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Veterinary Vaccines and Diagnostics

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CONVENER : **Dr A.K. Srivastava**, Director and Vice-Chancellor, National Dairy Research Institute, Karnal

EDITORS : **Dr Rajendra Prasad**
: **Dr P.S. Pathak**

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Tel : (011) 25846051-52; Fax: (011) 25846054
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VETERINARY VACCINES AND DIAGNOSTICS

PREAMBLE

In India, the Animal Husbandry sector makes significant contribution to the agricultural GDP. This sector has further potential to grow if livestock and poultry management is addressed properly. However, the animal health has not received due attention that it deserves. The XIth Five Year Plan has included ambitious programs to increase the outlays for control of animal diseases. The present domestic animal health industry, which is of about Rs 1,000 crore, is only 4.5 percent of the total estimated domestic pharmaceutical market. Similarly, the domestic market for animal vaccines is only of Rs 180 crore as compared to Rs 1,600 crore for human vaccines. The low market for animal vaccines in India is due to relative paucity of cost-effective vaccines for prevention of livestock diseases, since the expensive research oriented pharmaceutical industry in western countries does not find this to be an attractive market from commercial point of view. Besides, the public investment in research and development of vaccines for animals in India is meager. Recognizing the importance of veterinary vaccines as prophylactics in controlling animal diseases, National Academy of Agricultural Sciences organized a **Brain Storming Session on "Veterinary Vaccines and Diagnostics"**. The objective of this brain storming session was to draw the future roadmap for laying a national policy on veterinary vaccines and diagnostics for effective management of diseases in livestock population in the country. The Brain Storming Session was convened by Dr. A.K. Srivastava, Director and Vice-Chancellor, National Dairy Research Institute at Karnal on July 10-11, 2009 and was attended by policy makers, scientists and representatives from private sectors. The deliberations and discussions at the Brain Storming Session brought out the following observations and recommendations:

Present status

India has a network of 27,562 Polyclinics / Hospitals / Dispensaries and 25,195 Veterinary Aid Centers (including Stockmen Centres and Mobile Dispensaries), which are supported by about 250 disease diagnostic laboratories. Vaccines against important livestock and poultry diseases are produced in the country at 26 veterinary vaccine production units. Of these, 19 are in the public sector and 7 in the private sector.

IVRI and other laboratories in the country have developed world-class diagnostics/diagnostic kits against some important animal diseases like PPR (Monoclonal antibody based competitive ELISA kit, Monoclonal antibody based sandwich ELISA kit), Rinderpest (Monoclonal antibody based competitive ELISA kit), FMD (LPB ELISA kit, DIVA Kit, diagnostic reagents and vaccine quality assurance reagents), Bluetongue (recombinant BTV VP7 protein antigen-based indirect ELISA, whole viral antigen based indirect ELISA), Brucellosis (for sero-prevalence studies) and some other viral, bacterial and parasitic diseases. However, there are no manufacturers for these diagnostics in the country due

to high investments and inadequate demand on account of low purchasing power of livestock owner.

Dairy Livestock

Foot-and-mouth disease, Hemorrhagic septicemia, Brucellosis, *Peste des petits ruminants*, Infectious Bovine Rhinotracheitis, etc have significant adverse economic impact in livestock sector. Harmonization of FMD vaccine production and quality control parameters are important to produce uniformly good quality vaccine for use in FMD control campaign launched by the government of India, which has now been expanded to cover 121 districts in the country. The critical parameters include : (1) enhancement in duration of protective immune response of the current FMD vaccine to the extent of 9-12 months and elucidating the role of the T-cell response, (2) evaluation of structural and non-structural proteins for possible use as antigen in serological tests to replace whole virus antigen, and (3) development of "Lab-on-site" or "pen-side" test for detection of FMD virus as well as stereotyping by the side of the animal.

Research programme on the development of safe and potent new generation cost-effective FMD vaccine e.g. HAD-5 vectored vaccine is suggested. The economic impact of brucellosis in dairy industry is considerable because of reproductive disorders. The RB-51 vaccine is a suitable marker vaccine for control of brucellosis. Calf-hood vaccination followed by boosters before each breeding service is very effective in reducing the brucellosis in endemic herds/villages. Since, developing a novel vaccine through recombinant DNA technology takes several years, currently available live attenuated vaccines should be tried judiciously as they are adequate to control brucellosis. Further, since RB-51 strain of Brucella is having capability of differentiating between infected and vaccinated animals, it can also be used in adulthood vaccination. In addition, the current status of Cotton Strain 19 vaccine utility needs to be reviewed. Considering the merits and demerits of RB-51 and S-19 vaccines, calf-hood vaccination with S-19 vaccine followed by RB-51 vaccine could be a better strategy.

Surveillance of Brucellosis/ IBR should be initiated by using DIVA test(s) and efficacious marker vaccines at the earliest in the interest of dairy industry. Regular screening of breeding bulls (cattle/buffalos) by available indigenous ELISA should be made mandatory to test all animals, prior to their procurement for a farm. If possible, IBR sero-positive bulls should be castrated. However, semen from IBR sero-positive bulls may be used after screening for BHV-1 virus. Marker vaccine with gE deletion mutant strains of BHV-1 is reported to be the vaccine of choice and therefore, work on development of marker vaccine and companion diagnostic test needs to be taken up on priority.

Anthrax, a zoonotic disease, is endemic in some states resulting in heavy economic losses. Recombinant, nontoxic mutants based vaccine, DNA vaccine, plant based edible vaccine and B cell epitopes of PA as peptide-based immunogens could be good alternatives for anthrax vaccine. It was also brought out that mere vaccination with either conventional

or recombinant anthrax vaccine is not the total solution to the problem and therefore, along with vaccination, management practices including proper disposal of animal carcass by burial without postmortem examination should also be strictly implemented.

Hemorrhagic septicemia (HS) is another one of the most important disease of dairy animals. The need of the time in control of HS is to improve the duration of protective immune response up to 12 months. Further, strict implementation of HS vaccine as mandated should be enforced with a clause of "punitive measures" if laxity in vaccination coverage is proved.

Piggery

Classical swine fever (CSF) is the major disease resulting in high morbidity and mortality in pigs. Following steps are recommended for the control of swine fever: (1) strengthening of existing diagnostic laboratories to adopt new technologies for confirmation of CSF, (2) control of recurrent outbreaks of CSF by prophylactic vaccination, and (3) ensuring adequate doses of quality vaccine and its availability round the year.

Piggery being the livelihood of people in NE states, measures for controlling CSF disease should be taken up on a war footing. Lapinized vaccine, being used currently in the country, is usually short in supply. The disease is still rampant on an alarming scale and there is an urgent need for a better vaccine, preferably cell culture attenuated lyophilized vaccine. Indian Veterinary Research Institute (IVRI) should hasten the process to make the cell culture vaccine for CSF and make available in the market in near future.

Sheep and Goat

Regular vaccination against important diseases of sheep and goats could reduce the mortality from 12-14 % to 4-5% in small ruminants. IVRI has already developed live attenuated cell culture based vaccines for *Pest des Petits Ruminants* (PPR), goat pox, sheep pox (new sheep pox vaccine using Srinagar isolate, which is better than RF strain vaccine), and thermostable live attenuated PPR vaccines using sheep and goat strains of PPR virus. Further the combination of vaccines viz. PPR+sheep pox combined vaccine, PPR+goat pox combined vaccine have also been developed by IVRI, Mukteswar. Process should be hastened by IVRI for transfer of technologies of these vaccines so that these are manufactured as per the requirement of the country. Further, there is need to develop effective marker vaccines against PPR, Bluetongue, ET, Sheep pox, goat pox, JD and CCPP. The confirmatory diagnostic DIVA tests should also be developed for important sheep and goat diseases.

Poultry

Avian influenza (H_5N_1) is an important devastating disease of poultry, having zoonotic implications. In India, the disease struck for the first time in 2006, when Maharashtra state experienced HPAI due to H_5N_1 virus. The disease was controlled by "stamping out"

policy. Subsequently, a vaccine was developed using indigenous strain of virus by High Security Animal Disease Laboratory, Bhopal. The WHO has also opined that the Indian strain of AI virus was highly immunogenic. The strategy for controlling the ingress of AIV across the border from north eastern states of India (West Bengal, Assam and Tripura etc.), which is at the high risk region due to porous border with Bangladesh, is to be finalized soon. Option of using vaccine in the border area is to be evaluated by an Expert Group. Although heterologous vaccine, which differentiates between vaccinated and infected birds is generally used, the Avian influenza vaccine incorporating the homologous strain is not advisable as it does not differentiate whether the AIV antibodies are due to vaccination or due to infection. Accordingly, it is desirable to develop a new AI vaccine employing reverse genetic technology which should lead us in developing a DIVA strategy that can be used for differentiation between vaccinated and infected birds.

In spite of availability of quality vaccines, Newcastle disease (ND) is a major problem in commercial poultry flocks due to immune-suppression factors. The Marek's HVT vectored vaccine with ND virus 'F' gene insert is now successfully commercialized in North and South America (the region endemic for both MD and ND). Therefore, this vaccine should also be considered for its use in India. It has advantage of one simple application *vs* more than 8 applications of conventional ND vaccine(s) in layer and breeder stocks.

Chicken infectious anemia (CIA), an economically important viral disease of poultry, is wide spread in India. Internationally, the vaccine against CIA has been successfully used in breeding stock to prevent the vertical transmission of virus in the progeny (commercial broilers and layers) and, therefore, it may also be considered for its use in India. The need for developing efficient diagnostics and vaccine for CIA and other poultry diseases, using indigenous isolates of the etiological agent(s), is also warranted.

Infectious bursal disease (IBD) is also a concern as it lowers the poultry productivity considerably. Vaccines are now available in the country. IVRI has also developed a thermostable vaccine for IBD. Technology of such vaccines should immediately be transferred so that commercial manufacture can be taken up.

General Issues

Recent advances in protein biochemistry, molecular biology and immunology can facilitate development of suitable vaccines against parasitic diseases in near future. Further recent advances in structure-function relationships of parasite antigens should be exploited to translate these findings in vaccine development. Peptide based diagnostics can be used for more specific assay that can also be developed in "pen-side" test format for use in field. Further, new instrumentations and technologies like mass spectrometry and clinical proteomics should be adopted for developing diagnostic assays for pre-clinical diagnosis, understanding molecular basis of disease pathogenesis, and identification and characterizqation of pathogens for diagnosis and reposition purposes. Nanoparticle based diagnostics and vaccines, DNA vaccines, RNA interference (RNAi) in controlling viral

infections and application of biosensors and micro-arrays in disease diagnosis need attention on priority.

RECOMMENDATIONS

A. Policy

1. A National Network for Disease Surveillance and Monitoring (livestock and poultry) needs to be established in the country with the main objective of coordinating the state AH / Veterinary departments, Department of AHD & Fisheries, Ministry of Agriculture, GoI, ICAR and State Agricultural/Veterinary Universities.

2. Effective mechanisms for **inter-state**, **inter-laboratory** and **inter-sectoral** networking and collaboration for lab support and standardization of diagnostic test procedures need to be worked out to reduce the incidence of diseases, particularly in cattle and buffaloes.

3. All the state AHD/Veterinary departments must establish **Disease Surveillance & Monitoring System Cell / Centre** adequately supported by specialist veterinary officers / veterinary scientists having sufficient expertise in the subject. **A separate cadre of veterinarians trained in disease diagnosis work should be created in each state so that these trained scientists are always available for this specific work.**

4. In order to cater to the needs of poultry health in the context of SPS under WTO and Food Security, the Division of Poultry Science needs to be established in each veterinary college with adequate facilities to manage poultry health aspects including research, disease diagnosis and surveillance. At present, there is no lab which is specific for poultry health.

5. Adequate financial support from the government is suggested for a micro- or small-scale industry in this high risk area, as the production of diagnostics require relatively higher level of technical expertise than in vaccine production.

B. Research

1. Isolation and characterization of the target virus, prompt and accurate diagnosis of AI in new outbreaks/new areas employing new generation diagnostic assays including multiplex RT-PCR, Real-Time PCR, and mass spectrometry should be developed for agent identification and disease diagnosis. Development of marker vaccine and DIVA diagnostic test to differentiate vaccinated from infected birds, isolation and characterization of different avian pathogens using molecular tools need top most priority.

2. Enhancement in the duration of protective immune response of the current FMD vaccine to the extent of 9-12 months and to elucidate the role of the T-cell response / cell mediated immunity. Similar approach for HS vaccine is also required.

3. Evaluation of structural and non-structural proteins for their possible use as antigen in serological diagnosis of FMD and other infectious diseases to replace the whole virus antigen. This will also help in ensuring adequate biosecurity measures.

4. Development of "Lab-on-site" test for pen-side detection of FMD and many other microbial pathogens is essentially required.

5. Application of 'Biosensors' for animal disease surveillance.

6. Development of effective combinations of vaccines and vaccination against PPR, Sheep pox, Goat pox, Blue tongue, ET, Contagious ecthyma, and CCPP are priority.

7. Research work on development of IBR marker vaccine with gE defined strains of BHV-1 and companion diagnostic test are to be taken up on priority so that marker vaccine for IBR can be made available at the earliest.

8. Development of diagnostic reagents and tests/assays for various infectious diseases endemic in the country especially against BVD should not be delayed.

9. Microarray and metagenome sequencing in animal disease diagnosis and development of chips for detection of pathogens prevalent in India, should be taken up.

10. The present crisis of paucity of vaccine against Classical swine fever (CSF) should be addressed by ensuring adequate dose of vaccine, preferably freeze-dried-cell-culture vaccine round the year.

11. Development of suitable marker vaccine for brucellosis and feasibility of using RB-51/S-19 vaccine in Indian bovine population should be studied.

12. Structure-function relationships of protozoan and metazoan parasite antigens and development of vaccine against economically important protozoan and metazoans.

13. Development of efficacious vaccines and vaccination strategies against Coccidia, Salmonella and Mycoplasma infections in poultry should be taken into consideration.

14. Whole genome sequencing of microbial pathogens, which will help in understanding the details of molecular epidemiology and development of effective vaccines should be taken up.

15. Whole genome sequencing of important livestock breeds also needs to be taken up, as the vaccines efficacy also depends on the genetic make up of the animal.

C. Disease Surveillance and Control

1. Livestock diseases of economic importance should be identified on priority and an effective national eradication program should be launched along with the ongoing **FMD control program** already in progress. It is recommended that **PPR, Goat pox, Sheep pox, Swine fever, Fowl pox** and **Newcastle disease** should be added in phases in disease eradication program through mass vaccination.

2. **HS vaccination** using new generation HS vaccine should be taken up with the aim of achieving 100% immunization in the target herds by 2015.

3. Structured field trials with RB-51/S-19 vaccines should be undertaken to evaluate the appropriateness and efficacy of this vaccine for the control of brucellosis in India.

4. Keeping international norms for mass vaccination in background the option for mass vaccination programme against HPAI (H_5N_1) may also be weighed, especially for north-eastern region of India (West Bengal, Assam, Tripura etc.), which is endemic for the disease.

5. For accurate diagnosis of brucellosis RBPT, STAT, MRT, ELISA, PCRs along with suitable isolation techniques for the target pathogen(s) should be employed as a national policy.

6. Recurrent outbreaks of **Swine fever disease** in pig population should be controlled by effective vaccination program and disease management, especially in the north-eastern states.

7. Testing of all the breeding bovines farm stocks for BHV-1 infection (IBR/ IPV), along with other STDs, should be made mandatory, in view of considerable impact of the disease on animal health and productivity.

8. All the animal population susceptible to anthrax should be vaccinated with the currently available “anthrax spore vaccine”, especially in the endemic areas in different states. Further the management practices should also be standardized for controlling this disease in these tracts.

9. In order to reduce the menace of rabies in human and livestock populations, vaccinating the stray dogs against rabies must receive a serious consideration. It can be done by oral vaccination using recombinant vaccine baits.

ACRONYMS FOR THE ABBREVIATIONS USED IN THE TEXT

AH	Animal Husbandry
AHD	Animal Husbandry and Dairy
AI	Avian Influenza
AIV	Avian Infleunza Virus
BHV-1	Bovine Herpes Virus type 1
BVD	Bovine Viral Diarrhoea
BTV	Bluetongue virus
CCPP	Contagious Caprine Pleuro Pneumonia
CIA	Chicken Infectious Anemia
CSF	Classical Swine Fever
DIVA	Differentiating Infected from Vaccinated Animals
DNA	Deoxyribo Nucleic Acid
ELISA	Enzyme-Linked Immuno Sorbent Assay
ET	Enterotoxaemia
FMD	Food and Mouth Disease
F gene	Fusion protein gene
GDP	Gross Domestic Product
gE	Glycoprotein E
GoI	Government of India
HAD-5	Human Adenovirus Serotype 5
HPAI	Highly Pathogenic Avian Influenza
HS	Haemorrhagic Septicemia
HSADL	High Security Animal Disease Laboratory
HVT	Herpesvirus of Turkey or Turkey Herpesvirus
IBD	Infectious bursal disease
IBR	Infectious Bovine Rhinotracheitis
ICAR	Indian Council of Agricultural Research
IPV	Infections Pustular Vulvovaginitis
IVRI	Indian Veterinary Research Institute
JD	Johne's Disease
LPB-ELISA	Liquid-phase blocking- Enzyme-linked Immunosorbent Assay
MD	Marek's Disease
MRT	Milk Ring Test
ND	Newcastle disease
NDRI	National Dairy Research Institute
PA	Protective Antigens
PCR	Polymerase Chain Reaction
PPR	Pest des Petits Ruminants
RB-51	Rifampin-resistant mutant of *Brucella abortus*
RBPT	Rose Bengal Plate Test
RNAi	RNA interference
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SPS	Sanitary and Phytosanitary Measures Agreement
STAT	Standard Tube Agglutination Test
STDs	Sexually transmitted diseases
S19	Spontaneously attenuated *Brucella abortus* strain S19
WHO	World Health Organization
WTO	World Trade Organization

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