INDOVAX

INFECTIOUS BRONCHITIS – A MOVING TARGET
CONTEMPORARY CRISIS; CONTEMPORARY SOLUTION

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# HOW TO AIM AT
## MOVING TARGET OF INFECTIOUS BRONCHITIS

"IBV is a moving target" says Dr. Cavanagh a world renowned authority on Infectious Bronchitis. He says "The task before the poultry farmers and the poultry scientists is formidable but not impossible. There were never easy victories in field of vaccine development and all attendant methodology. The traditional approach together with support from impressive array of genetic engineering techniques is already busy in facing this formidable situation.

In preparing this note we have a utilitarian approach – it should provide insight in the nature of disease, its spread and attendant damages for our farmer brothers and some pertinent thoughts on "what best could be done" for our learned consultants and practicing veterinarians.

### Introduction :

It was customary to define Avian Infectious Bronchitis as an acute highly contagious viral respiratory distress accompanied by precipitous drop of egg production in laying flocks. The epidemiological picture has now changed. In addition to IB associated with respiratory distress and reproductive disorder, we are also witnessing nephrotropic, enterotropic and proventricular manifestations of the disease with severe losses.

### Aetiology :

Infectious Bronchitis virus belongs to **Corona virus group 3.** The virus is round to pleomorphic and it's envelop has club shaped surface projections and hence named as "Corono" (crown).

### Antigenicity - Mutation- Variant strains :

IB virus is single stranded RNA virus with 4 genes. (1) S- Surface protein, (2) M- Membrane protein, (3) E -Small membrane protein, (4) N – Nucleocapsid protein.

Out of this S. protein is not stable and is highly prone to variation. S1 protein has 500 amino acids. If there is change in sequence to the extent of even 2 – 3% it becomes a new virus type.

Now let us know the important role of S1 spike protein.

i. It induces virus neutralizing and HI antibodies.
ii. It is a major inducer of protective immunity.
iii. Minor differences in amino acid sequence on S1 protein (2 – 3%) can result in major antigenic difference between strains.

The new variants may cause unusual manifestation of disease.

The common IB strains reported in literature are:-

**Respiratory -**

- Massachusetts
- Connecticut
- Arkansas
- Georgia

**Nephrotropic -**

- T strain
- Gray
- Holte strain

Although H120 is still the most widely used vaccine all over the world but its protective scope appears to be diminishing with time. Currently several broiler and layer variants exhibit lower protection with H120 vaccine and newer vaccines e.g. attenuated 793B or 4/91 strains are being employed in several countries. These vaccine strains are not available currently in India. INDOVAX is continuing research in new variants to find a suitable protective type from indigenous isolates.

**A general understanding on classification of IB virus isolates :**

It would be a formidable task to take note of thousands and thousands of IB isolates. For sake of general understanding they could be filled in the table given below.

**Host :** It is generally considered that the chicken is the only bird that is naturally infected by IBV and in which the virus causes disease. All ages are susceptible but the disease is more severe in chicks. As age increases chick become more resistant to nephrogenic effects, oviduct lesions and mortality due to infection.

It is important to understand that proper protection of chicks is of paramount importance for successful poultry operation. A susceptible, inadequately protected chick can lose its oviduct; if infected in the early stage of life.

**Transmission :**

The virus spreads rapidly among chicken in a flock. The route is usually by inhalation of virus droplets produced by sick birds. Wind borne infections can infect surrounding farms. The incubation period is very short, **18 to 36 hours.** Birds become ill, production parameters are challenged and carrier birds become a nucleus of infection for other farms.

Movement of live birds and day old chicks(DOC's)

| Classification of IB Viruses<br>1. Object - what do they do? | | |
|---|---|---|
| **Scheme** | **Basis** | **Value** |
| **Serotype** | Virus neutralization | Traditional method of classification important to know for designing protection |
| **Pathotype** | Tropism<br>i. Respiratory<br>ii. Enterotropic<br>iii. Nephrotropic<br>iv. Proventricular<br>v. Uterotropic | Tropism is not serotype specific but some strains show tropism. Valuable to know for designing protection |
| **2. Object - will the vaccine protect?** | | |
| **Protectotype** | Cross protection in vivo | This is gold standard for designing a vaccine for a particular problem |
| **3. Object - what the virus is?** | | |
| **Genotype** | Molecular differences | Valuable to know epidemiology/ spread of disease / tracing of disease / valuable input for vaccine industry |



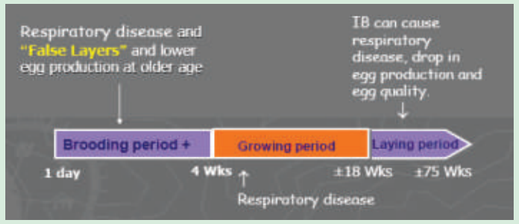
are potential source of introduction of IBV. However, the poultry trade takes conscious steps in proper disinfection of hatching eggs before setting them for hatching.

Vertical transmission usually does not occur in IB. But there are few reports voicing strong possibility of vertical transmission.

**Clinical syndrome :**

Avian infectious bronchitis is primarily a respiratory infection of chicks. Nevertheless, three clinical manifestation of IB are generally observed namely Respiratory disease, Reproductive disease and Nephrotic disease.

In general the age wise prevalence of disease is as under:-




**Respiratory Disease :**

Respiratory disease is most frequently observed syndrome of IB. In chicks between 2 to 6 weeks of age, main clinical signs are difficulty in breathing, tracheal rales, coughing and sneezing. Feed consumption is reduced which affects body weight. Respiratory disease is due to narrowing of the lumen of tertiary bronchi.

Secondary infections

with CRD and E. coli cause heavy mortality. Some chicks may develop nephritis. Few isolates of IBV can cause spectacular mortality due to nephritis, visceral gout and urolithiasis.

**Reproductive disorders :**

IBV at young age and or after maturity, both can lead to reproductive problems in hens. In adult birds clinical signs may not be present or may take the form of a mild respiratory disease. A decline in egg production varies according to stage of lay at infection and strain involved.

Typically declines are between 3 to 10% but reduction upto 50% have also been observed in some flocks. This is associated with smaller size eggs, inferior shell, soft shell, misshapen eggs and thin watery albumin

If IBV infection occurs when chicks are less than 2 weeks of age, permanent damage to oviduct may result. Such birds turn out as "false layers" and one may lose upto 25% adult hens as false layers.

Some layers may show pendulous abdomen with cystic remnant of right oviduct. Cystic oviduct may be filled with clear fluid exceeding 1000 ml.

**Why there is egg drop, misshapen eggs, discolored eggs, shell less eggs :**

Experimental IB infection of the oviduct of mature hens results in decreased height and loss of cilia from epithelial cells, dilatation of tubular glands, infiltration of lymphocytes and other mono nuclear cells, plasma cells, heterophils, edema and fibro plasia of the mucosa of all regions of oviduct.

In experimental intra tracheal infection of 34 weeks layers, virus could be detected in 3 – 6 days post infection. De-ciliation of epithelium occurred. Tubular glands were replaced by fibrous tissue.

In fact every portion of reproductive tract gets affected. A decline in shell quality occurs when oviduct is directly affected. This decline is reflected as cystic right oviduct, impacted oviduct and ruptured ova, poor egg specific gravity, reduced shell thickness, misshapen eggs, deterioration in shell pigment.

It has been noticed that some strains of IBV are uterotropic. In such cases albumin forming region is affected. There is disturbance in physiological rhythm of cells and consequently there is total cessation of egg formation.

## Diagnosis:

### i) Virus isolation :

Lung, trachea and caecal tonsils are ideal tissues for attempting virus isolation. In case of nephrotropic strains kidney tissues should also be examined for IB virus. Virus is isolated in 9 – 10 days old SPF egg embryo. It causes characteristic dwarfing of embryo. Virus isolation is a protracted exercise. It is necessary to give 5 – 6 blind passages before virus can be recovered. This is gold standard of IB diagnosis.

### ii) Agar Gel Precipitation Test (AGPT) :

It is a quick test. It needs high titred mono specific IB serum. One can use allantoic fluid from IB infected embryos as an antigen. Positive reaction is exhibited by line of identity.

Negative AGPT does not confirm absence of IB.

### iii) Enzyme Linked Immunosorbent Assay (ELISA) :

ELISA kits are available from IDEXX, KPL, Artec and other companies. Each kit has its own calibration, positive/negative values and system of interpretation.

ELISA shows IBV exposure but it will not identify the serotype involved.

A well protected flock will have high titre and low CV (Coefficient of variance) less than 25.

ELISA test can estimate MAB status, sero conversions as a result of vaccination/ infection.

In interpreting ELISA titre due consideration should be given to vaccination history and optimum performance traits of the breed.

### MAB – KEY FOR SAFE JOURNEY :

Maternal antibody is important for providing protection against IBV induced mortality.

| IB isolate number | MAB status | % Mortality |
|---|---|---|
| 1. | Negative | 37% |
| | Positive | 3% |
| 2. | Negative | 10% |
| | Positive | 0% |
| 3. | Negative | 27% |
| | Positive | 16% |

In one of the experiments reported in literature mortality during first 5 weeks in MAB positive and negative chicks when exposed at day 1 of age is recorded in above table.

Mortality depends on pathotype of virus. But MAB carrying chicks have less mortality and more chance of healthy survival. MAB can prevent spread of disease in internal organs.

It is important to remember that exposure to IBV during first 3 weeks of life can destroy the oviduct of the bird.

Highest titre and protection of progeny can be achieved by judicious use of infectious bronchitis inactivated vaccines.

### Control :

For any disease the methods available for control are two fold:-

1. Bio-security
2. Immunization

### 1. Bio-security :

Biosecurity should not be restricted to only hygienic measures by liberal use of variety of disinfectants. It should include verification of DOC's received on farm, vaccination history of parents, MAB profile of chicks, adequate down time in between two flocks. Rigorous bio-security measures should be adopted if earlier flock was infected with IB. Effective quarantine, developing a sentinel validation of disinfected shed, restricted entries and not keeping multiple age groups on the farm, together with laboratory aided vaccination programme. Let the bio-security emerge as a total plan to reduce virus bio-burden on the farm.



### 2. Immunization :

Poultry industry have got free availability of live vaccines and inactivated vaccines for this purpose.

### i) Live vaccines :

Mass strains H52 and H120 vaccines are commonly known to poultry industry. Let us know more about them.

The H strain of IB was one of the earliest live attenuated vaccines to be developed and is being used in most parts of the world for last 50 years.

H stands for the name of owner- Huyben- a poultry farmer in Holland. It was isolated from a broiler farm.

It was developed for use at both 52 (H52) and 120 (H120) vaccine levels. It has ability to provide heterologous cross protection against number of different serotypes. It has proved to be most enduring live attenuated vaccine. H120 is possibly most widely used live vaccine globally.

### An important work of Cook et al 1986 :

Chicks were immunized with H120 live IB vaccine on day 1 and were challenged thereafter with;

Mixed infection comprising of pool of E.coli and heterologous IB virus. Good cross protection was achieved following challenge with several but not all heterologous isolates.

Good protection was obtained against the strains viz. Holte, Iova, D207, Aust T, D 3896 and several UK isolates. Dr. Jackwood found that IB isolates possessing differences to the extent of 40% in sequence homology can yet be contained with H120. Dr. Kenn Ruda states "from the earliest discovery of the virus", mass strain has become truly a global virus. Variations have been shown but good cross protection is demonstrated by mass strain.

### ii) Inactivated vaccines :

It is necessary that the birds are earlier primed with live IB vaccines properly so that the subsequent administration of inactivated vaccines produce a spectacular secondary response.

Inactivated vaccines are intended for use in layers and breeders. The vaccines are administered by subcutaneous inoculation at 6 weeks and then boosted at 13-18 weeks of age, to pullets which have been previously primed with live attenuated vaccines. Inactivated vaccines provide high and uniform levels of antibodies that persist for longer periods than those induced by live vaccination. These high levels of antibodies are particularly useful in providing protection to the internal organs by preventing spread of the virus. In layers and breeders, inactivated vaccines provide protection against reduction in egg production, which might not always be afforded by live vaccination. In addition, in breeders, progeny chicks will be protected by maternally transferred antibodies. Progeny chicks that originate from breeders vaccinated with inactivated vaccines have high and uniform maternal antibody levels in comparison to those from breeders vaccinated with live vaccines only. Most of the inactivated vaccines are of one type, Mass type; however, bivalent vaccines that incorporate additional variant antigens may also be necessary. Inactivated vaccines are produced from IBV infected embryonic fluid, which is inactivated and usually formulated as oil emulsion vaccine.

It is possible to incorporate strategic strains in inactivated vaccine to afford tailor made protection in situations as Nephrotropic syndrome, visceral gout or in such situation where variants are reasonably suspected but can not be easily attenuated to offer a live vaccine.

### Consider this before introducing a new vaccine :

Although IB is distributed world wide, only certain strains can be considered global. Most variations in

genetic make-up of virus occur at best on regional basis. There can not be any one "world wide" successful product. In nature new variants are produced virtually daily. Nature in most cases eliminates these in process of natural selection.

Therefore, the strategy should be;

a) The correct use of vaccines including correct dosing, application, sensible introduction and withdrawal of antigens as appropriate, before blaming variants for the problem.

b) Attempt to use present vaccines or combinations thereof, to eliminate closely related variants. It has been shown recently in field tests (more effective than laboratory tests) differences of up to 40% can be controlled with present products.

c) The 3rd scenario, and it is rare, is the isolation of a true variant at least 70% different from the present known strains. In this case, cross-protection attempts will prove futile and a potential new vaccine may be required. It is important that pathogenicity tests are performed as a number of non pathogenic variants are known to exist. There is always an option to introduce such heterologus strains as one of the component of IB inactivated vaccine.

Dr. Jackwood has a valuable suggestion:

"Best results for controlling IB are achieved by using a vaccine strain that is identical or highly similar to the causative field strain (s)".

**Taming the moving target :**

The IB virus is vulnerable to high degree of mutation. But the foregoing survey of available knowledge does not ask for new vaccines at every stage. Fortunately H120 has been authenticated by several workers to give broad protection against most of the variants reported to be causing respiratory/ nephrotropic IB. There is a promising choice of incorporating strategic strains in inactivated vaccines. With the aid of these weapons in the armor of science, the moving target of IB can be humbled and tamed along with the aid of other laboratory supports, translating sera conversions at various stages:-

**Indovax at service of farmers -**

Indovax provides the broad spectrum H120 live vaccine in 3 presentations:

1) Newcastle disease Asplin F strain + Mass H120 live vaccine for day old chicks

2) Newcastle disease Lasota strain + Mass H120 live vaccine intended for growers, commercial layers & breeders

3) Infectious Bronchitis H120 monovalent vaccine for chicks, pullets and adult birds

Infectious Bronchitis killed vaccine in following presentations :

i) Monovalent IB killed vaccine

ii) Bivalent ND + IB killed vaccine

iii) Trivalent ND + IB + IBD killed vaccine

iv) Multi component 4 way ND + IB + IBD + REO killed vaccine

v) Infectious Bronchitis Inactivated vaccine with strategic isolates to contain specific nephrotropic, gout or reproductive problems

Indovax also provide services for MAB profiling and sero conversion monitoring for IB by ELISA method to its customers on basis of mutual understanding.

**Vaccination schedule:** There is no thumb rule for vaccination schedule. This is decided on the basis of (1) MAB, (2) Sero conversion, (3) Previous history of disease on farm, (4) Disease in surroundings, (5) Vulnerable age, (6) Pathotype of virus, (7) Success/ failure of earlier schedules, (8) Population handled. This work should be ideally left to poultry consultants.



(In general it is necessary to do repeated vaccination to keep protective titers)

A vaccination schedule is suggested for general guidance. It should be discussed with the poultry consultant and tallied and weighed with previous experience.

| Day/Week | Vaccine | Route |
|---|---|---|
| Day 1 | Mass H120 (Bronkichick) alone or Bronki-F | I/N and I/O |
| Day 25 | Mass H120 (Bronkichick) alone or Bronki-L | D.W. |
| Day 42-45 | Mass H120 (Bronkichick) alone or Bronki-L<br>ND+IB killed vaccine (Polyvax) | D.W.<br>S/C |
| Day 84 | Mass H120 (Bronkichick) alone | D.W. |
| Day 112 | ND + IB killed vaccine/ Polyvax (NB)/ (Trivalent or 4-way vaccine as per need) | S/C |
| Week 32 | Mass H120 alone or with Lasota<br>(To be repeated every 7 weeks till liquidation of flock) | D.W. |

**Contemporary crisis has a contemporary solution:**

The contemporary crisis in management of IB control lies in the problem posed by umpteen IB variants. We have to address this problem in a practical manner. We should judiciously use Mass H120 vaccine for priming and the booster consisting of IB inactivated vaccine. Vaccines properly formulated are contemporary hopes to over come this crisis.

**Contribution from Scientific team of INDOVAX**

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