Abstract Book

International Conference

28-29 February, 2024

VACCINE CONFERENCE
CEMB - 2024

Current Trends, Prospects and Opportunities in Vaccine Research

Unlocking a Healthier Tomorrow: The Crucial Role of Vaccines

Venue: Centre of Excellence in Molecular Biology (CEMB), University of the Punjab, Lahore, Pakistan

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Chief Organizer's Massage

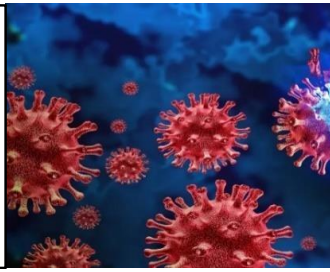
Dear Esteemed Colleagues,

Welcome to the abstract book of the International Conference on "Current Trends, Prospects, and Opportunities in Vaccine Research: Unlocking a Healthier Tomorrow," hosted at the Centre of Excellence in Molecular Biology (CEMB) at the University of the Punjab, Lahore, Pakistan, in partnership with the School of Biological Sciences (SBS) Lahore, National Center for Biotechnology and Genetic Engineering (NIBGE) Faisalabad, Atta-ur-Rahman School of Applied Biosciences (ASAB) NUST Islamabad, University of Veterinary and Animal Science (UVAS) Lahore, University of Agriculture, Faisalabad, Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, Cholistan University of Veterinary and Animal Sciences (CUVAS) Bahawalpur, Veterinary Research Institute (VRI) Lahore and Sindh Institute of Animal Health (SIAH) Karachi, Livestock Research KPK and Center for Advanced Studies for Vaccinology and Biotechnology (CASVAB), Quetta. As we convene on 28-29 February 2024, we aim to explore the crucial role of vaccines in shaping the future of global health. Vaccines stand as powerful tools, a testament to human ingenuity, and collective effort. Vaccines have significantly impacted public health by shielding us from devastating diseases, leaving a profound mark on generational well-being. Beyond human health, vaccines play a vital role in safeguarding animal health in the poultry and livestock sectors, ensuring food safety, and sustaining global food production. Vaccines mitigate economic losses for farmers and reduce the risk of zoonotic diseases, thus addressing serious public health threats. Throughout the conference, we will delve into current trends, prospects, and opportunities in vaccine research, recognizing the transformative potential of innovative approaches and technologies. Our discussions will encompass various critical topics, from novel vaccine platforms to emerging challenges including bottlenecks in the production of vaccines in Pakistan. I believe this gathering will foster collaborations and unite individuals dedicated to advancing humanity's welfare. Together, let us unlock the potential of vaccines to safeguard public health, enhance pandemic preparedness, and create a healthier world for future generations.

Thank you for your participation and valuable contributions.

Warm regards,
Prof. Dr. Moazur Rahman
Director, CEMB

INTERNATIONAL CONFERENCE Current Trends, Prospects & Opportunities in Vaccine Research, 2024



Scientific Program Day 1, (Wednesday; February 28, 2024)

09:10-10:15Hrs	**Registration of Participants**	
Opening Session		
10:15-10:30Hrs	**Guests to be Seated**	
10:30-10:35Hrs	**National Anthem**	
10:35-10:40Hrs	**Recitation of the Holy Quran**	
10:40-10:50Hrs	**Welcome and Overview of the Conference**	**Prof. Moazur Rahman** Director CEMB & Chief Organizer
10:50-11:00Hrs	**Genesis of CEMB**	**Prof. Sheikh Riazuddin** (Founding Director CEMB)
11:00-11:20Hrs	**Comments from Eminent Scientists and Prestigious Guests**	Prof. Dr. Shahid Baig; Prof. Muhammad Ali, Prof. Talat Naseer Pasha, Prof. Nasim Ahmad, Prof. Muhammad Sajjad, Mr. Asim Rauf CEO DRAP, etc.
11:20-11:30Hrs	**Address of Vice Chancellor PU**	**Prof. Khalid Mehmood** *Vice Chancellor, University of the Punjab, Lahore*
11:30-11:40Hrs	**Address by Guest of Honor**	**Prof. Javed Akram** Provincial Minister for Health
11:40-11:50Hrs	**Address by Chief Guest**	**Prof. Kouser Abdullah Malik** Federal Minister for National Food Security and Research
11:50-12:20Hrs	**Plenary Talk 1** Why is it important to	**Prof. Donald King** Pirbright Institute, UK

	monitor the quality of foot-and- mouth disease vaccines?	
12:20-12:30Hrs	**Distribution of Souvenirs and Group Photo**	
12:30-13:30Hrs	**Prayer and Lunch Break**	
Technical Session I Chair: Dr. Abdul Haq; Co Chair: Dr. Shahid Baig		
13:30-14:00Hrs	**Plenary Talk 2** Vaccination strategies for selected poultry pathogens	**Prof. Michael Hess** University of Veterinary Medicine, Austria
14:00-14:20Hrs	Progression in Indigenous Vaccine Development in Poultry Sector	**Dr. Khalid Naeem** Grand Pharma
14:20-14:40Hrs	DRAP and its current operational approach	**Dr. Zia Hussain** Additional Director DRAP / Chairman Gap Analysis Committee on VRIs / Chairman CALSD, DRAP, Pakistan
14:40-15:00Hrs	Development of vaccine against Peste-des-Petits Ruminant virus having ease of application and improved efficacy	**Dr. Salahudin Shah** Principal Scientific Officer National institute of Agriculture and Biology (NIAB)
15:00-15:20Hrs	Opportunities and Challenges in Foot-and-Mouth Disease Vaccine Development and Commercialization in Pakistan	**Dr. Wasim Abbas** Principal Scientific Officer National Institute for Biotechnology and Genetic Engineering (NIBGE)
15:20-16:00Hrs	**Tea break and Poster session**	
Technical Session II Chair: Prof. Dr. Munir Iqbal; Co Chair: Prof. Dr. Naeem Rashid		

16:00-16:30Hrs	DNA Vaccine: a promising vaccine platform for human and animal health	**Dr. Budiman Bela** Universitas Indonesia
16:30-17:00Hrs	Preparation of bivalent nanoparticle vaccines for Influenza virus subtypes H1N1 and H3N2 and evaluation of its immunological effect in mice	**Prof. Jing-yu Wang** Northwest A&F University China
17:00-17:20Hrs	Outlook- Animal vaccines sector in Pakistan	**Dr Sajjad Hussain** Veterinary Research Institute (VRI), Punjab, Pakistan
17:20-17:40Hrs	Production of Foot and Mouth Disease Vaccine and its Challenges in Pakistan	**Prof. Ali Raza Awan** University of Veterinary and Animal Sciences, Lahore, Pakistan
17:40-18:10Hrs	Structure-based vaccine design. ***(Virtual Talk)***	**Prof. Jason Mclellan** University of Texas, USA
Sceintific Program Day 2 (Thursday; February 29, 2024)		
09:55-10:00Hrs	**Recitation of Holy Quran**	
10:00-10:30Hrs	Improving the efficiency of Poultry vaccines	**Prof. Munir Iqbal** Pirbright Institute, UK
10:30-11:00Hrs	Genotype VII of Newcastle disease virus. A global approach for a new vaccine. ***(Virtual Talk)***	**Dr. Farid Hemmatzadeh** Associate Professor The University of Adelaide, Australia
11:00-11:20Hrs	**TEA BREAK**	
Technical Session III Chair: Dr Khalid Naeem		
11:20-11:50Hrs	Development of the typhoid conjugate vaccine being used in the childhood vaccine programme in Pakistan. ***(Virtual Talk)***	**Prof. Andrew Pollard** University of Oxford, UK

11:50-12:20Hrs	mRNA manufacturing, and analytical development ***(Virtual Talk)***	**Dr. Saif ur Rashid** Australia
12:20-12:40Hrs	Exploration of viral structural proteins for vaccine development	**Prof. Moazur Rahman** CEMB/SBS
12:40-13:00Hrs	Vaccine production setup at VRI Peshawar, its current status and future plan.	**Dr. Muhamad Ijaz Ali** Veterinary Research Institute (VRI), KPK, Pakistan
13:00-14:00Hrs	**LUNCH & PRAYER BREAK**	
Technical Session IV **Chair: Dr. Mazhar Iqbal; Co Chair Dr. Kashif Saleemi**		
14:00-14:30Hrs	Multiple subunit vaccine induces highly immunity protection against waterfowl *Chlamydia psittaci* infection	**Prof. Cheng He** China-ASEAN Innovative Academy for Major Animal Disease Control, China
14:30-1500Hrs	Important Zoonotic Diseases and Vaccines	**Dr. DERYA KARATAŞ YENİ** Necmettin Erbakan University, Turkey
15:00-15:15Hrs	Infectious Bronchitis virus ***(Virtual Talk)***	**Dr. Min Liao**
15:15-15:30Hrs	Current status of Vaccine Production in Baluchistan.	**Prof. Muhammad Shafee** CASVAB, University of Baluchistan
15:30-15:45Hrs	Vaccine Portfolio at Sindh Institute of Animal Health	**Dr. Nazeer Hussain** Director General, Sindh Institute of Animal Health, Pakistan
15:45-16:00Hrs	Computational Tools for Bacterial Protective Antigen Discovery	**Dr. Amjad Ali** National University of Sciences and Technology (NUST), Pakistan
16:00-16:10Hrs	**Discussion on the upshot of the Vaccine Conference**	
CLOSING CEREMONY		

International Speakers

Prof. Michael Hess
University of Veterinary Medicine, Austria

Dr. Saif ur Raisheed
BioCina, Australia

Dr. Min Liao
Zhejiang University, China

Dr. Farhid Hemmatzadeh
The University of Adelaide, Australia

Dr. Don King
The Pirbright Institute, UK

Prof. Cheng He
China-ASEAN Innovative Academy for Major Animal Disease Control

Prof. Sir Andrew Pollard
University of Oxford, UK

Dr. Budiman Bela
University of Indonesia, Indonesia

Dr. DERYA KARATAŞ YENİ
Necmettin Erbakan University, Türkiye

Prof. Jason McLellan
The University of Texas, USA

Prof. Munir Iqbal
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ABSTRACT INFO

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Why is it important to monitor the quality of foot-and-mouth disease vaccines?

Donald P. King, Ginette Wilsden, Clare Browning, Krupali Parekh, Simon Gubbins, David J. Paton, Anna B. Ludi

Although technically difficult and expensive to produce, it is estimated that more than 2 billion doses of foot-and-mouth disease (FMD) vaccine are used annually. Most FMD vaccines comprise chemically inactivated antigens prepared by growing large amounts of virus in cell cultures (such as BHK21) formulated with an oil or aqueous adjuvant. The WOAH Manual of Diagnostic Tests and Vaccines for Terrestrial Animals describes procedures that should be adopted to ensure the homologous potency of the product, and FAO and WOAH have produced guidelines for vaccine selection and vaccination monitoring. Nevertheless, the market is complex with regional differences in prevailing viruses, approaches to vaccination, and governance systems for vaccine quality control. In many countries, there is no standardization of vaccine strains and different vaccine manufacturers supply FMD vaccines derived from a wide range of different master-seed strains. Furthermore, the quality of FMD vaccines (defined by potency, antigenic relevance, antigen payload, and purity) is highly variable and the selection of an appropriate vaccine needs to consider heterologous responses elicited by the formulated product against the target viral lineages likely to be encountered in the field. Independent assessment of vaccines is extremely important; failure to do this has contributed to poor trust in FMD vaccine quality and a lack of investment in FMD vaccines. This presentation highlights a simple approach that has been developed to assess heterologous responses of FMD vaccines using regional relevant reference antigens. In contrast to vaccine matching, this system can be applied to multivalent formulated vaccines as supplied to customers and generates data that allows vaccines from different suppliers to be compared.

ABSTRACT
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Vaccination strategies for selected poultry pathogens

Michael Hess, Dipl. ECPVS

Vaccines are crucial components to support health and welfare of poultry with substantial consequences on economy. Knowledge about disease pathogenesis and the biology of the pathogen are needed to implement a robust vaccination strategy. In the actual presentation three different pathogens, fowl adenovirus, *Escherichia coli* and the parasite *Histomonas meleagridis* will be covered, to highlight different approaches for vaccine development. The re-emergence of those pathogens in recent years with very limited intervention strategies is a common characteristic for all of them. Worldwide, diseases due to fowl adenovirus (FAdV) infections – inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome (HHS) and adenoviral gizzard erosion (AGE) – in broilers increased substantially in recent years. It can be hypothesized that the severe impact on health and the lack of specific therapeutics leads to the untargeted use of antimicrobials in order to minimize losses. Variation in strains representing different serotypes is a substantial challenge to develop a broadly protecting vaccine. As a consequence of strain diversity autogenous vaccines are widely used in chicken breeders and even broilers depending on the epidemiological situation. In recent years we developed subunit vaccines based on the fiber protein and very good efficacy was demonstrated experimentally. However, the lack of cross protection is a severe disadvantage, similar to whole virus killed vaccines. As a new concept we developed chimeric fibers consisting of 2 different serotypes fulfilling the demand of a broad-based vaccine. In addition to successful demonstration of efficacy new knowledge on antigenic epitopes was revealed. *Escherichia coli* is frequently isolated from healthy birds with some avian pathogenic *E. coli* (APEC) strains being capable to induce colibacillosis, a global problem in poultry production. *E. coli* is probably the most extensively studied organism in poultry and a substantial number of publications show the high interest on the pathogen within the international scientific

community, especially in the field of poultry health. However, due to the zoonotic potential of some strains research on *E. coli* is also of interest for a wider community and together with the frequent use of antimicrobials to minimize suffering of animals and losses an important One Health issue. The substantial variation of strains together with the need for mass application via the respiratory tract is a substantial challenge for vaccine development. We have recently implemented radiation technology to inactive live bacteria but at the same time being able to induce immunity. In experimental animal trials the efficacy of such a concept was demonstrated opening a new option for vaccine development against this highly important pathogen. The protozoan parasite *Histomonas meleagrids* is the etiological agent of histomonosis (syn. Blackhead disease), a fastidious disease in turkeys which can lead to the complete loss of a flock. The disease is also of increasing importance in chickens, mainly broiler breeder and layers. With the recent ban of all prophylactic and therapeutic substances in the EU and the US the disease is a serious problem on welfare and health of animals. Albeit Paramomycin, an aminoglycoside antibiotic, has certain effects when given prophylactically it was shown in an experimental study that the application triggers antimicrobial resistance in the gut microbiota with consequences on One Health. The complicated nature of the parasite and its dependance on the presence of live bacteria for growth in vitro is a severe obstacle to develop a vaccine. In the last 2 decades we not only succeeded to establish a clonal culture of a virulent strain which we attenuated *in vitro*; we also developed the concept of a single bacterial strain-parasitic culture (syn. monoxenic culture). Although efficacy and safety data in turkeys and chickens support the idea to prevent histomonosis by vaccination, the low tenacity of the parasite with consequences on the vaccination technology are substantial challenges which remain to be solved. Overall, the pathogens mentioned above, ranging from viruses to bacteria and a parasite, emphasize different efforts to develop new vaccines for re-emerging poultry diseases which are characterized by the restricted availability of licensed therapeutics or vaccines, if available at all. All of them are of increasing importance in poultry production with severe consequences on health and welfare. Histomonosis is also a good example how legislation and the ban of drugs severely impacts health and welfare of animals. The frequent antimicrobial intervention for some of the pathogens targeted in the actual presentation highlights the importance of them in the One Health concept which goes beyond poultry health and production.

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DNA vaccine: a promising vaccine platform for human and animal health

Budiman Bela

The potential use of naked DNA as a vaccine platform remains appealing due to its good biocompatibility, stability and cost efficient production. The ability of DNA vaccine to stimulate both humoral and cellular immune responses and the advances in antigen design renders this platform as a versatile platform for various purposes of vaccination. Studies have shown that DNA vaccine has the potential to be used for prevention and treatment of infection, cancer, allergy and autoimmune diseases. Despite its effectiveness in small animal, however, DNA vaccination has been shown to be less effective in large animal. The use of DNA vaccine in human also faces a similar challenge with that of large animal in its effectivity to stimulate immune response due to low translation of antigenic protein as a result of low transfection efficiency. In contrast to mRNA vaccine platform that can be readily used for translation of the antigenic protein in the cytoplasmic compartment, naked DNA vaccine requires entry into the nuclear compartment for gene transcription prior to translation of its antigenic protein. Strategies to increase DNA vaccine safety and effectiveness consist of elimination of antibiotic use in DNA propagation, targeting to dendritic cells, needleless injection, electroporation, mini circle DNA, use of nuclear localization sequence for delivery into the nuclear compartment and increasing expression through design of DNA and mRNA structure. Guidelines of plasmid DNA vaccines for medical and veterinary use have been developed to ensure the safety and efficacy of this vaccine platform.

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Preparation of bivalent nanoparticle vaccines for influenza virus subtypes H1N1 and H3N2 and evaluation of its immunological effect in mice

Jing-yu Wang, Xuefeng Qi, Pan Tang, En- qi Du

Vaccination is the most effective and economical strategy to prevent and control the infection of human and animal influenza viruses. Most of the influenza A viruses (IAV) vaccines in the market are traditional chicken embryo whole virus inactivated vaccines, which have certain limitations. In recent years, Nanoparticle (NPs) vaccines have shown broad application prospects in the prevention of novel coronavirus, hepatitis B and other diseases. We obtained the HA gene sequence of human influenza virus subtypes H1N1 and H3N2 IAV pandemic strains through GISAID database, and constructed recombinant plasmids using homologous recombination method. The recombinant plasmid was transfected into Sf9 cells with the linearized baculovirus genome Bacmid, and the generation and expression of the recombinant baculovirus rBac-H1-HA-TM and rBac-H3-HA-TM were identified by PCR, WB and hemagglutination. We used Hi5 insect cell lines to express the recombinant baculovirus HA protein of H1 and H3 subtypes, and through the replacement of high concentration PS80 buffer in the affinity purification process, the HA protein and PS80 were co-incubated on the lentil affinity column, and the hydrophobic transmembrane domain of the stem of the tripolymer HA was stably bound to the hydrophobic core of PS80 detergent. This in turn forms the nanoparticles at the core of the detergent. SDS-PAGE analysis showed that the purity of purified HA nanoparticle protein was > 85%. Dynamic light scattering (DLS) analysis showed that the particle size of the nanoparticles ranged from 28 nm to 68 nm. Transmission electron microscopy (TEM) analysis showed that the HA head domain was prominent outside the nanoparticles. Hemagglutination experiment showed that the coagulative activity titer of purified nanoparticle protein was > 211. Bivalent nanoparticle vaccines (BNV) with H1 and H3 subtypes immunized Balb/C mice and showed that the BNV group showed a

balanced Th1/Th2 immune response, while the BNV+CPG1 adjuvant group tended to have a stronger Th1 immune response. The results of Balb/C mice challenge test showed that BNV vaccine had good protective effect and could effectively reduce the gross and microscopic lung lesions caused by virus infection.

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Multiple subunit vaccine induces high immunity protection against waterfowl *Chlamydia psittaci* infection

He Cheng, Li Qiang, Chen Siyu.

Chlamydia psittaci (*C. psittaci*) is threatening to the animal industry and human beings. An increasing human psittacosis is frequently reported due to close contact with ducks, pigeons, and parterres. Although major outer membrane protein (MOMP) of *C. psittaci* was commercialized as the recombinant vaccine for layers in 2006, its partial protection hampers implementation for ducks and pigeons. In our pioneer study, an inactivated *C. psittaci* EBs vaccine with chitosan-Vibrio cholera ghost (VCG) adjuvant showed a promising immunity against *C. psittaci* infection in SPF chickens by inducing a high Th-1 cellular immune response and consistent high antibody levels. However, no vaccine is available against waterfowl. In the present study, our hypothesis is that multiple subunit vaccine adjuvanted with VCG and temperature sensitive chitosan gel might trigger highly protection via intranasal route. Initial safety test, ducks aged 7-day, 28 day and 180 day were inoculated intranasally with the multiple subunit vaccine (Pmp17G, Pmp19G, Pmp20G, Pmp21G, MOMP) and observed for 2 weeks. Both the performance of ducks' growth and egg production were not affected. Moreover, no severe inflammation was observed in local inoculations. Subsequently, we did assess multiple vaccine efficacy, both 7-day-old ducklings and 90-day-old layer ducks were immunized the vaccine twice with an interval of 14 days. Post immunization, ducklings with the multiple vaccine were found to yield higher IgG antibody levels with a consistent increase, which was comparable to the inactivated EB vaccine. After the challenge with virulent *C. psittaci*, lower bacterial excretion and less pathological lesions of target organs were determined in the multiple subunit vaccine compared to those of other groups. Regarding layer ducks, both the inactivated EB and multiple subunit vaccine induced higher IgG antibodies than the commercial MOMP vaccine. However, the multiple subunit vaccine induced less inflammation than the inactivated EB vaccine in the layer ducks, suggesting that the inactivated EB vaccine and multiple subunit

vaccine were alternatives to the commercial MOMP vaccine for layer ducks. The combination of multiple subunits can prove a promising approach to developing a vaccine against *C. psittaci* and stopping its transmission from animals to humans.

ABSTRACT INFO

Improving the efficiency of poultry vaccines

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Munir Iqbal

Poultry vaccines play a crucial role in mitigating the impact of avian viral diseases, such as avian influenza and Newcastle disease. However, these viruses continually undergo genetic changes, leading to vaccine failures. Moreover, commonly used inactivated virus vaccines result in reduced immunity in chicks due to interference from maternally derived antibodies (MDAs). Additionally, these vaccines do not allow easy differentiation between infected and vaccinated animals (DIVA).

To address these challenges, we have developed novel approaches that identify antigenic epitopes for integration into viral antigens, enhancing the breadth of vaccines. We have also created Targeted Antigen Delivery Vaccine (TADV) technology, selectively delivering vaccine antigens to chicken antigen-presenting cells (APCs), inducing faster, stronger, and broader cross-reactive immunity in chickens. The TADV-H9N2 avian influenza vaccine was developed containing recombinant haemagglutinin (rH9HA) fused with a single-chain fragment variable (scFv) antibody specific to chicken APCs (CD83). Chickens with varying MDA levels (MDA-, MDA+, MDA++) were vaccinated with TADV (rH9HA-CD83), untargeted rH9HA, or inactivated H9N2 virus vaccines. Antisera from vaccinated birds were analyzed for vaccine antigen (H9HA)-specific antibodies using haemagglutinin inhibition (HI) and virus neutralization (VN) assays. The TADV-H9N2 vaccinated chickens showed the most potent H9HA-specific immune responses at a faster rate compared to untargeted rH9HA or inactivated H9N2 virus vaccines. Moreover, chickens vaccinated with TADVs demonstrated complete protection from clinical diseases, with significantly reduced virus shedding upon challenge with the H9N2 virus.

Furthermore, our TADVs induced broader cross-reactive antibodies against H9N2 antigenic variants. In summary, our data demonstrate that targeting antigens to the APC receptor CD83 improves the efficacy of poultry vaccines, and their potency remains unaffected by the presence of MDAs in chickens.

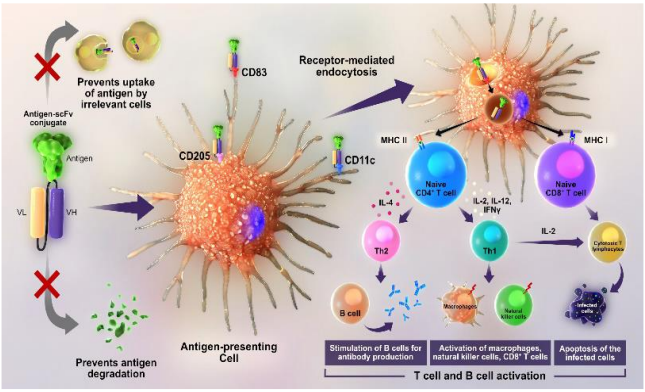

Figure: Schematic representation of antibody-based antigen targeting. The antigen is conjugated to antibodies specific APC receptors e.g., CD83. Binding between antigen-antibody conjugate and APC receptor initiates receptor-mediated endocytosis, activating CD4+ T cells and inducing antibody responses. Depending on the targeted APC receptor, the antigen-antibody conjugate can also activate CD8+ T cells.

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Development of the typhoid conjugate vaccine being used in the childhood vaccine program in Pakistan

Andrew Pollard

Enteric fever is a major cause of morbidity in childhood across South Asia and especially in school-age children, where access to clean water and adequate sanitation is limited. Studies conducted across Nepal, Bangladesh and Malawi have helped define the size and importance of typhoid and paratyphoid infections as contributors to childhood illness and use of antibiotics. Use of a controlled human infection model of typhoid infection in Oxford accelerated the development of a typhoid conjugate vaccines by giving definitive evidence of its efficacy and supported recommendations for its use by the World Health Organization in 2017. Efficacy studies in South Asia showed that the vaccine could prevent 80% of childhood typhoid among children under 15 years of age over the subsequent 2 years. For comprehensive control of the disease, bivalent conjugate vaccines are now in development which will cover both typhoid and paratyphoid, and if shown to be protective could further improve the health of children in Asia and Africa. In Oxford a COVID19 vaccine was developed in partnership with AstraZeneca and became one of the most widely used vaccines in the world, preventing more deaths than any other product according to one report.

ABSTRACT INFO

Genotype VII of Newcastle disease virus. A global approach for a new vaccine

Putri Pandarangga[1,2*], Phuong Doan[2,3], Rick Tearle[5], Wai Low[5], Yan Ren[5,] Hanh Thi Hong Nguyen[2], Niluh Dharmayanti[4], and **Farhid Hemmatzadeh[2]**

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Newcastle Disease Virus (NDV) genotype VII (GVII) becoming predominant strain of NDV in poultry industry. It causes high mortality even in vaccinated chickens with a common NDV genotype II vaccine (GIIvacc). The pathogenesis of the NDV-GVII is different than the other velogenic strains and mainly cause lymphocyte depletion and mortality within first 24 hours of infection in infected birds. To overcome this, killed GVII vaccine has been used to prevent NDV outbreaks. However, the debate about vaccine differences remains ongoing. The spleen transcriptomes from vaccinated chickens reveal that GVIIvacc affected the immune response by down regulating neuro-inflammation. It also enhanced a synaptogenesis pathway that operates typically in the nervous system, suggesting a mechanism for the neurotrophic effect of this strain. The down-regulated immune system regulation correlated with protecting the nervous system from excess leukocytes and cytokine activity. In contrast, GIIvacc halted apoptosis via PERK/ATF4/CHOP as part of the unfolded protein response pathway but did not affect the expression of the same synaptogenesis pathway. Thus, the GVIIvacc only be used in countries where GVII is the driver of NDV outbreaks. Additionally, molecular signatures may also help in crafting new vaccines that activate particular immune system genes to combat NDV outbreaks.

ABSTRACT INFO

mRNA and beyond

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The market of biopharmaceuticals is on the rise. Revenue of the worldwide pharma industry is expected to cross 1.6 trillion USD in 2023 and biopharmaceuticals accounting for a third of the global market. To date, only a small portion of the human genome has been drugged which represents only 0.05% of the human genome. The four letters "ATGC" have the potential to save our lives. Targeting these four letters could expand the proportion of human genome for therapeutics. Nucleic acid modalities, DNA- and RNA-based technologies, offer promising approaches in gene therapy and represent an auspicious alternative to conventional therapeutics because of their high potency and efficacy, safe administration and capability for rapid development and low-cost manufacture. In this talk recent developments, current challenges and future directions in the end-to-end manufacturing process, analytical testing for critical quality attributes, formulation, and delivery system in nucleic acid modalities are discussed.

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Important zoonotic diseases and vaccines

Derya Karatas Yeni

Zoonotic diseases have threatened human and animal health since ancient times; It is a global problem that can result in death and has many serious consequences. The main causes of zoonotic diseases are bacterial, viral or parasitic agents. In some studies; Every year, 2.5 billion cases and 2.7 million people die from zoonotic diseases in the world. 61% of diseases seen in humans are of animal origin, diseases that need to be notified are zoonotic, and 5 new human diseases emerge every year, 3 of which are zoonotic. Over 90% of foodborne illnesses in humans result from consuming animal products, while 80% of potential bioterrorist agents have zoonotic origin. Zoonoses reported to date in Türkiye; 37 of them are bacterial, 13 fungal, 29 viral, 28 parasitic, and 21 of these infections are among the high priority zoonotic diseases in Europe. Türkiye is situated geographically in Eurasia, making it susceptible to the intercontinental movement of animals, particularly through bird migrations, and exposing humans to potential zoonotic infections. Climatic conditions, vegetation structure, wildlife and especially the protection area for migratory birds are highly variable. The geographical structure also provides suitable habitats for various vector arthropods, such as blood-feeding insects and ticks, throughout all four seasons of the year. Most zoonotic diseases can be prevented with preventive vaccines. Many vaccines are produced to protect against zoonoses in the world and in our country. Zoonotic and non-zoonotic vaccines produced in our country. Some of these are Clostridial vaccines, *E. coli*, *Pasteurella multoicida, Mannheimia haemolytica, Mycoplasma agalactiae, Mycobacterium paratuberculosis, Corynebacterium pseudotuberculosis, Rhodoccocus, Moraxella bovis, Brucella abortus* S-19, *B. melitensis* Rev.1, *Bacillus anthracis, Mycoplasma capripneumonia* and ORT., foot and mouth, blue tongue, rotavirus, coronavirus, sheep-goat pox, sheep-goat plague/PPR, LSD, ecthyma, mycotic and protozoan vaccines. Turkey is self-sufficient in the production of animal vaccines. The robustness of our veterinary vaccine manufacturing system has, in turn, enabled the development of human vaccines for the COVID-19 pandemic. To safeguard public

health and animal well-being, disease and epidemic prevention and control require coordinated efforts among national and international stakeholders. Multidisciplinary and expert researchers engage in developing advanced research projects and vaccine studies specifically geared towards controlling zoonotic diseases. Administrative and political decisions impacting global climate change, urbanization, land use, and industrial and agricultural pollution must align with ecological and epidemiological insights into diseases. To ensure the health of people in a society, it is imperative that animals are healthy first.

National Speakers

Dr. Khalid Naeem Khawaja
Grand Pharma, Pakistan

Dr. Zia Hussain
Additional Director DRAP/ Chairman Gap Analysis Committee on VRIs / Chairman CALSD, DRAP, Pakistan

Prof. Dr. Ali Raza Awan
University of Veterinary and Animal Sciences, Lahore, Pakistan

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Progression in indigenous avian vaccine development

Khalid Naeem Khawaja

Use of poultry vaccine in poultry started in Pakistan in mid 60's. Initially, vaccine was only introduced against two major diseases of backyard poultry, such as Newcastle Disease and Fowl Pox, soon followed by more organized vaccination activities in commercial poultry. Poultry vaccine production at different public sector institutions also started between 1960 to1975. However, continuous expansion in the commercial poultry led to the import of multiple types of live and killed avian vaccines. The first time introduction of Hydropericardium Syndrome (HPS) among broilers in 1989 led to the involvement of private sector in vaccine production in this country. Despite limitations of developing a vaccine against a new pathogen for which no vaccine was previously available globally, this unique event led to the better understanding of the concept of using homologous vaccines. This resulted in the development of more such vaccines using locally selected seed-virus in subsequent years at the time of avian influenza (AI) H7 outbreak of 1995, AI H9 outbreak of 1999 and AI H5 outbreak of 2005. These efforts led to the successful control of most of the above listed poultry diseases. Currently, along with the conventional egg-based & Cell-culture based vaccine production techniques the procedures of genetic engineering are being employed for the local production of poultry vaccines in public and private sectors in this country. Better regulatory and production controls are being developed to support the local vaccine production using the concept of Homologous vaccine. More work on studying the antigenic variability of local pathogens and/or their potential as vaccine candidate is further desirable through closer interaction between R & D institutions and avian vaccine producers.

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Exploration of viral structural proteins for vaccine development

Nazeer Hussain Kalhoro

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DRAP and its current operational approach

Zia Hussain

DRAP was established under the DRAP Act 2012. DRAP is under the process of WHO accreditation, and various operational guidelines have been prepared in collaboration with WHO. Digitalization of the operations of DRAP has upgraded its working capacity. Presentation on the topic shall give insight into the working of DRAP, including the approval process of vaccines and other biological products.

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Opportunities and challenges in foot-and-mouth disease vaccine development and commercialization in Pakistan

Mazhar Iqbal, Waqar Rauf, **Wasim Abbas**

The foot-and-mouth disease (FMD) is highly transmittable and economically lethal disease of cloven-hoofed animals. There are more than 93.9 million head of cattle and buffalos in Pakistan. In Pakistan, 35-40% of the animals are exposed to FMD virus before reaching to the age of one year. Animal health is prime factor which ensures the food safety of dairy products. The occurrence of FMD is a major hurdle to the export of animal and dairy products. Due to FMD, the domestic markets of the country suffer an estimated loss of 690 million USD/annum. To control the disease currently strategies "test and slaughter" and/or "vaccination" are being used. The use of either one or both approaches is decided on the epidemiological situation of outbreaks. Currently, inactivated trivalent vaccine (O, A, Asia 1) has been practiced in FMD eradication programs in Pakistan. However, the control and prevention of FMD by vaccination remains unsatisfactory in the country. FMD challenge requires a coordinated effort involving government agencies, research institutions, industry stakeholders, and international partners. By overcoming these hurdles, Pakistan can strengthen its ability to control FMD outbreaks and contribute to the overall improvement of animal health and agricultural productivity.

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Outlook- animal vaccines sector in Pakistan

Sajjad Hussain

The agriculture sector, in Pakistan, contributes 19.3% share of the total GDP out of which 60.6% is from the livestock sector which is a dynamic sector and encompasses various activities, including animal disease prevention and control measures, animal breeding and genetics, milk and meat production, nutrition of animals, export of livestock products and other basic services for animals and public health. The OIE notifiable animal diseases incidence is increasing day by day. Veterinary vaccines are the only Hobson's choice for disease prevention and controlling measure to combat the endemic diseases. There is a vast potential for local and foreign industry in the animal vaccines sector. However, this sector is performing below its prospective competence. The key challenges are mainly related to less quantity of vaccines available with compromised quality standards, limited coverage of the vaccines due to lack of financial assistance, inadequate resources, and least contribution of local and private sector into animal vaccine production and import.

For a long-lasting sustainable livestock sector in Pakistan, the emphasis should be given to the implementation of the stringent policies, cogent preventive measures through increase in quality veterinary vaccine production, and involvement of local private industry into animal vaccines sector.

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Production of foot and mouth disease vaccine and its challenges in Pakistan

Ali Raza Awan

Foot and Mouth Disease (FMD) is a lethal viral disease of hoofed animals, including Buffalo, Cattle, Sheep, Goat. FMD is prevalent in Pakistan. The disease is highly contagious where virus replicates and spreads rapidly. This disease results in the economic burden due to production drop of dairy & meat industry and causes severe losses to the livestock farmers. Sometime the damage is so severe that results in the loss of farmers business. The impact includes calf mortality, reduced milk production, abortions in pregnant animals, treatment expenses, reduced weight gain and significant distress. Vaccination at the mass level is the only remedy to control this disease. Presently the population size of the cattle and buffalo in Pakistan is more than 100 million. Therefore, an annual requirement of 200 million doses of FMD vaccine are required for the immunization of the animals. Currently, Pakistan imports 10–15 million doses of FMD vaccine per year; domestically produced vaccines make up only 10% of this amount, leaving a significant deficit. An important first step, in bridging this gap, is the establishment of Training Center for Biologics Production Facility (TCBP), University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan, which has been recently approved by the Drug Regulatory Authority of Pakistan after a long marathon of efforts. Initially, it has been anticipated to produce 02 million FMD doses per annum and will also be increases gradually. This facility will be the source for promoting local industry for production of quality FMD vaccines as well as produce technical human resource in the field of vaccinology. With an initial production target of two million doses annually, the production capacity can be enhanced to 10–15 million doses annually to minimize the existing gap. Exports of livestock products especially meat and milk products can only be possible if the indigenous animals would be declared immunized and disease free.

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Exploration of viral structural proteins for vaccine development

Moazur Rahman [1,2,3*], Majid Ali Shah[3], Soban Tufail[3], Maryam Zafar[2,3], Muhammad Saleem[2], Aamir Shehzad[3], Mazhar Iqbal[3]

The poultry sector plays a pivotal role in the economy of Pakistan and worldwide, meeting the food demand for the rapidly growing population. Current investment in this sector is over 1000 billion rupees in Pakistan and over 4000 billion US$ in global market with an annual increase of > 5%. However, the poultry industry is more vulnerable to infectious diseases than any other enterprise in livestock. Inclusion-body hepatitis hydropericardium syndrome (IBH-HPS) and infectious bursal disease (IBD) are such economically important diseases which cause sudden death of infected birds. IBH-HPS and IBD are caused by fowl adenovirus-4 (FAdV-4) and infectious bursal disease virus (IBDV). To this end, to develop modern recombinant vaccines against IBH-HPS and IBD, viral structural proteins namely penton base, hexon and fiber-2 of FAdV-4 and VP2 of IBDV were explored. The efficacy of subunit vaccines targeting the immunogenic regions of penton base, hexon-virus like particles and VP2 (protrusion domain-VP2) was evaluated in chicken giving variable level of protection ranging from 50 to 100% against the respective pathogen challenge. Moreover, human adenoviral-5 based viral vector vaccines targeting the hexon, fiber-2 and VP2 were generated. It has been observed that VP2-adenoviral vector vaccine significantly reduced the lesion in bursa of Fabricius of chicken upon challenge with infectious IBDV. We anticipate that the aforementioned studies will contribute towards developing local solutions for the respective diseases.

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Vaccine production set up at VRI Peshawar, its current status and future plans

Muhammad Tariq Zeb[1], **Muhammad Ijaz Ali[2]**

Veterinary Research Institute (VRI), Peshawar since its creation in 1949 as Pakistan Animal Husbandry Research Institute (PAHRI), apart from research and disease investigation activities is also engaged in Vaccine Production against some of the diseases of high economic significance, considering that the loss of an animal or a bird to a preventable infectious disease is socially appalling, economically impoverishing and ethically unacceptable. Vaccines are the most cost-effective cheapest medical intervention known to prevent death and disease and ultimately improve health and production of animals. Ensuring the provision of safe, effective vaccine is crucial for the animal health. The Biological Production Centre of the VRI, Peshawar is currently producing four bacterial and two viral vaccines and the demand of these vaccines is on the rise due to expanding livestock sector. The Vaccine Centre always tries its best to cope with the situation but unable to fulfill the 132 million doses requirements of the targeted animal population of the provinces due to less available space, declining efficiency of machinery & equipment, conventional production practices and lack of training opportunities of the related technical staff in latest techniques & practices in vaccinology. Vaccinating animals with high quality vaccines is not only the key to control many animal diseases but the quality vaccine production is also serves as revenue generating activity. The Institute has therefore put emphasis on:

- Conduct research on improvement of Vaccines, introducing new practices in antigenic sequencing developing tailored vaccines, their large-scale production and design strategies for effective vaccination of livestock in the province.
- Re-designing/ re-structuring & rehabilitation of the existing laboratories building
- Further Improving quality control procedures to meet the global standards
- Establishment of state-of-the-art cold storage / cold chain facility.
- Certification of the processes.

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Computational tools for bacterial protective antigen discovery

Amjad Ali

Accelerating the discovery of Bacterial Protective Antigens (BPA) or Bacterial Vaccine Candidates (PVCs) is crucial for combating infectious diseases effectively. We introduce a suite of robust computational tools designed to streamline the identification process of PVCs within microbial pangenomes. Our tools, VacSol, PanRV, B-Vac, VacSol-ML, and B-Vac-AI, offer user-friendly interfaces and employ advanced algorithms to expedite the screening and selection of potential vaccine candidates. VacSol utilizes a comprehensive database and sophisticated algorithms to predict BPA candidates based on various antigenic properties. PanRV employs comparative genomics to identify conserved regions across multiple strains, aiding in the discovery of broadly protective antigens. VacSol-ML harnesses machine learning techniques to enhance the accuracy of BPA prediction, incorporating diverse genomic features for improved performance. B-Vac-AI integrates artificial intelligence algorithms to further optimize vaccine candidate selection, leveraging big data analytics for enhanced predictive modeling. By automating and optimizing the analysis process, our computational tools significantly reduce the time and labor traditionally associated with identifying PVCs. Moreover, the user-friendly nature of our analysis packages makes them accessible to a wide range of researchers, fostering collaboration and advancing vaccine discovery efforts. In conclusion, the development of VacSol, PanRV, VacSol-ML, and B-Vac-AI represents a significant advancement in the field of vaccine informatics. These tools empower researchers with efficient and effective means to identify BPA and PVCs, ultimately contributing to the development of vaccines against bacterial pathogens and the improvement of public health outcomes worldwide.

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Current status of Vaccine Production in Baluchistan

Muhammad Shafee, Muhammad Zahid Mustafa and Zafar Ahmad

Balochistan, the largest province of Pakistan, has different ecological zones from, lofty mountains to plains and deserts to coastal areas. It shares two international borders with Iran and Afghanistan. More than 70% of the population depend upon livestock rearing for their livelihood. The major livestock are sheep (46%) and goat (23%) of the country population in addition to cattle in some patchy areas. Center for Advanced Studies in Vaccinology & Biotechnology (CASVAB), the former Veterinary Institute of the Province is the only Center for biologics production in the province. It works under the administrative control of University of Balochistan, involved in eight different bacterial and viral vaccines in addition to academic activities for higher studies in four disciplines viz, (Microbiology, Molecular Biology & Biotechnology, Nutrition and Toxicology and Physiology). Bacterial vaccines include Enterotoxaemia (ETV), Black Quarter (BQ), Hemorrhagic Septicemia (HS), Contageous Caprine pleuropneumonia (CCPP), and Anthrax Spore vaccine). Three viral vaccines include Pesti des petits ruminants (PPR), Sheep pox, and Goat Pox. The centre is also running some pilot projects on vaccine production such as combo vaccine against HS and BQ. Moreover, the lumpy skin disease vaccine is in the final stage through local field isolate. Although 12-13 million vaccine doses are produced annually and supplied to the livestock department, it still gives limited animal health coverage. There is a dire need for new vaccine development and fold production of vaccines to extend the animal health control program and improve the socioeconomic status of the poor farmers of the province.

ABSTRACT INFO

Development of vaccine against Peste-des-Petits Ruminants virus having ease of application and improved efficacy

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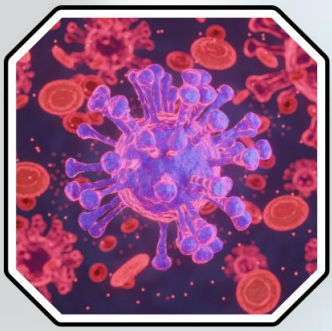
Peste des petits ruminants (PPR) is a viral infectious disease of sheep and goat. Animal populations in Middle East, Africa and Asia are severely affected by this disease. High mortality and morbidity rates result in huge economic losses. Small Ruminants Morbilli Virus (SRMV) is the causative agent of the disease, which belongs to family Paramyxoviridae. Vaccination is being used as principal method for control of PPR. Attenuated vaccine having PPRV/Nigeria/75/1 is considered as effective vaccine in the region. This study was focused to develop an ocular vaccine formulation against PPR and compare its efficacy with sub-cutaneous vaccine. Different methods of cell culture/ viral propagation, including roller bottles and Celcradle system has been used to propagate the vaccine strain. Experimental mucosal vaccines were formulated having different compositions of cryo-protectants and viral concentrations. Experimental trials of the vaccines were conducted. Pre and post-vaccine blood and serum samples were collected. Samples were processed to determine the Serum (IgG) antibody titers against PPR Virus by ELISA and VNT. Expression analysis of immune markers was also done to determine the immune response of the different vaccines. The results of serological tests revealed that 100ul dose of ocular vaccine having trehalose and gelatin as stabilizer with viral titer of 104 TCID50 /animal is suitable for an optimum immune response. It was also revealed that sera of vaccinated animals have neutralizing antibodies against the virus. Moreover, it was found that sera of experimental groups vaccinated either by subcutaneous route or through ocular route have almost same pattern/ response of (IgG) antibody titers. Studies are in progress to determine IgA antibody titers of vaccinated animals to determine if ocular vaccine will provide better protection as compare to the sub-cutaneous vaccine. Moreover, field trials are also required to further confirm its ease of application under field conditions in PPR control programs.



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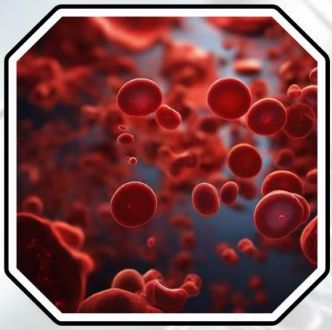

ABSTRACT INFO

Development of recombinant adenoviral vector vaccines against inclusion body hepatitis–Hydropericardium syndrome for poultry

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Inclusion body hepatitis–hydropericardium syndrome (IBH-HPS) is a communicable disease of poultry caused by fowl adenovirus serotype 4 (FAdV-4). This disease infects chickens at an early age of 3-6 weeks, causing immunosuppression and ultimately mortality of up to 100% of infected chickens in the flock. This project is aimed to develop safe and effective adenoviral vector vaccines against IBH-HPS to mitigate the economic losses inflicted to poultry industry due to this disease. To this end, highly immunogenic proteins (fiber-2 and hexon) from a local strain of FAdV-4 were isolated followed by their individual insertion into pAdTrack-CMV vector under CMV promoter to generate pAdTrack-CMV-fiber-2 and pAdTrack-CMV-hexon vectors. The developed vectors were co-transformed along with pAdEasy-1 adenoviral backbone vector into AdEasier-1 (BJ5183-AD-1) E. coli cells to carry out homologous recombination by adopting the human adenovirus-5 (hAd5) based AdEasy technology. The potential recombinant clones were linearized to liberate the inverted terminal repeats (ITRs) and utilized for lipofectamine-mediated transfection followed by infection to amplify the viral titer in Human Embryonic Kidney (HEK-293T) cell line. The immunization studies will be carried out in chicken followed by immunogenicity analysis. The adenoviral vector vaccines thus prepared will serve as effective vaccine candidates in controlling the disease (IBH-HPS).

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Current trends, prospects and opportunities in vaccine research

Hussain Ibraheem

Vaccination helps in preventing various diseases. Despite the success of vaccines, there are still various hurdles in vaccine development and distribution. The conventional methods which include poor health services, genetic variabilities of pathogens and the manipulation of host immune response by some pathogens are few of them. To combat these challenges, non-viral vaccination technologies and viral vector platforms are new strategies. Viral vectors deliver the antigen using a non-pathogenic viral carrier and exhibits a strong adaptive immune response while non-viral vaccination technologies include DNA, mRNA and bio-material based vaccines. The use of genetic engineering and NGS technologies in vaccine development increase the revolutionary potential of new vaccine types. Another rapidly emerging approach involves the integration of multi-omics data-sets, single-cell genomics and epigenetic profiling of immunity underlie both the innate and adaptive immune systems. As new methods and technologies are developed to treat a range of diseases, it is expected that the worldwide vaccination market will rise significantly in the upcoming years. The creation of nucleic-acid vaccines has demonstrated significant promise in the treatment of HIV and cancer. Additionally, they are looking into developing vaccines for diseases like diabetes and asthma that have not yet been addressed.

ABSTRACT INFO

Structural informatics approach for designing an epitope-based vaccine against the brain-eating *Naegleria fowleri*

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Naegleria fowleri, a parasite commonly referred to as the "brain-eating amoeba," is the cause of Primary Amoebic Meningoencephalitis (PAM), a serious and sometimes fatal brain disease. After contracting this parasite, the likelihood of a patient recovering is extremely low. The survival rate for this potentially fatal virus is 5%. *N. fowleri* causes a serious, sometimes fatal infection, yet there is no effective treatment to prevent or inhibit it. In this study, developing a vaccine candidate that could be able to fight *N. fowleri* infection is essential. The goal of the current study was to use reverse vaccinology and immune-informatics techniques to produce a multi-epitope subunit vaccine against *N. fowleri*. Several methods were used to predict the T- and B-cell epitopes. Toxicological, antigenicity, cytokine-inductivity, and allergenicity analyses were performed on the epitopes to choose those that could elicit immunological responses mediated by T- and B-cells. The epitopes were combined with adjuvants and linkers to create three distinct vaccine constructions. When the modeled vaccinations were docked with immune receptors, vaccine-1 exhibited the most affinity for binding. The binding affinity and stability were verified by molecular dynamic simulations and normal mode analysis. The immunological profile was created by immune simulations and the expression probability of the vaccine design in *Escherichia coli* strain K12 was confirmed by in silico cloning. By using immuno-informatics and reverse vaccinology techniques to produce a possible vaccine, this work presents a novel preventive strategy against the brain-eating amoeba. Primary amoebic meningoencephalitis may be prevented with significant potential thanks to this study, yet more investigation is needed to see how effectiveness of the proposed vaccine.

ABSTRACT INFO

Development of phylogenetic tree of hepatitis C virus envelope (E) protein for study of vaccine design

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Hepatitis C virus remains a significant health concern globally, with diverse genotypes and variants that challenge vaccine development. In this study, we aim to contribute to the understanding and design of effective HCV vaccines by focusing on the envelope (E) protein, a key target for the immune systems response. Our research employs cutting-edge molecular biology techniques, including MEGA-X software, to sequence the genome of HCV genotype 3a strains obtained from Gene bank of the National Centre for Biotechnology Information (NCBI). The main goal of this study is to compare the variant and conserved regions of the envelope protein of HCV genotype 3a prevalent in Pakistan to strains from all over the world. This comparative research will help us identify significant genetic changes, conserved epitopes and possible vaccine targets. We hope to obtain insight into the evolutionary links between diverse viral genotypes by developing a complete phylogenetic tree that include HCV strains from Pakistan and other regions of the world. The phylogenetic tree constructed as a result of our research will be a useful tool in determining the genetic diversity of HCV E protein and its epidemiology. This information will be critical in developing vaccination against hepatitis C virus.

VC-P5

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Transformation of *Lactuca sativa* L. with ESAT-6 antigen from *Mycobacterium tuberculosis*

Iqra Mubeen[1], Muhammad Suleman Malik[1], Neelam Batool[1], Fatima Ijaz[1], Zarafshan Shahid[1], Kiran Saba[2], Mohammad Tahir Waheed[1]

Tuberculosis (TB) is an infectious disease of humans, caused by *Mycobacterium tuberculosis*. TB is a prominent cause of death worldwide. The available anti-tuberculosis vaccine is ineffective to prevent latent TB in adults along with many other disadvantages. The production of novel, safe, high yield, and cost-effective vaccines is required to reduce the burden of TB, especially in developing countries. Plant transformation technologies have great importance to develop plant-derived edible vaccines. In the current study, we optimized tissue culture conditions and Agrobacterium-mediated transformation of an edible plant *Lactuca sativa* (cvs. Iceberg and Grand Rapids) with ESAT-6 antigen from *M. tuberculosis*. The nodal explants of Iceberg were used for stable transformation, while leaves of Grand Rapids were transiently transformed using Agrobacterium-infiltration. The transgenic status as stably transformed Iceberg and transiently transformed Grand Rapids was confirmed by conventional PCR. The copy number of integrated ESAT-6 gene in the nuclear genome of Iceberg was two as calculated through qRT-PCR. The expression of ESAT-6 antigen in both cultivars was confirmed by Dot blot analysis. Western blotting showed 10.75 kDa monomeric ESAT-6 protein. Altogether, this research will help to establish a basis for the production of plant-derived edible vaccine against TB.

ABSTRACT INFO

Zoonotic risk assessment of *Escherichia coli* isolates from bovine fecal matter; A diverse genomic approach to estimate their potential risk to the surrounding ecosystem

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Escherichia coli (*E. coli*) plays a crucial role in One Health, particularly in human-dominated environments such as dairy farms. The infectious potential of 87 *E. coli* isolates from bovine fecal matter using whole-genome sequencing was assessed in the current study. Most of the isolated strains belonged to phylogroup B1, with prevalent serotype O8 and dominant sequence type ST10. Pan vs core-genome analysis revealed a dynamic genome showcasing capacity for adaptability and functional variety. Screening of virulence genes indicated the occurrence of ExPEC (Extraintestinal pathogenic *E. coli*), DEC (Diarrheagenic *E. coli*), and hybrid (ExPEC + DEC) strains. Among ExPEC isolates, UPEC (Uropathogenic *E. coli*) and SEPEC (Sepsis-associated *E. coli*) sub-classes were identified based on the presence of their characteristic genes and phenotypic characteristics. Understudy ExPEC isolates showed zoonotic potential as they can cause urinary tract infection and sepsis in mouse models. All isolates were multidrug-resistant as displayed resistance to three or more different classes of antibiotics. In summary, this comprehensive genomic analysis of *E. coli* from bovine feces highlights the potential risks to animals and humans in farm environments, emphasizing the importance of surveillance programs, hygiene measures, and the development of effective vaccines against *E. coli* to mitigate the spread of infectious and antibiotic-resistant bacteria.

VC-P7

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Sero-prevalence of *Peste des Petits Ruminants* in Sindh from December 2021 to November 2022

Benazir Kanwal*[1,2], Urooj Zafar[2], Nazeer Hussain Kalhoro[1]

Transboundary Animal Diseases are of great concern due to their serious consequences which include huge economic losses. Among TADs, PPRV has found to be the most prevalent in Asian countries. The outbreaks of the disease occur throughout the year while the disease incidence is generally more during the peak winter in Pakistan. The main objective of this study was to determine the sero prevalence of PPR in goats and sheep in Sindh province of Pakistan for the year Dec. 2021 to Nov. 2022. Method: 1548 blood samples of non-vaccinated sheep and goats showing symptoms of nasal oral discharge, difficulty in breathing and diarrhea were collected. Samples were processed for the detection of PPR antibodies in the blood via ELISA. Results: revealed that the sero prevalence of PPR was gradually increasing both in goats and sheep from the month of September till February while a decline in the sero positivity observed from the month of March till August. The disease incidence is generally more during the peak winter in Sindh Pakistan. Conclusion: Extra sanitary measures in winters might be helpful to control the spread of disease as during winters, the small ruminants likely to migrate to the other areas for food and survival.

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Identification of novel drug targets and vaccine targets using in silico techniques for bacteria *Rickettsia rickettsii*

Fizza Arshad, Asifa Sarfraz, Mohibullah Shah*

Rickettsia rickettsii is the cause of Rocky Mountain spotted fever (RMSF), an acute febrile illness spread by ticks for which no effective therapeutics are available. Death rates can reach 20-30% percent; therefore, it is necessary to investigate new drug and vaccine targets to combat this infection. In this study, we used subtractive proteomics and reverse vaccinology techniques to find novel drug targets and create possible *R. rickettsii* vaccines based on core genome analysis. To create a complete core genome, whole-genome sequencing data from several *R. rickettsii* strains were combined. Eight novel, pathogen-essential, human non-homologous and druggable drug targets were found by comparative genomics. To identify the potential proteins for a multi-epitope vaccination, extracellular and outer membrane proteins were submitted to physicochemical, allergenicity, and antigenicity analyses, developed two effective vaccine candidates by utilizing conserved antigens. Molecular docking and MD simulation studies showed that the vaccines interacted strongly and steadily with human immune receptors. Strong immunological responses are ensured by immune simulation; vaccine design is cloned in bacteria. The development of eight therapeutic targets and two stable vaccinations against *R. rickettsii* was made possible by in silico research, demonstrating the influence of computational methods on the discovery of treatments for infectious diseases.

ABSTRACT INFO

Growth kinetic analysis of Pakistani isolated *Peste des Petits Ruminants* (PPR) vaccine strain on cell culture

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Peste des Petits Ruminants (PPR) is a disease that poses a significant economic threat especially to nations that rely profoundly on small ruminants. The necessity of effective disease control and eradication program has been emphasized by the WHO and OIE by 2030. In order to contribute in the national PPR eradication program, one of the local PPR virus (PPRV) isolates that had already been undergone 65 serial passages was used. The research aimed to determine the optimal harvesting time for maximum virus yield and to gain valuable insights into the strain specific replication kinetics of a Pakistani isolated PPR vaccine virus by monitoring infectivity titer and cytopathic effects (CPEs). One-step growth curve was established to determine the replication kinetics by quantitative estimation of infectivity titer at various time points using microtiter plate titration method. The peak virus titer for CFV was 6.5 log10 TCID50/ml observed at 72 to 88 hpi. Prominent CPEs i.e. cell aggregation and syncytia evident after 47 hours of infection. The study concludes that infectious titer of vaccine strain remains stable for an extended period (72 to 88 hpi), describes the CPEs of the isolated strain and further advancing our understanding of the virus's dynamics in an environment.

VC-P10

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In silico designing of an effective multiepitope-based vaccine against SARS-CoV-2

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In silico vaccine designing involving the prediction of antigenic regions in the viral proteins, has recently gained much consideration because of its numerous advantages. These include rapid screening and detection of several antigen candidates which can activate the immune system leading to the generation of a stronger immune response. This method utilizes high-throughput proteomics data that is easily accessible through public databases to identify highly effective antigen candidates based on their immunogenicity profiles. This immunoinformatic analysis is aimed at the development of a broad-spectrum multiepitope-based COVID-19 vaccine comprising the two structural proteins of SARS-CoV-2 i.e., spike and nucleocapsid. For this purpose, various bioinformatics tools were taken into consideration and strict standards were set for the selection of potential B-cell and T-cell epitopes of spike and nucleocapsid proteins. Consequently, highly immunogenic, non-allergic, non-toxic, and species-specific B-cell and T-cell epitopes were predicted. The potential epitopes were inserted at the tip and C-terminus of hepatitis B virus-like particle and their structures were predicted by ChimeraX Alphafold. These structures were further screened based on their favored regions in the Ramachandran plot. Now to experimentally verify the immunogenic potential of predicted epitopes, their production in prokaryotic systems followed by immunization in animal models is in progress.

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Immunological characterization of SARS-CoV-2 nucleocapsid protein and intrinsically disordered region for vaccine development

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Nucleocapsid protein of SARS-CoV-2 is highly stable and conserved having almost 90% amino acid sequence similarity with that of SARS-CoV. The immunogenicity and expression of nucleocapsid protein of many coronaviruses is quite high and abundant during the infection. The nucleocapsid protein serves as a representative antigen to generate T-cell response and induces SARS-specific T-cell proliferation and cytotoxic activity in a vaccine setting. Additionally, intrinsically disordered regions (IDRs) are highly abundant in SARS-CoV-2 proteins, making up to 51% for the nucleoprotein. IDRs bind to the host proteins and are associated with viral infectivity and pathogenicity. Thus, both the nucleocapsid protein and its IDRs are promising targets for vaccine development. This study is aimed at the immunological characterization of nucleocapsid protein and IDRs. The proteins were recombinantly expressed in E. coli and purified using Ni affinity chromatography. BALB/c female mice were immunized with 20 µg of purified proteins. Animals were sacrificed after 8 weeks and sera was harvested followed by ELISA to demonstrate the immunoreactivity of recombinant proteins. The results showed the production of antibodies against both the proteins. The results emphasize that use of these antigens in combination with other antigens can lead to the development of a broad-spectrum vaccine against SARS-CoV-2.

ABSTRACT INFO

The future of vaccine: using plants for oral vaccination against infections

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The challenge of developing efficient vaccinations for numerous infectious diseases remains a significant concern in global health. Plant-based systems have emerged as a promising solution due to their affordability, scalability, and reduced risk of human pathogen contamination. Traditional injectable vaccines, while effective, face challenges in distribution, storage, and administration. The research of oral, edible vaccines against Newcastle disease virus using algal chloroplasts and maize nuclear genome signifies a model shift in vaccine production. The maize-based vaccine expressing F and HN proteins demonstrated significant increases in cytokine expression (IL-1, IL-2, IL-6, IL-8, IL-10, IL-15, Interferon-α, Interferon-β, Interferon-γ, and CCL3) indicating robust stimulation of cell-mediated immune responses comparable to commercial vaccines, suggesting its potential efficacy in protecting against NDV infection. Additionally, integration of the NDV HN gene into *Chlamydomonas reinhardtii*'s chloroplast also showed promising results, highlighting the potential of transgenic algae-based vaccines in inducing significant immune responses for NDV protection. As part of a multidisciplinary team, I contributed to groundbreaking research, resulting in chewing gum containing CTB-ACE2. Our approach efficiently inhibited viral entry into cells and reduced SARS-CoV-2 virus count in COVID-19 saliva samples. In conclusion, integrating plant-based systems and virus-trapping proteins offer immense promise for affordable, scalable, and widespread protection against infectious diseases.

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A novel immune-informatics approach for developing a poly-epitope vaccine against FMDV, exploiting structural VP proteins

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The high mutation rate of the widespread virus FMDV having seven main serotype, causes both acute and chronic illnesses in cattle. Vaccine development is essential against this lethal virus. Employing the immune-informatics approach for efficiency and efficacy we designed an in-silico polyepitope vaccine (PEV) targeting structural and immunogenic components (VP1, VP2, VP3, and VP4) of FMDV. 7 CTL, 3 HTL, and 12 B-cell epitopes were selected through screening for antigenicity, non-allergenicity, Interferon simulation, and non-toxicity. To modulate immunity, these epitopes were linked using the proper linkers and a CTB adjuvant. The stability, hydrophilicity, and solubility of the PEV were demonstrated by the physiochemical examinations that were conducted after the structure prediction. Molecular docking and MMPBSA with excellent stability and compactness confirmed by MD simulation provided insights into the interactions and stability of the vaccination, TLR3, and TLR7. A robust immune response was shown using in-silico immune simulation. An E. Col system will efficiently manufacture FMDV-PEV since codon optimisation and cloning in an expression vector were carried out. More experimental validations may verify the efficacy, safety, and immunogenicity profile of FMDV-PEV.

ABSTRACT INFO

Development of indigenous recombinant human adenovirus 5-based vector vaccines against SARS-CoV-2 and its emerging variants

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To combat the coronavirus disease, a vaccine is urgently needed. In the current study, the highly efficacious and cost-effective viral vector vaccines were developed against local SARS-CoV-2 and its variants. For this purpose, the WT/consensus sequence of spike (S-perfusion) and the nucleocapsid (N-gene) of SARS-CoV-2 was amplified and cloned into shuttle vectors under CMV promoter. The generated shuttle vectors pAdTrack-CMV-S-perfusion and pShuttle-CMV-N-gene was confirmed by restriction analysis and verified by DNA sequencing. For generation and production of S-perfusion/N-gene adenoviral vector vaccine, linearized shuttle vector was co-transformed along with backbone vector into AdEasier-1 *E. coli* strain. After homologous recombination, the potential recombinants were re-introduced into recombination deficient *E. coli* (DH5α) strain to generate the stable clones. Potential recombinant clones (pAdEasy1-CMV-S-perfusion and pAdEasy1-CMV-N-gene) were utilized for transfection and infection into Human Embryonic Kidney (HEK293T) cells. The transfection efficiency of generated vaccine candidates was monitored by tracking GFP expression and cytopathic effects under fluorescence microscopy. Moreover, the S-perfusion/N-gene-based adenoviruses will be used for immunization/challenge studies in the mice/hamsters and will serve as promising vaccine candidate against coronavirus disease.

VC-P15

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Preparation and comparative immunological evaluation of whole cell and subunit typhoid vaccine candidates

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Typhoid fever, a major health concern globally, particularly affects developing countries with high morbidity and mortality rates across all age groups. The rise of drug-resistant *Salmonella enterica* serovar Typhi strains raises significant health concerns. With no recent antibiotic discoveries, the focus has shifted to prophylactic treatment. This study compares three typhoid vaccine classes; whole-cell vaccines, including formalin-killed and heat-killed variants, subunit crude outer membrane proteins (OMPs) and recombinant 46 kDa OMP. Immunological evaluation included humoral and cell-mediated responses, along with protective efficacy assessment. The humoral response was measured by determining antibody titers through ELISA, with the WHO pre-qualified Typhoid Conjugate Vaccine (TCV) as the positive control. Cell-mediated immune response was evaluated by relative quantification of the expression of cytokine genes using real-time PCR. The protective efficacy was assessed with live challenge after following the immunization schedule. Results highlighted the crude OMPs extract as the most potent vaccine candidate, with enhanced immunogenicity and 100% protective efficacy. The WHO-listed Typhoid Conjugate Vaccine (TCV) exhibited lower antibody titers but provided strong protection (95%). While formalin-killed cells elicited a robust humoral response, but with limited protection. This study concludes that OMPs are a promising vaccine candidate, surpassing the reliability of the WHO-listed TCV in Pakistan.

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Genetic engineering of *Spinacia oleracea*: Unveiling the OmpK antigen for a novel plant-based vaccine against fish vibriosis

Iqra Elahi, Muhammad Suleman Malik, Mohammad Tahir Waheed*

Vibriosis, caused by *Vibrio anguillarum*, is a deadly hemorrhagic septicemic disease that affects aquaculture, causing considerable economic losses. Innovative, safe, and stable vibriosis vaccines are needed to eradicate it. Edible plants are cheap, easy to grow, yield a lot of antigens, and can be kept at room temperature, making them ideal bio-factories for vaccine antigen expression. Bioinformatics tools showed that outer membrane protein K (OmpK) is immunogenic, non-allergic, and non-toxic, making it a promising vibriosis vaccine antigen that is conserved in all *Vibrio* species. The docking analysis revealed the OmpK model's interaction with TLR-5. We optimized seed sterilization using 0.2% HgCl2 and tissue-culture protocols with full MS media containing BAP 5mg/l and IAA 0.5mg/l for *Spinacia oleracea*. The OMPK gene was expressed in *Spinacia oleracea* using Agrobacterium-mediated nuclear stable and transient transformation. The integration of the transgene was confirmed by PCR, and the copy number was determined by qRT-PCR. The monomeric form of the OmpK protein was identified by western blotting, and the expression was further confirmed by ELISA. This study is the first to suggest that OmpK antigen expression in edible plants could pave the way for a sustainable, low-cost, and efficient edible plant-based vaccine against vibriosis.

ABSTRACT INFO

Structural and functional elucidation of cell culture adapted amino acid substitutions in foot-and-mouth-disease virus capsid

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Foot-and-mouth-disease serotypes O, Asia-1, and A is the most prevalent FMDV serotypes in Pakistan. FMDV-suspected samples were collected and virus propagation was performed in LFBK αVβ6 cells. VP1 region was sequenced and phylogenetic analysis was done. FMDV serotype O's an emerging lineage, O/ME-SA/Ind2001e was identified and adapted in BK-21 cells against heparan sulfate glycosaminoglycans. The P1 region of FMDV capsid was sequenced and amino acid substitutions were identified. Molecular analysis identified N17D, N143K, and V144L amino acid substitutions in VP1 and I23T amino acid substitution in VP2 capsid protein. N17D substitution in VP1 has been widely studied, it functions in stability of viral RNA in acidic conditions, ultimately leading towards enhanced capsid stability. N143K and V144L substitutions were located in GH-loop of VP1 which contributes to antigenic site 1 as well as receptor binding site for integrins. Capsid modelling revealed these substitutions caused disturbance of GH-loop conformation suggesting reduced binding with integrin to support altered binding with heparan sulfate. In conclusion, this study characterizes the substitutions responsible for O/ME-SA/Ind2001e FMDV adaptation in BHK-21, facilitating the chemically inactivated FMDV vaccine production process.

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Unveiling the roots of COVID-19 vaccinophobia: A comprehensive survey analysis of key influencing factors

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Vaccinophobia is an absurd fear of vaccines among people which hinders the accomplishment of global vaccination campaigns, serving as a significant challenge to cope with severe pandemics like COVID-19. The aim of this research was to conduct an in-depth analysis of the factors that influence vaccinophobia focusing on the complicated relationship between public health and the COVID-19 vaccine. Using a 46-item online questionnaire, a survey with 275 participants (70% females) was conducted. Statistical analysis, employing the Chi-square test, revealed a substantial correlation between revealed a significant correlation of vaccinophobia with fear of infertility (P value = 0.0003), and that of developmental disorders (< 0.0001). Values for genome alteration, inadequate testing, having hidden microchip and their use as bioweapons (< 0.0001) respectively. A positive correlation was observed between vaccinophobia and health background of people (P-value=0.002) and impact of vaccine hesitant individuals on others (< 0.0001). Paradoxically, people's vaccinophobia conflicted with their trust in government policies (60%, P = 0.0002) and healthcare providers (81.8%, P = 0.0004). These findings highlighted the strong need for public awareness against the circulating myths that lead to vaccinophobia.

VC-P19

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Development and evaluation of a *Salmonella gallinarum* ghost vaccine for protection against fowl typhoid in chickens

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This study presents the creation of a promising fowl typhoid (FT) vaccine candidate, an indigenous *Salmonella gallinarum* ghost. This was achieved through the isolation of purified *Salmonella gallinarum* strain from local samples and overnight incubation in LB media containing 7% Tween 80 followed by a rapid drop in Ph to 3.6 using lactic acid for one hour. The ghost's formation, characterized by tunnel formation and loss of cytoplasmic contents, was confirmed through electron microscopy. No living cells were found post tunnel formation 7 days of incubation in LB media and streaking on LB agar. The vaccine's safety and efficacy were tested on chickens divided into five groups for vaccination and one negative control, each receiving the vaccine through different methods. The birds were vaccinated on day 7 and had a booster dose on the 21st day post-vaccination and showed no adverse reactions or signs of FT. They were later exposed to a virulent *Salmonella gallinarum* strain 108 cells after seven days of booster dose. All vaccinated groups showed significant protection against the virulent strain, based on mortality and post-mortem lesions, compared to the non-vaccinated control group. The group D ELISA results showed that the vaccine also induced a high systemic IgG response in all vaccinated groups. The qualitative confirmation was performed using a locally prepared plate agglutination kit. These results suggest that the *Salmonella gallinarum* ghost is a safe, highly immunogenic, and effective non-living bacterial vaccine candidate against FT.

ABSTRACT INFO

Mapping of prevalent strains of Clostridial species in livestock of Punjab to develop multivalent vaccine

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Livestock is a major contributor of food security throughout the world including Pakistan. It faces the challenge by encountering fatal diseases like Enterotoxemias, Lamb Dysentery and Black Quarter caused by *Clostridium perfringens* types A, B, D and *Cl. chauvoei* respectively. These fatal diseases affect sheep, goat, lambs and cattle population of Pakistan leading to huge economic loss. At present, Enterotoxemia and Black Quarter vaccines are being produced separately using imported strains. During the current study, 1350 samples (intestinal contents, rectal swabs, liver and muscle tissues) were collected from sheep, goat and cattle of Punjab province. These samples were examined microscopically and then processed for bacteriological culture and inoculation test. The Polymerase Chain Reaction (PCR) confirmed 35 isolates of *Clostridium perfringens* type A, 07 type B, 16 type D and 08 *Clostridium chauvoei* by targeting alpha, beta, epsilon and Phospholipase-A genes. The confirmed isolates were sequenced for accession numbers and phylogenetic analysis. After standardization, these confirmed local isolates will be incorporated for the production of multivalent Clostridial vaccine. This vaccine will enhance the livestock production by preventing multiple clostridial diseases through a single dose contributing to improved food security and economy.

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Unvaccinated backyard poultry, a concern for commercial sector and public health

Ahmad Shakoor Bhutta

The backyard poultry keeping In Pakistan is an age-old practice, commonly in rural households. It not only helps in poverty alleviation but also contributes towards food security and eradication of malnutrition. Although backyard poultry birds are considered very hardy and disease-resistant, they can always be a source of maintenance and spread of diseases. Backyard poultry always lacks preventative vaccination, proper veterinary consultancy, and biosecurity practices. Due to these reasons, history has witnessed many reported examples of the emergence of disease outbreaks from backyard poultry flocks, like the ones from India and Serbia recently, with strain variation in ND and AI viruses respectively. Thus, unattended and unvaccinated backyard poultry flocks can be a potential risk for the commercial sector of poultry and a public health concern at the same time because of certain zoonotic infections. More focused research on backyard poultry is needed to study the disease patterns and genetic diversity of various avian pathogens in these birds. Along with that, dedicated regulations for disease monitoring in backyard poultry and improved practices for disease prevention are required.

ABSTRACT INFO

Designing a multi-epitope vaccine targeting HIV-1 using computational tools

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Despite the progress made in the twenty-first century, the global AIDS epidemic continues to pose a substantial challenge, requiring the development of a vaccine that is both safe and efficacious. Primarily, insufficient immune responses led to the failure of prior vaccine trials. By employing immunoinformatic approaches which have demonstrated promise in the development of vaccines against a variety of rapidly mutating pathogens, this study intends to resolve these shortcomings by suggesting a potential vaccine. To achieve this, the LANL (Los Alamos National Laboratory) database was queried for all HIV-1 polyprotein and protein sequences. To forecast epitopes, a consensus sequence was produced. Antigenic, non-allergenic, conserved epitopes that induced T- and B-cell responses, in addition to IFN- production, were chosen. Dual vaccine constructs, HIV-1a (adjuvant-free) and HIV-1b (adjuvant-containing), were generated by combining these epitopes. It was determined that both multi-epitope vaccines elicited cellular, humoral, and innate immune responses, were stable, antigenic, and non-allergenic. Furthermore, both constructs were subjected to TLR-3 attachment and in-silico cloning. Our findings indicate that HIV-1b exhibits greater promise than HIV-1a. Experimental validation is required to confirm the safety and efficacy of both constructs, in addition to their in vivo effectiveness in animal models.

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Epidemiological trends and challenges of infectious bronchitis virus in South Asia: A comprehensive review

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The global poultry industry faces significant challenges due to the highly impactful Infectious Bronchitis Disease. Various strains of the disease are prevalent in different regions worldwide. This review distinguishes itself by conducting a dedicated exploration of the similarity index between the Infectious Bronchitis Virus (IBV) in Pakistan and strains found in specific South Asian countries. The uniqueness lies in a comprehensive phylogenetic analysis. The review makes a substantial contribution by revealing the genetic group distribution of avian coronaviruses, serving as a valuable resource to improve vaccine production. The emphasis on vaccine matching becomes essential, highlighting the necessity to align vaccines with the ever-evolving nature of IBV strains in the chosen South Asian regions. This forward-looking strategy not only tackles existing challenges in IBV management but also lays the groundwork for proactive vaccine development, ensuring sustained efficacy against emerging viral variants. The data regarding IBV types provided here is sourced from published manuscripts and submissions to Gene Bank on a global scale.

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Development of marker-free transgenic *Chlamydomonas reinherdtti* expressing conserved immunogenic determinants of dengue virus

Samia Afzal, Muhammad Hassan, Momina Afzal, Iram Amin, Muhammad Shahid, Muhammad Idrees

Dengue is among the most severe infectious diseases that affect millions of people annually. It may go on its own or may result in a medical emergency with fatal consequences for the patients. It has been a challenging endeavor for the researcher to create an effective dengue vaccine due to the existence of different serotypes of the dengue virus (serotypes 1 to 4), each carrying the potential to facilitate an antibody-dependent enhancement complex mechanism. Inadequate understanding of immune responses to dengue disease, absence of appropriate animal disease models, and insufficient assay techniques to detect responses generated by the body following infection or vaccine administration hinder the development of the vaccine and drug. A cost-effective, highly efficient, tetravalent vaccine is required that can elicit immune responses against different serotypes and mitigate the epidemic.

ABSTRACT INFO

A comparative analysis of diagnostic tools: In-house IELISA and ID Vet Kit for *Peste des Petits Ruminants* virus, in Pakistan

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Peste des Petits ruminants (PPRV) belongs to *Morbillivirus*, a febrile viral disease mainly impacting sheep and goats. *Morbillivirus*, which causes PPR, is a Paramyxoviridae virus with a high fever, conjunctivitis, mucopurulent ocular and nasal discharge, and respiratory symptoms. In severe cases, bronchopneumonia and diarrhea are fatal. This study evaluated the diagnostic efficacy of an in-house IELISA. We compared it with a commercially available ID vet kit, for its sensitivity and specificity. The study comprised the data of 35 goat/sheep farms distributed throughout Pakistan. A total of 400 blood samples and 35 swabs/tissues were collected during the investigation period 2021-2022. Following the initial screening by RT-PCR, one PPRV isolate, was selected for the preparation of antigen. Further, it was used to develop an IELISA. A known positive serum, having viral neutralization titer, VNT (1:128) was used for IELISA. Lastly, the IELSA was compared with a commercial ID vet kit for its sensitivity of 90.00% and a specificity of 82.50% was recorded. Finally, this study presents a cost-effective solution for sero surveillance of *Peste des Petits Ruminants* Virus (PPRV) in Pakistan and proved to be an alternative to cELISA for sero surveillance of PPRV in Pakistan.

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Production of onco-viral peptide-based therapeutic vaccine candidates against human papillomavirus type 16

Ayesha Akram, Shazia Rafique

Human papillomavirus (HPV) causes genital warts leading to a severe condition known as cervical cancer which has been one of the leading causes of death in women after breast cancer. The high prevalence and deadly effects of HPV show the importance of vaccines against this infection. Only three vaccinations, out of several being tested, have been licensed by the FDA: Cervarix ®, Gardasil ®, and Gardasil 9 ®. All these are prophylactic vaccines that are only effective before the exposure of the agent and are also costly. So, attention has been ultimately diverted toward therapeutic vaccine development. An E6 and E7 peptide-based vaccine approach will help provide CTL-based immunity against HPV. The proposed study plan involves creating mutant E6 and E7 proteins which have been shown to have lower binding affinities with their targets p53 and pRB. Mutations will be introduced through site-directed mutagenesis following transformation in DH5-α cells. Mutants will be confirmed by sequencing. Protein expression will be analyzed by transfecting these mutants in mammalian cell line followed by western blotting. Further, this cell line will be co-transfected with the E6 & E7 mutants and p53 and pRB respectively to check their effect on these tumor suppressor genes. Protein purification will be performed using chromatography. Future prospects involve inoculating these mutant proteins of E6 and E7 in animal models and analyzing the expression levels of p53 and pRB after infecting animal models with HPV.

VC-P27

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Swiss albino mice model to study immunopathology of foot-and-mouth disease virus

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There is a dearth of research on the tissue tropism of the FMD virus (FMDV) in Pakistan despite the availability of research data on the molecular characterization of the virus due to continuous genetic mutation of the virus in the field and leading to evolution of new strains. The Swiss albino mice was used as a cost-effective animal model instead of cattle. Field isolates of the foot-and-mouth disease virus Serotype A were confirmed by ELISA and further adopted on BHK-21 cells for the current study. Two groups of mice, A and B (n = 80) were arranged and 40 Swiss albino mice (group A) were experimentally infected intraperitoneally with FMD virus Serotype A. The trial continued for a total of 28 days. Lesions were seen in the mice's feet and mouth after different time intervals and recorded. In this study, liver, lungs, trachea, kidneys and spleen were the targeted organs for gross, histological and immunopathological abnormalities. The lesions were noted at various day intervals: 1, 2, 3, 14 and 28. Congestion along with hemorrhages of liver, kidneys and spleen was a constant significant histopathological feature observed in the whole study up to 28 days. Edema and cell swelling were also observed after 4 days of infection in liver. There were hemorrhages, bronchial edema, and alveolar emphysema in the lungs with deciliated tracheal epithelium. Immunohistochemical studies showed that the virus was present in the alveolar lumen and bronchial epithelia of lungs, hepatocytes, white pulp of spleen and convoluted tubules of kidneys. The results raised the possibility that in order to accommodate financial constraints for additional research on foot-and-mouth disease, laboratory animals might be used instead of large animals. The study concluded tissue tropism in the vital organs i.e. lungs, liver, kidney and spleen of Swiss albino mice.

ABSTRACT INFO

Optimization of cultural conditions in synthetic media to augment foot and mouth disease virus growth

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The livestock sector is of paramount importance in Pakistan, constituting 60.6% of overall agriculture and contributing 11.7% to the national GDP. It serves as a lifeline for 8 million rural families, accounting for 35-40% of their income. Unfortunately, Foot and Mouth disease (FMD) disease result in significant losses due to production decline and high mortality in ruminants. The efficacious vaccination plays a key role in the FMD control strategy. Mostly, FMD vaccines are produced on BHK-21cell line which needs essential growth media for its growth and maintenance while amino acids are the necessary components of these growth media. In this context, the study aims to enhance the cellular growth and ultimately viral contents in the harvest of FMD virus serotype O by improving the cytopathic changes in the BHK-21 cell line after its infection in growth Media i.e. DMEM in different ratios of amino acids including L-Arginine, L-Lysine, L-Serine, L-Threonine, L-Valine. The findings revealed the most significant CPE for serotype O in the harvested material with a two-fold concentration of valine and later it was confirmed by RT-PCR and OD value through ELISA i.e > 0.1.

VC-P29

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Development and comparative evaluation of cost-effective oil-adjuvanted avian influenza and Newcastle disease combo vaccine using Eolane and Ictyolane as oil adjuvants

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Newcastle disease virus and Avian Influenza (H9) viruses are causing predominant diseases in poultry birds which leads to substantial loss to poultry industry. A study was conducted to evaluate and compare four oil adjuvanted vaccines against Avian Influenza and Newcastle Disease in poultry by using four different oil adjuvants: (i) Eolane-150 (TOTAL, PARCO), (ii) Ictyolane -11 (IctyoDev, France), (iii) Montanide ISA-70 MVG (SEPPIC, France), (iv) Coralvac RZ 506 (Coral innovative solutions, Turkey). Avian Influenza virus H9 and New Castle Disease virus (Mukteswar) strains were used for combo vaccine preparation and adjuvants were used as per manufacturer's instructions. These vaccines were evaluated in commercial broiler and backyard desi poultry for immunogenicity by using Indirect ELISA. The antibody titer was measured in all groups prior to vaccination and then on fortnightly basis up to 54 days in broiler and on monthly basis up to 365 days in backyard poultry. The ELISA results indicated that the antibody titer induced by AI+ND Combo vaccine adjuvanted with Ictyolane-11 and Coralvac RZ 506 were higher than Eolane-150 and Montanide ISA-70 concluding that AI+ND combo vaccine prepared with Ictyolane-11 and Coralvac RZ 506 are cost-effective oil adjuvants for poultry.

VC-P30

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Preparation of *Aeromonas hydrophila* fish vaccines and evaluation of hematological and immune response via different vaccine delivery routes at Veterinary Research Institute, Lahore

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Aeromonas hydrophila is the causative agent of skin ulceration, pale gills, blot and high mortality in farm fish in Pakistan causing high economic losses to the fish farming industry. Three *Aeromonas hydrophila* oil adjuvanted fish vaccines administered via different delivery routes. Following vaccines were prepared: (i) Ah inactivated injectable vaccine adjuvanted with Montanide ISA 763 AVG (SEPPIC, France), (ii) Ah inactivated Immersion vaccine adjuvanted with Montanide IMS1312 VG PR (SEPPIC, France), (iii) Ah inactivated feed based oral vaccine adjuvanted with Ictyolane 52H (IctyoDev, France). Adjuvants were used as per the manufacturer's instructions. All three vaccines were subjected to sterility tests and safety testing in fish and were found satisfactory. Blood samples were drawn from all groups before vaccine administration and 21 days post-vaccination and subjected to hematological analysis. The hematological values indicated no significant difference ($p>0.05$) in hematological parameters before and 21 days after vaccination. Serum samples were collected on 0, 21th and 42th day of vaccination and will be subjected to indirect ELISA for antibody titer estimation against *Aeromonas hydrophila* bacterium.

VC-P31

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Development and comparative evaluation of homologous live and inactivated lumpy skin disease vaccine for cattle at VRI Lahore

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Lumpy skin disease is a viral disease that affects cattle and causes high fever, nodules on the skin leading to death and causes high economic losses due to production losses. In this study, we aimed to develop and compare two types of Lumpy Skin Disease (LSD) vaccines for cattle (1) live gel-based vaccine (2) inactivated oil-based vaccine. The live gel-based vaccine was formulated using Montanide Gel (01 PR from SEPPIC, France), while the inactivated oil-based vaccine was formulated using Montanide ISA-50 V2 (SEPPIC, France). To evaluate the efficacy of these vaccines, cattle were divided into two groups having three animals in each. Group A received the LSD gel-based vaccine via subcutaneous (S/C) route at a dosage of 2 mL per animal with a TCID50 log10(3.5) per dose. On the other hand, Group B received the LSD oil-based vaccine via intramuscular (IM) route at the same dosage, with a TCID50 log10(7) per dose. To measure the antibody response, indirect ELISA was performed with ID Screen® capripox double antigen multispecies kit (ID VET, France). Prior to vaccination, the serum antibody titers were seronegative according to the indirect ELISA results. The ELISA results revealed that both groups showed positive antibody titers for up to 350 days post-vaccination.

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Evaluation and commercialization of cost-effective oil adjuvanted haemorrhagic septicaemia black quarter combo vaccine for cattle and buffaloes

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Pasteurella multocida causes swelling in pharyngeal and brisket region while high fever, swelling in neck, shoulder or gluteal regions, and lameness are caused by *Clostridium chauvoei* and both these organisms are highly fatal for large animals. A study was conducted for evaluation of cost-effective Haemorrhagic Septicaemia- Black Quarter (HS+BQ) combo oil adjuvanted vaccine at government and private livestock farms for immunogenicity by using Indirect Haemagglutination test (IHA). Forty Sahiwal cattle calves and ten Nili-Ravi buffalo calves were divided into two groups (A & B) having n=20 Sahiwal cattle calves and n=5 buffalo calves in each group at Livestock Experimental Station Shergarh District Okara. Group A was subjected to HS+BQ combo vaccine adjuvanted with Eolane-170 oil adjuvant while Group B was subjected to HS+BQ combo vaccine adjuvanted with Montanide ISA-50 V2 oil adjuvant. Antibody titers were measured by using IHA test before the start of trial and after 107 days post vaccination. The IHA results showed no significant difference ($p > 0.05$) in antibody titer induced by both vaccines. HS+BQ vaccine adjuvanted with Eolan-170 oil adjuvant is cost-effective. Moreover, both vaccines were subjected to n=623 cattle and buffaloes at farmer level which showed good safety level in animals and acceptability among livestock farmers.

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Development and production of VP2 viral vector vaccine for infectious bursal disease

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Infectious bursal disease virus (IBDV) is a non-enveloped dsRNA virus. Among the two serotypes of virus, serotype 1 infects the bursa of Fabricius causing immunosuppression in chickens. In Pakistan, a mortality rate of 60% has been estimated in chickens infected by IBDV. VP2, a major structural protein, constitutes the outer capsid of the virus is recognized by the neutralizing antibodies and a target for vaccine development. Among different type of vaccines, viral vector vaccines exhibit better efficacy against IBDV. The aim of this study is to produce and commercialize VP2-based viral vector vaccine that provides complete protection in chickens against local as well as the circulating strains of IBDV in the field. For this purpose, the production of VP2-based viral vector vaccine in adherent HEK293T and suspension HEK-293F has been optimized by tracking the expression of green fluorescent protein followed by its titration through TCID50 and qPCR assays. Immunization of chickens with various doses of VP2 viral vector vaccine followed by challenge studies using local IBDV isolates is under-progress. Moreover, the development of virus neutralization test using antisera produced against VP2 viral vector vaccine will also be performed.

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Exploring novel candidates of genes through De novo assembly of *Hyalomma* tick's Sialotrancriptome for the development of vaccines against ticks and tick-borne diseases

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Hyalomma anatolicum, a multi-host tick, primarily affecting cattle and is an important vector of various bacterial, protozoal, and viral diseases. *Theileria annulata* parasite persistence in tick populations is attributed to transovarian and transstadial transmission, rendering it difficult to eradicate from endemic environments. Tick saliva consists of physiologically active molecules that are responsible for tick attachment, like local inhibition of inflammation, coagulation, angiogenesis, and immune camouflage. Sialotrancriptome analyses in ticks by the use of next-generation sequencing (RNA-seq) have effectively accelerated the research of vaccine-candidate molecules. In this study, we performed de novo mRNAseq on the Illumina platform in an attempt to identify differentially expressed potential vaccinal candidates in *H. anatolicum* in response to *T. annulata* infection. Followed by the validation of gene expression using qPCR. We found 6 differentially expressed genes in the presence or absence of *T. annulata* in the ticks. So, the results showed possibilities for the discovery of novel therapies and the improvement of our knowledge of tick-host interactions.

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The frequency of bacterial infections carried by vectors in the blood samples of wild rodent species captured in Saudi Arabia

Abdul Ghafar, Furhan Iqbal

The most diverse order of mammals is called Rodentia, and rodents pose a risk to human health since they are known to harbor more than 60 zoonotic illnesses. The purpose of this study was to report on the molecular prevalence and phylogenetic evaluation of different blood-borne bacterial pathogens (*Anaplasma ovis*, *Anaplasma phagocytophilum*, *Anaplasma marginale*, and *Bartonella spp*.) in blood samples from four wild rodent species that were captured in Saudi Arabia's Al Makhwah province between August and October of 2020. Among the species were Rattus rattus (N = 3), *Myomys yemeni* (N = 6), *Acomys dimidiatus* (N = 18), and Meriones rex (N= 27). The findings showed that 9/54 (16.6%) rats had amplified Msp4 gene of *Anaplasma ovis*, while 2/54 (3.7%) mice had amplified rpoB gene of Bartonella spp. Among the rat species that were enrolled, *Anaplasma phagocytophilum* and *Anaplasma marginale* were not found. The most heavily infected rodent species was *Meriones rex*. Rodent blood samples were found to include *Anaplasma ovis* and *Bartonella koehlerae*, as validated by DNA sequencing and BLAST analysis. Based on a phylogenetic study of both pathogens, the isolates from Saudi Arabia were shown to be closely related to each other and to isolates reported from other places across the world. The incidence of both bacterial infections was not limited to a specific rodent species or sex, according to risk factor analysis (P >0.05). In conclusion, we are revealing for the first time that rodents in Saudi Arabia can contract *Bartonella koehlerae* and *Anaplasma ovis* infections. To minimize bacterial illnesses humans and animals, similar large-scale investigations are advised in all of Saudi Arabia's unknown areas regarding the incidence and prevalence of bacterial pathogens among mice living close to human homes.

VC-P36

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Investigating the potential of membrane protein of SARS-CoV-2 as an antigen by *in silico* tools

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Novel coronavirus (COVID-19), the contributing factor of SARS-CoV-2 has globally placed the socio-economic burden. Both market and literature statistics of this pandemic evoked us to synthesize an effective vaccine candidate. Using genomic and proteomic approaches, in-silico identification of antigens is an important step in vaccine design. Here, by using the various immune-informatics tools we determined the highly immunogenic and antigenic Major Histocompatibility (class I, II) and B-cell epitopes followed by addition of linkers and adjuvants. Various chimeras of potential vaccine candidates were analyzed for secondary and tertiary structures, on the basis of which physicochemical properties were determined and docking were performed. The vaccine construct was proved to be, stable, immunogenic, antigenic, non-allergen, and non-toxic. Among all the TLR receptors, the vaccine construct has shown the most effective binding against the TLR8 receptor. Further, IgG1 antibodies are produced when immunologic simulations were performed.

ABSTRACT INFO

Conjugate and whole-cell killed vaccine preparation against poultry *Salmonella enterica* serovar Typhimurium and its immunogenicity evaluation in mice

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Poultry is one of the largest and rapidly growing industries in Pakistan and salmonellosis is among the most prevalent bacterial infections in poultry. A preventive vaccine is needed against food-borne zoonotic *Salmonella enterica* serovar Typhimurium and was aimed in this study. The O-specific polysaccharide (OSP) antigen of *S.* Typhimurium was directly conjugated to two carrier proteins: diphtheria toxoid (DT) and bovine serum albumin (BSA) by reductive amination whereas, whole-cell killed vaccine candidates were prepared using formalin and heat. Direct conjugation of *S.* Typhimurium OSP with DT is novel. Immunogenicity of all the prepared vaccine candidates was found significantly better ($P<0.05$) in immunized mice groups than in the control saline group. Conclusively, the antibody titers against whole-cell killed vaccines were significantly higher ($P<0.05$) than conjugate vaccine candidates. Among DT and BSA conjugates, the antibody titers elicited by BSA conjugate were significantly higher after the first and second doses ($P<0.05$), whereas no significant difference ($P>0.05$) was found after the third dose of immunization. We report the use of DT as a carrier protein for direct conjugation with *S.* Typhimurium OSP using reductive amination chemistry. The OSP-DT conjugate elicited an adequate immune response comparable to the OSP-BSA conjugate.

VC-P38

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Genetic characterization of fowl adenoviruses; Analysis of major capsid proteins of fowl adenoviruses

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An emerging trend of hydropericardium hepatitis syndrome (HHS) associated with fowl adenoviruses (FAdVs), have caused huge economic losses to the poultry industry in the last two decades worldwide. Current study focuses on the genetic characterization of FAdVs circulating in poultry population and major capsid protein analysis among different serotypes of FAdVs. Phylogenetic analysis of the major capsid proteins supports the division of FAdVs into the recognized FAdV-A, B, C, D, and E species and among them FAdV-C (serotype-4) and FAdV-D (serotype-11) are currently prevailing in indigenous poultry population. Moreover, the molecular analysis of the hexon gene of FAdV-4 revealed 99.86% and 99.68% identity at nucleotide and amino acid level respectively with FAdV-4 representative reference strains, and 72.88% and 78.92% similarity at nucleotide and amino acid level respectively with FAdV-11 reference strains. Based on hexon gene similarity, high inter species diversity was found between strains representing different genotypes (FAdV-4 and FAdV-11).

VC-P39

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Immunogenicity of A Candidate DNA Vaccine against COVID-19

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The ongoing threat to global health posed by the SARS-CoV-2 pandemic, particularly with the emergence of variants such as Omicron and its sub-lineages, accentuates the need for continuous efforts in COVID-19 prevention. The urgent demand for effective COVID-19 vaccines on a global scale has prompted the exploration of innovative approaches. In addition to that World Health Organization (WHO) has also advocated the development of indigenous solutions by developing countries for sharing the burden of disease control, in case of next pandemic. In response to the rapid outbreak of the SARS-CoV-2 pandemic, DNA based vaccines are being explored as an alternative to traditional vaccines. In this research, several DNA constructs based on codon-optimized spike, envelop and membrane coding regions were designed and tested. Subsequent analysis in BALBc mice focused on assessing neutralizing antibodies, and cellular immune responses against SRAS-CoV-2 from various waves. The findings indicated the ability of DNA vaccine to generate cellular and humoral immune response. These results suggest that DNA vaccination holds promise as a compelling approach for safeguarding against COVID-19.

VC-P40

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Thermostable Nano-Vaccine against Newcastle Disease; A Review

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Newcastle disease (ND) is a viral disease that is a major threat to poultry industry. It also affects more than 240 species of birds worldwide. Pakistan poultry industry is facing serious problem due to spread of ND. ND can be prevented by vaccinating chickens. Although most chickens in commercial farms in Pakistan are vaccinated, ND still occurs frequently. Pakistan uses indigenously manufactured and imported live Newcastle disease virus (NDV) vaccines, such as LaSota and Mukteshwar virus strains, respectively. All of these are sensitive to heat and need to be kept in a cold chain to maintain their protective antigenic titers. This reliance on an end-to-end cold chain from manufacturing to delivery to vaccination sites presents logistical challenges, particularly in remote areas with inadequate cooling infrastructure or unreliable electricity supplies. Nowadays, nanotechnology has become a new method for vaccine preparation because of its encapsulating and immunomodulatory properties. The use of nanoparticles (selenium, ZNO, etc.) as an adjuvant in vaccine preparation enhances the immune response and leads to better protection against virulent strains of Newcastle disease virus. These nanoparticles can mimic the natural structure of viruses, providing a more effective immune response. The successful development of a novel nano-based ND vaccine for Newcastle Disease virus could facilitate disease control measures, improve chicken health and welfare, and lead to increased production and profitability of poultry farming.

VC-P41

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Immunoinformatic identifies Apigetrin and Kaempferol as promising adjuvants for Lumpy Skin Disease vaccine

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Lumpy Skin Disease (LSD) poses a significant global threat to cattle, causing considerable economic losses, with no specific treatment or subunit vaccine currently available. This study employs an innovative immunoinformatic approach to design a multi-epitope protein targeting the putative IMV envelope protein of LSDV. Our study identified the IMV envelope protein as antigenic, non-homologous to bovine proteins, and highly conserved among LSDV isolates. Employing molecular docking, we screened 1000 lead compounds and identified the top 5 with the highest binding affinities. Furthermore, physicochemical properties and drug-likeness attributes highlighted two flavonoids, such as Apigetrin and Kaempferol, as promising candidates with notable docking scores (-7.00 kcal/mol and -6.1 kcal/mol, respectively). To validate the stability of the interactions, molecular dynamics (MD) simulations were conducted, demonstrating the stability of the Apigetrin and Kaempferol complexes with the putative IMV envelope protein over a 100 ns. The simulations revealed low root mean square deviation (RSMD) values and residue fluctuations (RMSF), confirming the stability of these complexes. This study emphasizes the high affinity interaction between the modeled subunit vaccine candidate and identified phytochemicals. Additionally, we explore the immunological potential of Apigetrin and Kaempferol, discussing their efficacy as vaccine adjuvants based on immunological information against Lumpy Skin Disease (LSD) in cattle.

ABSTRACT INFO

Vaccines-Bridging the Gap for One Health; A Review

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The One Health approach establishes the interconnections between human, animal, and environmental health. It offers a comprehensive framework for addressing global health challenges. Zoonotic diseases are significant threat to global health and are transmitted from animals to humans. Vaccination is a crucial measure in preventing the spread of zoonotic diseases. A large-scale vaccination campaign for dogs can effectively prevent rabies, while poultry vaccination can prevent H5N1 avian influenza, significantly reducing the risk of disease transmission to humans. In addition, livestock vaccination is essential to ensure food safety and public health, prevent economic losses, and reduce the risk of disease transmission to humans through the food chain, such as brucellosis. Vaccination programs have been developed to reduce the spread of diseases in wildlife populations, such as rabies in wild carnivores. These programs help maintain ecosystem balance and protect biodiversity. Successful implementation of the One Health vaccination program requires interdisciplinary collaboration between experts in human health, veterinary and environmental sciences.

VC-P43

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Developing a novel protein epitope-rich domain vaccine against avian pathogenic *Escherichia coli* through subtractive proteomics: a step towards advancing antimicrobial resistance control

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Avian Pathogenic Escherichia coli (APEC) poses a significant threat to the poultry sector, causing substantial financial losses and endangering avian well-being. Despite its impact, developing a safe, effective vaccine against APEC has remained challenging. This study analyzes the core genome of 171 APEC genomes, using a subtractive proteomics approach to identify three highly antigenic proteins (MtrD, HofQ and PhoE). We further predicted protein domains in these three proteins and selected those domains with a higher concentration of epitopes (B-cell, CTL, and HTL epitopes). Three different vaccine constructs were developed, and the structural stability was confirmed by molecular docking analysis. The APEC-Vac's structure showed strong interactions with Toll-like receptors 4, 5, and 15, suggesting its potential to stimulate an immune response in chickens. Additionally, in-silico reverse translation and codon optimization techniques indicated APEC-Vac's potential for high expression levels in E. coli K12 strains. Wet lab experimentation is needed to confirm these findings. This study lays a foundation for future research and offers promise in addressing APEC challenges in the poultry sector.

VC-P44

B-Vac-AI: a machine learning framework for bacterial vaccine antigen prediction

ABSTRACT INFO

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The significant global health threat posed by bacterial infections and fuelled by antimicrobial resistance results in high mortality rates. Although vaccination is the most effective defence, the resource-intensive nature of its development presents a significant challenge. The development of vaccines requires identification of antigenic potential vaccine candidates (PVCs) from the targeted pathogen. Conventional laboratory techniques for identifying PVCs are expensive, difficult, and time consuming, whereas computational reverse vaccinology approaches utilizing Machine Learning methods can provide a faster and cost-effective method. This study presents VacSol-ML, which utilizes computational reverse vaccinology techniques with Machine Learning advancements to efficiently analyse bacterial genomes for potential vaccine antigens. The tool employs an ensemble model that integrates two robust algorithms Neural networks and Support Vector Machine (SVM). The model was trained using the biological and physicochemical properties of a dataset containing bacterial protective antigens and non-protective proteins, to predict potential vaccine candidates against various pathogenic bacterial infections. Eight ML algorithms were trained on the training dataset and model's performance was evaluated using a separate test dataset. Neural networks demonstrated an accuracy of 94%, while SVM achieved an accuracy of 96%, surpassing other machine learning classifiers utilized in the comparative assessment. Through Stratified 5-fold cross-validation, it was revealed that SVM maintained an average accuracy of around 97%, whereas Neural Networks maintained an average accuracy of 91%, indicating consistent and linear performance across all iterations for both models. The final ensemble model of VacSol-ML demonstrated exceptional performance when benchmarked against previously published machine learning and rule-based tools on an external dataset. This outstanding performance makes VacSol-ML a valuable tool in accelerating vaccine development. It is accessible to the public through both a web server and standalone version, encouraging collaboration among researchers. VacSol-ML web-based and standalone frameworks are available at http://bvacai.mgbio.tech/

VC-P45

Design of personalized neoantigen mRNA vaccines against breast cancer patients

in Pakistan based on next-generation sequencing data

Kayode Raheem, Muhammad Muddassar

Breast cancer is the most common malignancy among women in Pakistan, with an outrageous increase in the incidence and mortality rate. Because of decreased effectiveness and lower survival rates, there is an urgent need for developing novel, personalized vaccines with enhanced activity. The development of a personalized mRNA vaccine illustrates a promising approach to combating the prevalence of breast cancer. We used the whole exome sequencing (WES) data of Pakistani tumor samples to predict immunogenic neoantigens. The WES data was aligned to the GRCM39 reference genome using BWA. The genome analysis toolkit (GATK) was employed for variant calling to identify somatic mutations. Using immune-bioinformatics tools, we predicted cytotoxic CD8+ T cell epitopes and helper CD4+ T cell epitopes within the identified neoantigens and designed vaccines by assembling the fusion of CTL neoepitopes, helper sequences, and adjuvant together with linkers. The Insilco ImmSim algorithm was used to examine the immune responses of the vaccine. The vaccine design was further explored by checking its simulation effect on the immune system, its physiochemical characteristics, its binding to specific immune system molecules, and cloning into an appropriate vector. By examining these factors, we aimed to ensure the vaccine's safety, stability, and ability to bind tightly to specific immune cells (class I MHC molecules), ultimately triggering a comprehensive immune response against breast cancer cells.

VC-P46

ABSTRACT INFO

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Immuno-informatics-aided poly-epitope vaccine design for combatting uro-pathogenic *Escherichia coli* Infections

Zaara Ishaq[1], Amjad Ali[2]

Newcastle disease (ND) is a viral disease that is a major threat to poultry worldwide and can cause severe economic losses to the poultry industry. It also affects more than 240 species of birds worldwide. Pakistan poultry industry is facing serious problem due to spread of ND. ND can be prevented by vaccinating chickens. Although most chickens in commercial farms in Pakistan are vaccinated, ND still occurs frequently. Pakistan uses locally produced and imported live Newcastle disease virus (NDV) vaccines, such as LaSota and Mukteshwar virus strains, respectively. All of these are sensitive to heat and need to be kept in a cold chain to maintain their protective antigenic titers. This reliance on an end-to-end cold chain from manufacturing to delivery to vaccination sites presents logistical challenges, particularly in remote areas with inadequate cooling infrastructure or unreliable electricity supplies. Nowadays, nanotechnology has become a new method for vaccine preparation because of its encapsulating and immunomodulatory properties. The use of nanoparticles (selenium, ZNO, etc.) as an adjuvant in vaccine preparation enhances the immune response and leads to better protection against virulent strains of Newcastle disease virus. These nanoparticles can mimic the natural structure of viruses, providing a more effective immune response. The successful development of a novel nano-based ND vaccine for Newcastle Disease virus could facilitate disease control measures, improve chicken health and welfare, and lead to increased production and profitability of poultry farming.

VC-P47

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Binding Selectivity Analysis of AURKs Inhibitors through Molecular Dynamics Simulation Studies

Ghulam Fatima, Numan Yousaf, Muhammad Muddassar

Aurora kinases (AURKs) are promising targets for cancer therapy. Using molecular dynamics simulations, this work examined the binding selectivity of three inhibitors against two proteins AURKA and AURKB. The three inhibitors were VX6, MPY and HPM. The findings demonstrate that these inhibitors distinctly favor AURKB over AURKA in terms of interactions. A detailed analysis of binding free energy decomposition showed that certain residue pairs are significantly implicated in specific binding of inhibitors against the AURKA and AURKB. Moreover, analysis of trajectories of the molecular dynamics showed that these inhibitors change the internal dynamics of the protein structures, that in turn affects the selectivity of these structures against AURKA and AURKB. In order to target AURKA and AURKB in cancer treatment, this work offers important insights that might lead to the creation of new inhibitors with improved selectivity.

ABSTRACT INFO

Pharmacophore-based virtual screening for novel inhibitors targeting lux protein in quorum sensing: a drug design approach

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Antibiotic resistance poses a significant threat to modern medicine, with quorum sensing proteins which plays a pivotal role in promoting resistance mechanisms among bacteria. Quorum sensing proteins regulate various bacterial behaviours, including virulence factor expression, biofilm formation, and drug resistance, by releasing chemical signals known as autoinducers. Autoinducer-2 (AI-2) is considered a universal signalling molecule found in both gram-negative and gram-positive bacteria. The synthesis of AI-2 involves LuxS-mediated cleavage of S-ribosyl homocysteine (SAH) to produce (4,5 dihydroxy-2,3-pentanedione DPD), which rearranges to form AI-2. Inhibition of LuxS activity presents a potential strategy to mitigate antibiotic resistance. To identify potential LuxS inhibitors, pharmacophore-based virtual screening and molecular docking were employed. The LuxS protein from Salmonella typhi selected for further investigation. Pharmacophore models were generated, and databases were screened. Subsequent molecular docking simulations identified three hits with high binding affinities, which were further validated through molecular dynamics simulations for 100ns. The findings provide insights into potential therapeutic interventions for preserving antibiotic efficacy and overcoming challenges posed by antibiotic resistance in clinical settings.

ABSTRACT INFO

New Trends of Proteomics-Based Vaccination Against Brucellosis under One-Health Perspective

Iahtasham Khan[1*], Riaz Hussain[2], Muhammad Farooq[1], Shahzad Ali[3], Muhammad Saqib[4], Muhammad Kashif [5], Abid Hussain Shahzad[5]

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Brucellosis, a zoonotic disease caused by Brucella bacteria, poses a significant threat to human and animal health. Conventional vaccines have limitations, prompting exploration of proteomics-based vaccination. This study investigates its potential to transform disease prevention within the One-Health framework. Proteomics has emerged as a potent tool in vaccine development, offering detailed insights into pathogen-host interactions. It identifies crucial immunogenic proteins for robust immune responses. Advanced proteomic techniques pinpoint specific antigens with potential as brucellosis vaccine candidates. The One- Health perspective, acknowledging the interconnectedness of human, animal, and environmental health, is vital in combatting zoonotic diseases. Proteomics-based vaccination aligns seamlessly with this holistic approach, targeting both human and animal populations to break transmission cycles and reduce zoonotic risks. Proteomics integration enhances the precision and efficacy of immunization, customizing vaccines based on regional Brucella strains and host responses. This adaptability is crucial in areas with multiple Brucella species. The study highlights proteomics' potential in discovering novel vaccine adjuvants and delivery systems, enhancing immunogenicity and stability. Additionally, proteomic techniques identify biomarkers indicating vaccine efficacy, enabling rigorous monitoring and evaluation. In conclusion, combining proteomics with vaccination strategies against brucellosis is a promising frontier in disease prevention. This approach, grounded in the One-Health perspective, has the potential to revolutionize the fight against zoonotic diseases, safeguarding human and animal populations. The identified keywords encapsulate this innovative approach to brucellosis control.

VC-P50

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Enhanced immunogenicity of epitope Pres 32-53 inserted in the immune-dominant region of hepatitis B core molecule

Imran Riaz Malik[1], Moaz ur Rahman[2], Javed Anver Qureshi[3]

Chronic hepatitis B infection is the worldwide problem and is the fundamentally a main cause that give rise to liver cirrhosis and hepatocellular carcinoma. Upto 4% of the world inhabitant are suffering from chronic hepatitis B and annually almost 80000 infected with this particular disease develop advanced stages. Hepatitis B is the most prevalent disease in Pakistan despite the availability of vaccine and antiviral drugs against this disease. The therapeutic vaccine can be nowadays an attractive approach to overcome on the infection of hepatitis B. The several studies suggest that key target for cellular response is the hepatitis B core gene. We found that this chimeric protein using hepatitis B core gene as a carrier for the amino acid residues 32-53 primed both HBcAg specific T cells and antibodies to PreS1. This will produce both cellular and humoral response to inserted region. In future, this chimeric protein may be used as potential candidate for vaccine development in order to prevent the hepatitis B infection.

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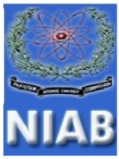

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