean. The most sensible way of controlling this disease is to apply effective biosecurity and to vaccinate the fish.

The use of Bacillus subtilis as a vehicle for antigen delivery is a relatively new approach in human vaccinology and has been shown to have a significant adjuvant effect when used to display model antigens such as H5N1, tetanus toxin, ovalbumin (OVA) or HIV gagp24. A great deal is already known about the genetics of the bacterium, and it is relatively straightforward to genetically manipulate the bacterium and integrate stable constructs into its chromosome. Foreign antigens have been expressed on the surface of B. subtilis, both as vegetative cells and spores, and both have been used as delivery vectors for vaccines with antigens displayed on their surface.
A B. subtilis display system was used here to anchor the full coat protein (CP) of betanodavirus genotype RGNNV onto the surface of vegetative B. subtilis cells. We have confirmed that the proteins were correctly displayed on the surface of the bacterium using ELISA, western blotting, confocal microscopy and proteomic analysis of the bacterial ShaveOME, a technique that analyses the proteins displayed on the surface of the bacterium. The latter showed significant coverage of many bacterial surface proteins together with the CP peptides of Betanodavirus, and strong staining of the bacterium was obtained with monoclonal and polyclonal antibodies against the virus by confocal microscopy; both techniques confirmed the display of the Betanodavirus CP on the surface of the B. subtilis. Vaccination trials are currently being undertaken in which European seabass have been vaccinated with the inactivated B. subtilis and efficacy is being examined by experimental infection.

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Assessment of an enhanced bacterin vaccine for Streptococcus agalactiae in Nile tilapia (Oreochromis niloticus) based on an iron-dependent regulator gene knock-out mutant

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Streptococcus agalactiae, along with other Streptococcus spp. causes streptococcosis in fish. This is a major disease problem, particularly for the tilapia farming. In intensive aquaculture systems S. agalactiae can lead to levels of mortality between 60 and 95 %. Such high levels of mortality can have severe economic repercussions and in 2008, the disease was estimated to account for losses in the region of US$250 million to the aquaculture industry. Although commercial vaccines are available for this disease, based on formalin-inactivated bacterin vaccines, the aim of this study was to produce an enhanced bacterin vaccine based on technology successfully developed for other pathogens like Streptococcus suis or Corynebacterium pseudotuberculosis. Briefly, knockout of the regulatory gene dtxR-like, which controls the expression of virulence–associated genes in response to environmental metal concentration, allows expression of proteins normally produced in vivo and enhances the protective efficacy of the bacterin vaccine.

In this study, a strain of Streptococcus agalactiae (GBS) collected from a disease outbreak in tilapia farm in Canada [NCBI: NKPZ00000000] was used. The iron dependent regulatory gene (idr) in the genome, analogous to the dtxR, scR or troA genes was identified. A construct was then created by introducing 1kb flanking regions of the target idr gene into thermo sensitive plasmid pGhost+9 with an erythromycin resistance gene for selection. The idr gene was knocked out by allele replacement and mutant candidates were selected by PCR screening. Extracellular protein profiles were identified by LC-ESI- MS/MS analyses and its comparison shows enhanced expression of secreted proteins by Δidr mutant in comparison to the corresponding wild type.

The efficacy of formalin inactivated wild type bacterin was compared to the mutant bacterin. Unlike Δidr mutant vaccines for other pathogens, the GBS Δidr mutant did not offer any enhanced protection in vaccinated tilapia experimentally infected with Streptococcus agalactiae.

Combination vaccines targeting iron metabolism and mid-gut proteins in Rhipicehalus appendiculatus provides highly effective potential vaccine against tick infestation

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Rhipicephalus appendiculatus (Family; Ixodidae) has been found to parasitize livestock and wild animals such as buffalo and antelopes and is widespread in Africa. It transmits the diseases, such as theileriosis, Nairobi sheep disease and Thogoto virus in addition to causing anaemia, tick toxicosis and severe damage to the ears that become predisposed to bacteria infections and other tick-borne diseases such as anaplasmosis, babesiosis, cowdriosis which often occur in the same geographical regions. These tick-borne diseases, particularly East Coast Fever (E.C.F) caused by Theileria parva cause great looses to livestock farmers in Africa. The commercial BM86 vaccines (Tick GARD™ and Gavac™) provided alternative tick control from chemical acaricides, for a number of Boophilus tick species but are not effective against R. appendiculatus and the search for an effective vaccine against this tick is a priority for the livestock industry. Two bacterially expressed antigens from R. appendiculatus, Ra86 and Ferritin 2 (raferr2) were evaluated against R. appendiculatus in rabbits. Two overlapping fragments (N-terminal and C-terminal) of Ra86 were PCR amplified and expressed in pQE30UA vector in E. coli. The insoluble protein was purified using 6xHIS Tag fusion. Eight New Zealand white rabbits were vaccinated using 50 µg mixture of Ra86N and C in adjuvant and immune response monitored by ELISA and Western Blot. Using 50 R. appendiculatus adult females on rabbit ears a 12-33% reduction in individual tick feeding parameters was recorded between control and vaccinated. Vaccination of rabbits with full-length Ferritin 2 (raferr2) antigen showed significant reduction in dropped ticks, mean tick weight and egg weights of immunized versus controls (ANOVA, $P<0.05$). A combination of Ra86 and raferr2 antigens could result in reduction of ticks in successive generations in vaccinated animals.

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An integrative approach towards control of the cattle tick, Rhipicephalus microplus

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An effective working solution to manage both the increasing number of acaricide-resistant Rhipicephalus ticks and transmission of tick-borne diseases is lacking and requires immediate attention. At the University of Pretoria, five research areas are integrated to provide an overarching strategy for the control of these ticks, using Rhipicephalus microplus and R. decoloratus as model species. These include rapid acaricide resistance diagnostics, tick vaccines, phylogeography, bovine immunology and drug screening platforms for Babesia divergens. Timely diagnosis of acaricide resistance is vital as it guides farmers and governments towards selection of appropriate acaricide(s) and the identification of target areas for first-stage field vaccination trials. To date, known SNPs have been confirmed at 108 farms in South Africa and new SNPs have been identified for amitraz resistance diagnostics. A high prevalence of resistant alleles against amitraz and homozygous resistant to pyrethroid-based acaricides were shown with highly resistant farms along the east-coast of South Africa. With regards to tick vaccines, a multi-antigen vaccine that reduces the number of engorged Rhipicephalus microplus ticks by some 78% in bovines, with no viable eggs and offspring has been validated in small-scale vaccine trials. Phylogenetic studies of R. microplus indicate that South African and South American geographical strains clade together. As low genetic diversity and no genetic population structure could be observed, using conventional markers, a panel of microsatellites for phylogeography was developed. Results obtained for sub-Saharan Africa can now guide the design of cross-protective vaccines across geographical areas. In regards to vaccine formulation, the bovine immune response to R. microplus was determined from skin, blood and lymph node tissues from Friesian, Brahman and Bonsmara cattle during the entire life cycle of R. microplus. The latter provided insight into specific stages of immune suppression by R. microplus in bovines, such as suppression of T and B cell maturation and migration of immune cells to lymphoid tissues. The latter have also been validated and expanded on using a murine model, which now pave the way forward to evaluating adjuvants for improvement of vaccine formulations. In conclusion, combining all the avenues of research that includes genetic diversity of R. microplus in sub-Saharan Africa (including acaricide resistance status), parasite modulation of host immune responses, optimized vaccine production and formulation will aid in a comprehensive approach towards control of the cattle tick, R. microplus.

Expression of recombinant proteins at different locations in Bacillus subtilis

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Bacillus subtilis is a model organism for Gram-positive bacteria, which is considered as a GRAS organism. This bacterium has been extensively used for the production of recombinant proteins at industrial scale by biotechnology companies. This report focuses on the construction of plasmid-based vectors for expression of recombinant proteins in different locations in B. subtilis. First, we investigated the use of plasmid-based vectors to develop expression vectors, which exhibit structural stability. Second, a new series of IPTG-inducible synthetic promoters, Pgrac was generated, in which promoter Pgrac100 could be useful for intracellular production of recombinant proteins in B. subtilis. These expression vectors could be converted into inducer-free expression, which allowed production of recombinant proteins without the addition of the inducer. Third, the secretion expression vector could be easily generated by introducing a secretion signal sequence after the promoter and ribosome binding site. Finally, a system for covalent immobilization of recombinant proteins on the surface of B. subtilis cells could be established.

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Prrsv Replicon With Diverse Tropism As A Vaccine Vector Candidate

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Porcine reproductive and respiratory virus (PRRSV) is a devastating virus in swine and is difficult to grow in cell culture partially due to a limited number of cell lines susceptible and permissive for it. Using glycoprotein G gene (GVSV) from a pantropic virus, vesicular stomatitis virus (VSV), to replace genes encoding the minor glycoproteins of PRRSV in FL12, a putative infectious clone of American serotype PRRSV, the resultant chimeric virus was recovered (FL12GΔ2-4). FL12GΔ2-4 can infect and complete its viral life cycle in different cell lines which originally unsusceptible to PRRSV such as BHK-21, PK-15, HEK-293, HeLa… The spreading of the virus was confirmed by immunofluorescent method and its expression of GVSV and PRRSV proteins was shown by Western blot and neutralization assay. Further investigation indicate that the chimeric virus is stable genetically and the pantropic effect of GVSV can also be observed when G protein is expressed from a separate DNA plasmid. Overall, our studies indicate that GVSV can be incorporated into PRRSV virion irrespective of being provided in cis or in trans and the incorporation confers the virus to be able to infect and spread in multiple cell lines.

Cattle breed TLR2 polymorphisms impact on immune and potential subsequent vaccine response

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Background and objective: There is evidence that high yielding cows are strongly susceptible to infectious diseases such as bovine tuberculosis. Many pathogens are recognized by Toll-like receptors (TLRs) and the resulting activation is crucial for an adequate immune response. Polymorphisms within TLRs have been associated with resistance traits in livestock and TLR2 plays a major role in recognizing Mycobacterium (M.) bovis. By comparing TLR2 sequences of two popular dairy breeds, we investigated whether sequences differ between breeds, potentially leading to functional effects in response to a variety of ligands including mycobacterial species.

Methods: TLR2 sequences of Brown Swiss (BS) and Holstein Friesian (HF) cows were cloned, sequenced, and transfected into HEK 293T cells stably transfected with NF-κB inducible secreted embryonic alkaline phosphatase (SEAP) gene. Cells were stimulated using the triacylated lipopeptide Pam3CSK4, diacylated FSL-1, M.bovis and Pam3CYs-SSSNKSTTGSGETTTA, a synthetic Mycobacterium tuberculosis (Mtb) peptide. SEAP activity induced by NF-kB activation was measured by reading the optical densities after incubation with Quanti-Blue reagent.

Results: A total of 18 polymorphisms within TLR2 were detected across the breeds. Response to specific TLR2 stimulation represented by NF-κb activation differed between the breeds. Activation of TLR2 increased significantly in constructs containing the H326Q variant, found in the BS cattle breed, confirming that TLR2 function is altered by substitution of the positively charged H to uncharged Q. No TLR2 activation was measured upon stimulation with live M.bovis, but with the synthetic Mtb lipoprotein.

Conclusion: The stronger TLR2 activation in H326Q constructs found in the BS breed may lead to a stronger immune response, making this breed potentially more resistant to infections than the HF breed. Furthermore, the non –response to M.bovis in all constructs may suggest a species-specific adaption of the pathogen to the corresponding receptor of its main host. These findings may have an impact on breeding strategies and future vaccine development.

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