

22nd Indian Veterinary Congress, 8-9 April 2022, Udaipur

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Proceeding and Recommendation

of

International Satellite Webinar/Seminar

on

**INADEQUACIES OF VETERINARY VACCINES AGAINST EMERGING
INFECTIOUS DISEASES IN ANIMALS INCLUDING POULTRY**

XXIX Annual Conference

of

08-04-2022 to 09-04-2022



। पशुधनं नित्यं सर्वलोकोपकारकम् ।

at

Rajasthan College of Agriculture (MPAUT) Udaipur
Organized By College of Veterinary & Animal Science, Navania,
Vallabh Nagar, Udaipur (Rajasthan)-313 601



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PREAMBLE

The idea behind the International Webinar/Seminar on “Inadequacies of Veterinary Vaccines Against Emerging Infectious Diseases in Animals Including Poultry” is to address scientific reasons and explanations for the failure of immunological responsiveness against emerging diseases in animals and poultry. It was Benjamin Jesty, a farmer who observed immunity afforded milkmaids by the acquisition of cow pox; inoculated his wife and sons with cow pox material; proved that protection was provided by having the sons inoculated with small pox and exposing them to small pox; and used the technique in others. Edward Jenner was an English doctor and researcher who developed the world's first immunisation against smallpox. The terms “immunisation” and “inoculation” are derived from Variolae vaccae, a term (vaca means cow) coined by Jenner to describe cowpox. Edward Jenner became hero of Immunology and he is known a “Father of Immunology”. The immune system is robust to help host to fight invading microorganism by any mode of immune mechanisms e.g. innate immunity, antibody mediated and cell-mediated immune response.



We do not have vaccines to fight against emerging diseases like African Swine Fever Virus and Porcine Reproductive and Respiratory Syndrome (PRRS), H5N1 in poultry and many. The mutable virus also does not allow immune system to act if there is less or no cross protection among variants. The problem also lies in identifying the vaccinated and infected animals or poultry requiring a DIVA strategy. Empirically, vaccines were developed as formalized bacterin or inactivated whole microorganism adjuvanted with some adjuvants. In order to address the requirement for the development of vaccine, there is a need to understand mechanism of pathogenesis of microorganisms as to when and how fast a vaccine could be developed at the earliest. With this background of philosophy and objectives, an international webinar/seminar inviting relevant experts engaged in the study of these diseases is called for.

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Current Shortcomings in Controlling AI H5Nx by Vaccination–Lessons Learned

Teguh Yodiantara Prajitno

Senior Vice President Japfa Comfeed Indonesia

Group Director of Animal Health and Laboratory Services

Managing Director of Vaksindo Satwa Nusantara and Agrinusa Jaya Santosa

I am honoured to support you in all your efforts to provide a solution for both government and farmers to accomplish a sustainable control strategy for Avian Influenza in India. The key words are sustainable and control. We have learned that we cannot eradicate AI from the globe, and we need to learn to live with the virus, as migratory wild waterfowls form a major reservoir for Avian Influenza viruses, which harbor representatives of all 16 hemagglutinin subtypes and 9 neuraminidase subtypes. Consequently, the poultry industry globally will always be at risk of seasonal pattern of spill-over and re-introduction of Avian Influenza viruses via migratory wild waterfowls. On the other side, I speak here on as representant of the poultry industry, Avian Influenza viruses have been entrenched in the diverse poultry and marketing system of many countries. Due to the lack of a sustainable surveillance monitoring program and despite national stamping out policies, many countries are not able to eradicate the virus. I can offer my assistance in sharing the Indonesian experiences, where government and poultry industry were brought together by FAO to conceptualise an Indonesian control strategy focused on 9 elements back in 2005:



1. Biosecurity improvement
2. Vaccination in endemic areas
3. Depopulation in endemic areas
4. Re-shape the poultry marketing system
5. Surveillance (tracing)
6. Restocking
7. Stamping out in newly infected area
8. Public awareness and education
9. Monitoring and evaluation

By then, we realised that vaccination using heterologous vaccination is doomed to fail, as antigenically the vaccines from overseas (Europe, Mexico, and US) differed from the Indonesian circulating H5N1 strains. Consequently, the poultry industry suffered from significant losses from 2006-2010. On top of

that, infected broilers made its way into the live bird markets in the cities, causing the rise in human infection and human fatalities.

OFFLU, the network of avian influenza expertise, inaugurated jointly in 2005 by the FAO and the OIE (recently WOA), discovered in 2007 that there is an antigen mismatch between vaccine and circulating viruses and started the “Monitoring AI virus variants in Indonesian poultry and defining an effective and sustainable vaccination strategy” project, which introduced the antigenic cartography as a tool to select vaccine and challenge strain candidates. The project documented that the use of non-homologous vaccines in long-lived birds, such as egg-layers and breeders provided suboptimal protection and have caused a faster antigenic drift of the AIV, when experiencing concurrent challenge with the enzootic virus. In 2009 Japfa Comfeed Indonesia acquired Vaksindo Satwa Nusantara (the first veterinary vaccine manufacturer in Indonesia) and we introduced the concept of producing matching vaccine for the poultry industry in Indonesia, and other countries.

We looked at DIVA (differentiating infected from vaccinated animal) - vaccine technologies, using reverse genetic systems, but practically this strategy does not work, if AIV is already endemic. As DIVA vaccines provide suboptimal protection, and prolonged tracheal and cloacal shedding of the challenge virus strains, which can create new outlier strains that will evade vaccination protection. From the regulatory point of view every update in the vaccine seed requires new registration process, that will take several months to implement.

OFFLU presented by 2010 the final recommendation to the Indonesia government that vaccine using wild-type viruses, representing antigenically most AI viruses in Indonesia (antigenic match) and equipped with a high antigen content (antigenic mass) should be considered. Because the main task in an endemic situation is massively reduce viral load in the field, and in combination with stringent biosecurity and surveillance practices by the poultry industry, Indonesia has been able to control AI in poultry and human. From 2011-till now Indonesia faced the introduction of a new clade of H5N1 AIV (clade 2.3.2.1c) and H9N2, and in both case Vaksindo Satwa Nusantara was on the forefront to produce matching vaccine solutions, that help the country to control Avian Influenza. I have shared my view with the government of Myanmar, Bangladesh before, and it will be an honour for me to help the scientific community and as well the poultry industry of India.

Can we Align Veterinary Policies in the Absence of Harmonised Definitions?

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The foundations of robust policies are reliant on harmonized definitions allowing consistency of understanding. However, it is evident that there is a lack of harmonization of definitions in the antibiotic space and even the simplest of terms such as ‘antibiotic’ and ‘antimicrobial’ do not have a single harmonized definition at an international level. This lack of harmonization makes interpretation of policies in different geographies a difficult, sometimes impossible, task. This article proposes a set of definitions that could be adopted by international institutions such as the WHO, World Organisation for Animal Health (OIE) and Food and Agriculture Organisation (FAO) as well as regulatory agencies.



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The Quest of Host-Pathogen Interaction Mechanisms can guide African Swine Fever Vaccine Design

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African swine fever (ASF) is a fatal, contagious, haemorrhagic disease of domestic and wild pigs, and considered as one of the major threats to the global pig industry. Although ASF was reported long back in 1921 in Kenya but it has spread to Europe and considered as one of the emerging viral diseases in Asia as the first outbreak was reported recently in 2018 from China. India witnessed the first outbreak in 2020 from the north-eastern region causing massive economic loss. The causative agent of ASF is African swine fever virus (ASFV) of the genus *Asfivirus* and family *Asfarviridae*. It is a large icosahedral, enveloped dsDNA virus with an approximate diameter of 200 nm. The genome of ASFV is about 170-194 kbp which encodes more than 160 proteins, out of which 68 are structural proteins and the functions of many are still unexplored. The strains of ASFV have been classified into 24 genotypes and 8 serotypes.



Lack of a potential commercial vaccine against ASFV is one of the major obstacles in implementation of effective control programmes against the disease across the globe. The recent outbreaks of ASF in Asia and Europe have intensified global efforts to develop vaccines with a focus on new generation vaccines as well as on identification of promising vaccine target antigens. The major gaps and bottlenecks in the development of an effective vaccine against ASFV are the complexity of the virus structure with large genome (170-194 kbp), limited knowledge on virulent and immunogenic genes, large number of uncharacterized genes, scarce knowledge on host-pathogen interaction, limited understanding of the protective immunity, high genetic diversity (24 genotypes) of the virus as well as genetic variations among the strains. Although, the process of immune evasion by ASFV is not completely understood, yet several genes involved in the immune evasion through different pathways were identified which helped the scientific community to explore the possible vaccine candidates. Several vaccine candidates have been identified by different approaches, but many of them failed to induce protective immunity and previous studies have made it clear that single antigen vaccine is not sufficient to produce protective immunity against ASFV infection. Study carried out with inactivated

and subunit vaccines revealed induction of antibody responses, but these do not confer strong protection. Again, live attenuated vaccines can confer protection against homologous strains, but not heterologous strain of ASFV. Ongoing research findings suggested the key role for the innate immunity, natural killer cells and the cytotoxic activity by CD8 T-cells. Over all identified gaps in the development of vaccines such as side effects of vaccines, virus persistence, vaccine doses and other safety parameters warrant early attention. Improvements in the current and new vaccine candidates will require more extensive analysis of viral genes that should be deleted to build more effective deletion mutants. It will also require further study of ASF pathogenesis and interferon-mediated induction of viral genes. Another important issue is the lack of stable cell lines for development of a live attenuated virus vaccine and scale up of vaccine production, optimization and manufacture. Optimized delivery systems that can induce a protective immune response and development of accompanying DIVA tests are needed. Recently a few experimental vaccine candidates have been identified which may have potential application in near future. A vaccine candidate with seven genes deleted has shown protective immune response; on the contrary, a recently reported single gene deleted vaccine against ASFV (Δ I177L) is also gaining attention as it could impart protection against heterologous strains. Again, a pool of eight antigens cocktail incorporated in a viral vector found to induce protection against the virulent strain. Hence, in ASFV vaccine developmental process, more focus should be given to identify gene deleted vaccine candidates as well as in formulation of potent and effective multi-antigen based vaccine candidates in the form of recombinant subunit vaccine or DNA vaccine.

Recommendations

- 1 To monitor genomic diversity of circulating ASFV, a regular temporo-spatial gene sequencing should be carried out.
- 2 A research and development thrust must be given on ASF vaccine development following newer vaccine approaches and development of point-of-care diagnostics.

Current Status of Porcine Reproductive and Respiratory Syndrome (PRRS) in India and Scope for Vaccine Development

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Porcine Reproductive and Respiratory Syndrome (PRRS) is the most economically important disease of pig throughout the world. Economic losses due to this disease are about \$664 million annually in USA and it was estimated to be around €75,724 (slightly affected) to € 650,090 (severely affected herd) in Europe. This disease is prevalent in almost all pig producing countries of the world including Asia. India was free from PRRS till May 2013. First outbreak in India occurred in the state of Mizoram and reported to OIE on 26th June 2013. Subsequently four major outbreaks occurred in 2015, 2016, 2017 and 2018. PRRSV isolated from outbreak cases in India was characterized as highly pathogenic (HP-PRRSV)- PRRSV Type 2. Clinical outbreaks of PRRS Type 2 have also been reported from the states of Assam, Manipur, Meghalaya and Nagaland. Sero-prevalence of PRRSV has been reported from all the South Indian states/union territory except Puduchery. Sero-prevalence of PRRSV has also been reported from other states like Punjab, Odisha and Madhya Pradesh. It was also observed that HP-PRRSV from different outbreak cases (2013, 2015 and 2016) of Mizoram were genetically different in each outbreak rather than persistence of a single strain. Phylogenetic analysis of Indian PRRSV (2013 and 2015) had revealed similarities with two different strains of HP-PRRSV (Chinese 10 HEB-3 isolate, 2013) and 07QN isolates of Vietnam. While comparing the Indian isolate with classical PRRSV prototype VR2332, they showed only 87.87–88.54% sequence homology. Comparison of N protein amino acid sequences of HP-PRRSV with VR2332 showed consistent mutation at position 15D to N or K and 46 K to R in all the HP-PRRSV.GP5 protein showed consistent mutations at 39 positions from that of VR2332. There is no certified vaccine currently available against PRRSV in India. In the last three decades, the disease has evolved from classical form to most devastating highly pathogenic PRRS. Current vaccines developed are ineffective because they suffer from both immune evasion strategies of the virus and the antigenic heterogeneity of field strains. The efficacy of MLV vaccines against PRRSV is largely determined by the genetic background of the challenge virus, and strong to complete protection is obtained only in the case the challenge virus is nearly identical to the vaccine virus.



Recommendations

- ❖ As only a few studies have characterized PRRSV from India, more studies involving large pig population (covering major pig producing states of India) can be undertaken to know the current status of PRRSV in India.
- ❖ In India, an attempt may be made to develop vaccine from a well characterized, field isolate/ vaccine candidate that will possess high immunogenicity, confers broad protection and is safe.

Development of a Homologous Lumpy Skin Disease Virus Vaccine in India

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Lumpy skin disease (LSD) is causes significant financial losses to the livestock industry and is considered as a serious threat to the food security and poverty alleviation. Until 1984, LSD disease was restricted to sub-Sahara Africa but thereafter its transcontinental spread was observed in the Middle-East and Europe (OIE 2017). The disease was reported for the first time in India in November 2019 (Kumar *et al.*, 2021, Sudhakar *et al.*, 2020) and since then it has become endemic in the country. LSD virus (LSDV), the causative agent of LSD is a member of the family *Poxviridae*, genus capripox virus. Two other members of the capripox virus genus, sheep pox virus and goat pox have high degree of cross reactivity with each other. Therefore, as an emergency measure, the Department of Animal Husbandry and Dairying (DAHD), Govt. of India has recommended using goat pox virus-based vaccine to induce protective immune response (heterologous) against LSD in cattle. However, recent studies suggest partial protection in cattle when vaccinated with goat pox- or sheep pox virus-based vaccine but full protection against homologous virus (LSDV)-based vaccine (Hamdi et al 2020). Therefore, LSDV-specific (homologous) vaccine must be licensed for control and eradication of this economically important disease in the country. To develop an indigenous homologous LSD vaccine, ICAR-National Research Centre on Equines (NRCE) has taken the initiatives. The group at NRCE was the first to isolate LSDV in the country. In order to develop a vaccine, the original virus (LSDV/2019/ India/ Cattle/Ranchi) which was isolated from an infected cattle in primary goat kidney cells, was modified by sequentially passage in the African green monkey kidney (Vero) cells for fifty times (P50). As compared to the original virus (LSDV/P0), LSDV/P50 had some deletion/ fragmentation in some of its virulence genes. The modified virus (LSDV/P50) was found to be safe and immunogenic in the laboratory animals (mice/rabbits) and cattle. It's currently being evaluated for its efficacy in providing protection against LSDV challenge infection in cattle.



Recommendations

1. The recent and unprecedented spread of LSD in India has highlighted the need for better research efforts into this rapidly emerging pathogen.

2. In India currently the DAHD has recommended to use goatpox vaccine to induce a protective immune response in cattle against LSD. However, goatpox- and sheepox virus based vaccines provide a partial protection against LSD. Therefore, LSDV-specific (homologous) vaccine must be licensed for control and eradication of this economically important disease in the country.
3. Pen-side/high throughput diagnostic tests for LSD must be developed for rapidly screening large numbers of filed samples.

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Vaccine to Prevent SARS-CoV-2 Infection in Animals: Indian Perspective

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Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), the etiological agent of COVID-19 in humans has been shown to readily infect and occasionally cause death in cat, dog, deer, lions, tigers, leopards and minks (Murcia *et al.*, 2020, Perera *et al.*, 2020, Ruiz-Arrondo *et al.* 2020, Segales *et al.*, 2020, Zhang *et al.*, 2020). In India, SARS-CoV-2 Delta (identical to human Delta SARS-CoV-2 strains) infection has been reported in lions (Mathavarajah & Delleire 2020); other pet animals (dog, cat) are also at high risk due to their close contact with human population. Jumping of SARS-CoV-2 from humans to animals might accelerate its evolution and hence affect surveillance and control strategies of COVID-19 in humans. Therefore, implementing effective risk management measures to prevent the transmission of SARS-CoV-2 from humans to animals and then back to humans is a major task of veterinary services. We isolated the Delta variant of SARS-CoV-2 from a COVID-19 confirmed patient, followed by its genetic (whole genome sequencing) and antigenic characterization. The virus was sequentially passaged (n=10) until it produced sufficient viral titer in the Vero cells. The P10 SARS-CoV-2 (Delta) was used to prepare an inactivated (β -Propiolactone inactivated and aluminium hydroxide gel adjuvanted) vaccine termed Ancovax. The Ancovax was found to be absolutely safe and induced a potent neutralizing antibody- and cell-mediated immune response against SARS-CoV-2 in laboratory animals (mice and rabbits) and dogs.



Recommendations

1. Evidence of SARS-CoV-2 infection has been observed in animals (Tigers/dogs) in India. Large scale surveillance studies on SARS-CoV-2 infection is required in other domestic and wild animals
2. SARS-CoV-2 infection in animals might accelerate its evolution and may affect its surveillance and control strategies. Therefore, besides humans, suitable control strategies must be implemented to control SARS-CoV-2 infection in animal as well.
3. Ancovax prepared at ICAR-NRCE is safe and induces a potent antibody- and cell-mediated immune response in mice/rabbits and dogs and may be a suitable vaccine candidate for prevention and control of SARS-CoV-2 infection in animals.

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Development of an Indigenous Duck Plague Cell Culture Vaccine

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Duck farming occupies an important position next to chicken farming in India with the total duck population of 33.51 million. In addition to many commercial farms, duck keeping is an integral part of rural farming system that supports the livelihood of the small, marginal and landless farmers and poorer sections of our country. The leading states in duck population are West Bengal, Assam, Kerala, Jharkhand, Manipur, Tripura, Andhra Pradesh, Tamil Nadu, Bihar, Odisha and Uttar Pradesh. Among various diseases, duck plague (DP) is the most important viral disease reported from different parts of our country very often causing huge economic losses due to high morbidity and mortality. In India, duck plague was first reported in West Bengal during 1960's and thereafter, the disease was spread to many other duck-rearing areas in Kerala, Tamil Nadu, West Bengal, Assam and the outbreak of the disease occurs from time to time. An attenuated DPV vaccine, which was developed in Netherland by passaging the virus in embryonated chicken eggs during 1963, was imported to India during 1970's and since then vaccine production in our country is being made using this foreign strain ("Holland strain"). The vaccine preparation involves a cumbersome method by inoculating the vaccine virus in developing chicken eggs and then collecting and homogenizing the entire embryo, CAM and allantoic fluid and subsequently freeze dried. The vaccine is apparently in a crude form that contains extraneous substances like chicken embryo tissues and other egg proteins, which may unnecessarily burden the immune system of the vaccinated birds. At present, the vaccine is being produced by only 3-4 states, through this cumbersome process, which is not suitable for industrial scale production, therefore, fails to meet the need of the country. In order to address these limitations, an **indigenous duck plague cell culture vaccine (DPvac/IVRI-19)** has been developed at ICAR-Indian Veterinary Research Institute using a virulent duck enteritis virus (DEV/India/IVRI-2016), isolated from the field samples collected during an outbreak in in Kerala in the year 2015-16. The live attenuated cell culture based duck plague vaccine was developed by several serial propagation of the field isolate in the primary chicken embryo fibroblast (CEF) cell culture. The titre of



the vaccine virus is $10^{7.5}$ TCID₅₀/ml, so large number of doses can be prepared easily to meet the demand of the entire country. The vaccine has been tested for safety and potency as per Indian Pharmacopeia (IP-2018) guidelines. It has been found to be completely safe and affords 100% protection against challenge infection with the virulent virus. Further, the whole genome sequence analysis of this vaccine strain revealed nucleotide mutations in various genes, which might have resulted in attenuation of the virus. This indigenous cell culture based vaccine would be advantageous for industrial scale production and precisely pure form of the vaccine can be obtained with uniform titre from batch to batch. This vaccine is cost effective and has export potential also. The technology has patent application no. 202211014041 and the vaccine has been officially released by ICAR on 26.03.2022.

Recommendations

- The newly developed duck plague cell culture vaccine with an indigenous strain (DPvac/IVRI-19) can replace the existing vaccine being prepared from a foreign strain, thus fulfilling the 'Make in India' aspiration.
- Production of the vaccine and quality control should be at the national level (instead of individual states) to maintain uniformity and adequacy meeting the demand of entire country.
- Regular mass vaccination in all the duck rearing states to be mandatory and should be monitored by the A.H. departments of state Govt. and GOI. The vaccine may be provided free of cost to the poor and small/ marginal farmers.
- Age of vaccination to be decided appropriately, may be at the age of 3-4 weeks instead of the present practice of 8 weeks, as DP has been found to affect the young ducklings also in many outbreaks.



Session in progress