PHYTOVIR *Where nature meets nurture…*

Proprietary formula for viral problems in poultry birds

Technical support material for PHYTOVIR from TRÈSBIEN Biosynth Pvt. Ltd.

Adenovirus Diseases in Poultry

The first recognized adenoviral infections of birds were **quail bronchitis** and chicken embryo lethal orphan (CELO) viruses, which were known in 1949 and 1957, respectively. Later, **inclusion bodies in chicken livers** were described in 1963, followed by the isolation of a "new agent" of a disease called **"inclusion body hepatitis (IBH)"** in 1973. However, for many years the exact role of adenoviruses in causing avian diseases was unclear. Adenoviruses are suspected of playing a **secondary role** in causing many syndromes. For instance, the presence of immunosuppressive viruses, such as chicken anemia virus (CAV) or infectious bursal disease virus (IBDV) has been reported to enhance the pathogenicity of some adenoviruses to cause IBH. However, there is evidence that adenoviruses cause IBH without a requirement for other pathogens. Today, IBH has a worldwide distribution, affecting domestic species of all ages, and with indication that the incidence of the disease is increasing.

Classification of adenoviruses in poultry

Genus	Species	Disease
Aviadenovirus (Group I Adenoviruses)		
Chicken, quail	**Fowl adenovirus (FAdV)** 5 species A-E 1-12 serotypes	Inclusion body hepatitis, hydropericardium syndrome, gizzard erosions, quail bronchitis, *etc.*
Goose	**Goose adenovirus (GoAdV)** 1-3 serotypes	Aviadenovirus infection in geese
Duck	**Duck adenovirus B (DAdV 2)**	Aviadenovirus infection in duck
Pigeon	**Pigeon adenovirus B (PiAdV 2)**	Aviadenovirus infection in pigeon
Turkey	**Turkey adenovirus B (TAdV)** 1-2	Aviadenovirus infection in turkey
Siadenovirus (Group II Adenoviruses)		
Turkey	**Turkey adenovirus A (TAdV 3)**	Hemorrhagic enteritis (turkey)
Pheasant		Marble spleen disease (pheasant)
Chicken		Avian adenovirus splenomegaly (chicken)
Atadenovirus (Group III Adenoviruses)		
Chicken	**Duck adenovirus A (DAdV-1)**	Egg drop syndrome

Virology of Adenovirus

The adenoviruses are members of the family **Adenoviridae**, which is divided in four genera namely Mastadenovirus infecting mammals, and **Aviadenovirus, Siadenovirus,** and **Atadenovirus** infecting birds. The latter three genera are classified as Group I, II and III avian adenoviruses respectively. Adenoviruses are **icosahedral, non-enveloped double stranded DNA viruses** that range in size from 70 to 100 nm and have 252 capsomeres surrounding a core. Adenoviruses replicate in the nucleus, producing characteristic inclusion bodies. With respect to several characteristics of the virion, such as virus morphology or genome organization, which are relevant for diagnostic purposes, the avian adenoviruses are very heterogeneous. Consequently, the diagnosis of avian adenoviruses differs significantly among the three different groups.

Resistance nature against common biosecurity measures

Avian adenoviruses show remarkable resistance to heat inactivation although differences in sensitivity between strains have been recorded. **Some strains survive 60°C or even 70°C for 30 minutes**. The stability of these viruses to heat is greater when they are suspended in monovalent cations compared to divalent cations, as with other DNA viruses. They are resistant to lipid solvents and to pH 3 to 9. However, **adenoviruses are sensitive to formaldehyde**.

Serology

At least **12 serotypes of fowl Aviadenovirus** have been recognized on the basis of virus neutralization tests (with several strains in each serotype). These serotypes and the other aviadenoviruses share a common group antigen. Under a new classification scheme, which considers additional criteria, such as calculated phylogenetic distance and restriction fragment length polymorphism analysis of the genome, the 12 serotypes were assigned to one of **five virus species** i.e., Fowl adenovirus **(FAdV) A-E**. Only serotype 1 (Fowl adenovirus A or FAdV-A) has haemagglutination activity, but it agglutinates only rat red blood cells. Exposure to one serotype confers no immunity to other serotypes within the group I (NO CROSS PROTECTIVE IMMUNITY AMONG SEROTYPES FOUND). Similarly, infections with a strain of the group I will not protect against infections with viruses from the group II or III. For these reasons it is not uncommon to isolate two serotypes from the same bird and a broiler flock may have four or more serotypes present. There is also little protection between the 12 serotypes of aviadenoviruses. Considerable exchange of serotypes may occur when commercial flocks are made up of the progeny of several parent flocks. At sexual maturity a bird may have been infected with the majority of the 12 recognized serotypes.

Virus excretion

Following experimental infection of specific-pathogen-free (SPF) chicks in the first days of life, using natural routes of exposure, **initial growth of fowl adenoviruses occurs mainly in the intestinal epithelium**, followed by a viraemia, and the presence of the virus in many organs (liver, kidney, respiratory tract, bursa of Fabricius, spleen and bone marrow). However, **in the field, infections with**

aviadenovirus are not normally detected during the first few days of life although isolations from 3 weeks onwards are common. In naturally occurring infections, aviadenovirus is excreted in the feces for about 3 weeks, with the peak excretion occurring between 4 to 7 days after infection. Certainly, birds can excrete one serotype in spite of high levels of neutralizing antibody to other serotypes.

Carrier status

The isolation of an adenovirus from the appropriate organ (e.g., the trachea of a bird suffering from tracheitis) does not necessarily means that it is the etiological agent of the disease. Such an isolate may be also a latent virus reactivated by the disease process. **Birds can be carriers during all their life**. In laying hens, aviadenoviruses may be transmitted through the egg, particularly around the **time of peak egg production**. Presumably, the stress associated with egg production or the increased level of sex hormones at this time causes reactivation of the virus.

Spread

Chicks hatching from infected eggs may excrete the virus in feces from hatching although virus excretion is often detected in the flock at 2-4 weeks of age. Horizontal spread of virus through all excretions is possible with the highest titers being in feces. Aerial spread between farms occurs when cleaning of depopulated houses takes place and the dust created transmits infection between farms. Spread by fomites, such as egg trays and egg trolleys, personnel, and transport can also occur. Following natural infection the incubation period of the virus ranges from 24 to 48 hours.

Disease types

Although aviadenoviruses have been isolated from a number of clinical conditions, there is no clear evidence for a primary role in disease causation. However, aviadenoviruses have been most commonly associated with **IBH** (mainly types D and E), **hydropericardium syndrome** (type C), **gizzard erosions** (type A), and **respiratory diseases**. Aviadenoviruses have also been suspected as a cause of egg production

Image-1: IBH. Birds showed lethargy, huddling with ruffled feathers and lack of appetite

problems in laying hens (**EDS**) and **viral arthritis/tenosynovitis**.

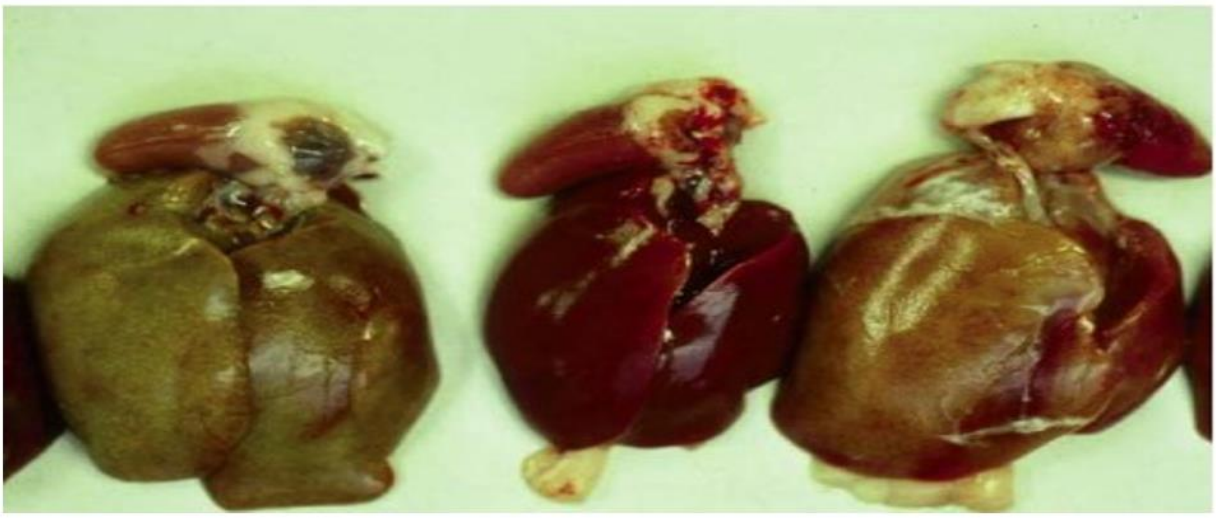
Inclusion body hepatitis (IBH)

Inclusion body hepatitis of chickens was first described in the U.S. in 1963. Since then, the disease has been reported worldwide. A sharp rise in severity and occurrence of IBH has been reported. This disease is usually **seen in 2 to 3 week-old broilers** (sometimes as young as **4 days to 7 weeks of age**). Other species, such as pigeon, guinea-fowl, psittacines, or turkey may be affected. Naturally occurring outbreaks have been associated with a wide spectrum of serotypes. **Aviadenoviruses are primary pathogens for IBH** although co-infection with **IBDV or CAV has been reported to increase pathogenicity**.

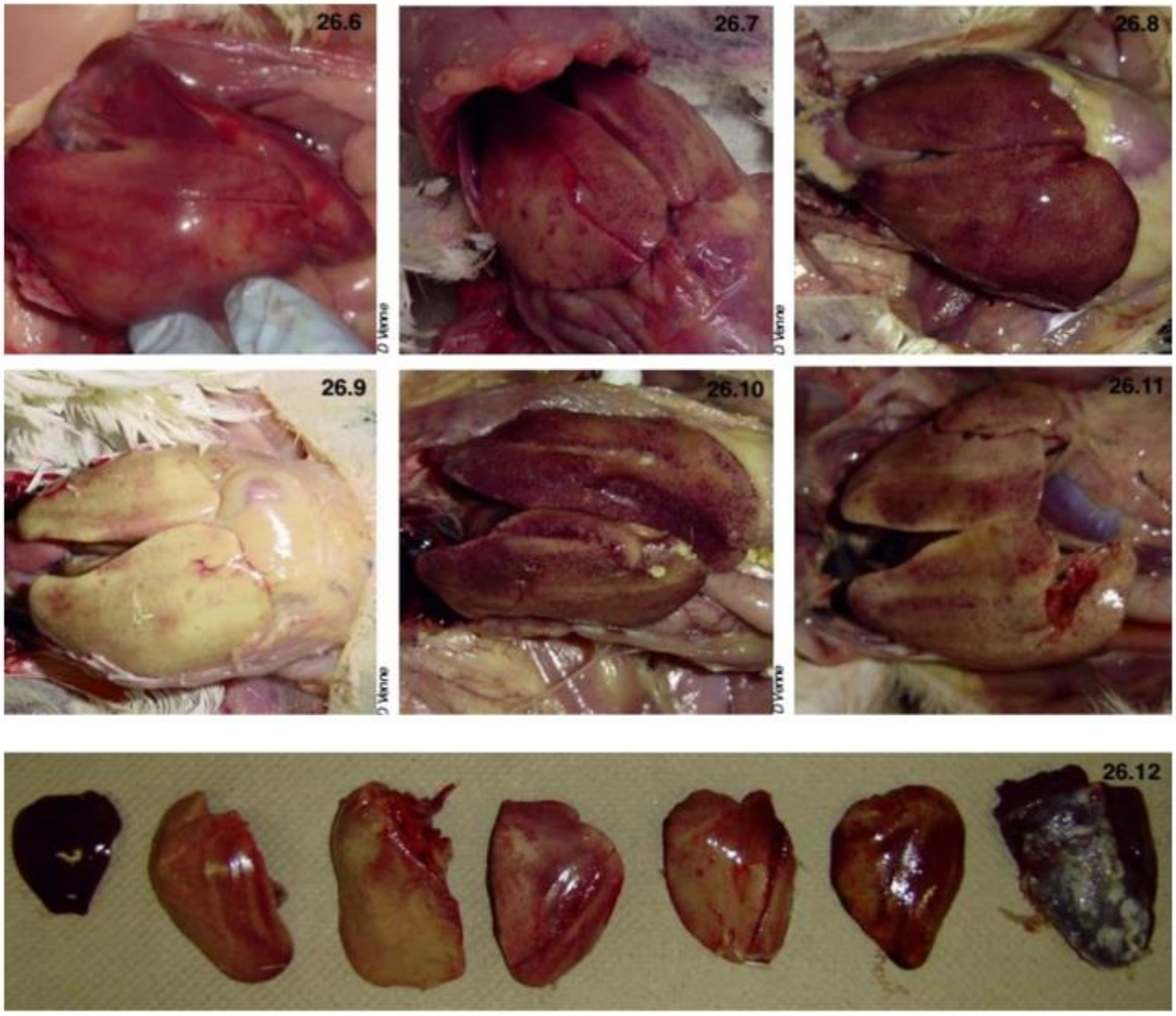
IBH is characterized by a **sudden increase of mortality** that generally peaks within 3 to 4 days and ceases within 9 to 14 days. Mortality normally ranges from **2 to 10%**. However, there have been outbreaks in which mortality has **reached 30%** depending of the pathogenicity of the virus, immune status of the affected birds, and concurrent secondary infections. Clinically, the birds show **lethargy, huddling with ruffled feathers, stooping, inappetence, and yellow and mucoid droppings** may be seen. Overall feed conversion and weight gain are usually depressed.

Post mortem lesions in IBH

Gross lesions in dead birds include an **enlarged, pale, and friable liver** sometimes with **necrotic foci**. **Hemorrhages** are frequently seen in the liver and sometimes in **leg and breast muscles (which usually considered as sign of Gumboro)**. The kidneys are enlarged, pale, and mottled with multiple hemorrhages. In some cases, hydropericardium can be observed. Necrotizing pancreatitis and intranuclear **inclusion bodies have also been reported** in some outbreaks,

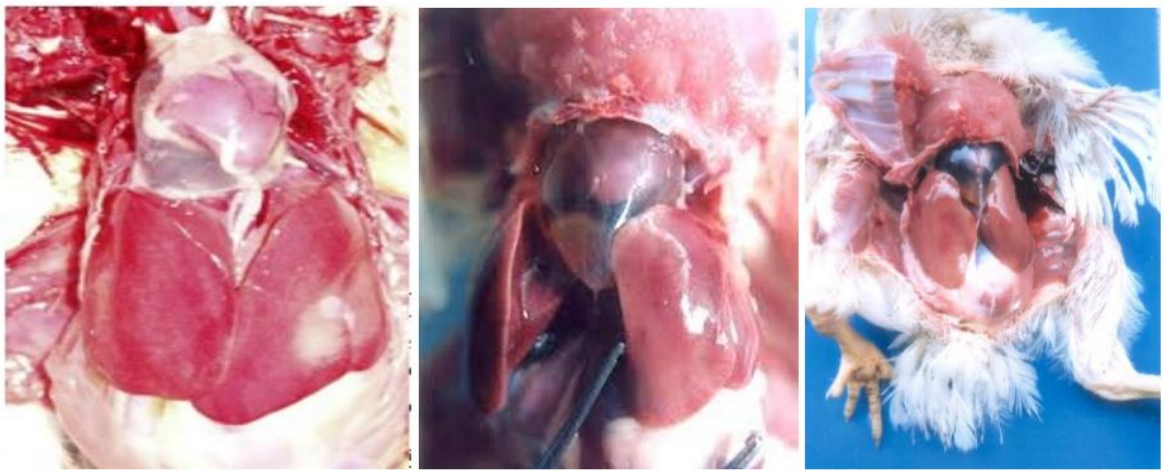


particularly in guinea fowl. In addition, **enlarged spleens and thymus atrophy** can be seen in most dead birds. Anemia, **icterus of the skin and subcutaneous fat, hemorrhages in various organs**, and **bone marrow degeneration** are usually present, but vary in severity. In some cases, **gizzard erosions** can be seen. Microscopically, there are necrotic focal lesions in the gizzard. In the liver, eosinophilic (or basophilic) inclusion bodies are present in the hepatocytes.



Hydropericardium syndrome

This condition was first recognized from the village of Angara near Karachi in Pakistan in 1987 hence named “Angara” disease. The disease is similar to IBH with higher mortality ranging from 20 to 80% in broilers. Hydropericardium syndrome is **characterized by the accumulation of up to 10 ml of fluid in the pericardium**. Aviadenoviruses belonging mostly to serotype 4 have been implicated in the causation of this condition. Hydropericardium syndrome affects mainly broiler chicks **3 to 6 weeks of age** and is caused by FAdV-4. With a course of 7 to 15 days, Hydropericardium syndrome is mainly characterized by rapidly increasing mortality. During the last stages of disease, affected birds exhibit **dullness, depression, ruffled feathers, huddling, ventral recumbency, and closed eyes.**



Hydropericardium syndrome is frequently associated with IBH.

Pathology of pericardial fluid

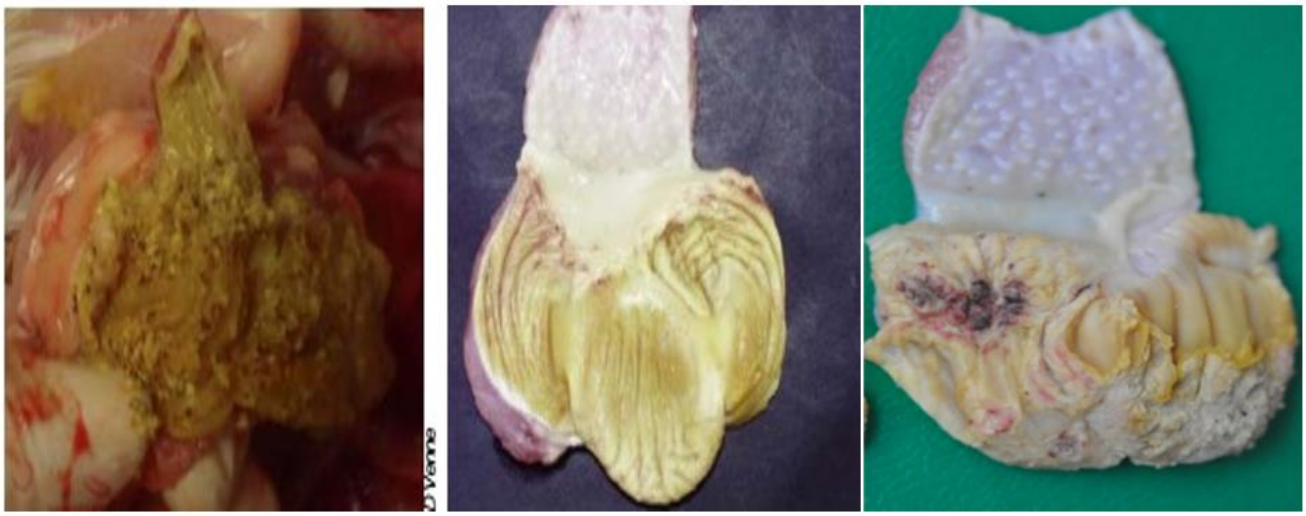
Fluid in the pericardial sac may accumulate due to transudate, inflammatory process in the pericardium, shunting of blood from the ventricles or large vessels into the pericardial cavity. Pathological fluid in the pericardial sac does not cause major hemodynamic disorders until the intra pericardial pressure is normal. This condition is treated mainly pharmacologically. Severe tamponade may result in cardiac arrest due to electromechanical dissociation. Cardiac tamponade may be due to all causes of fluid accumulation in the pericardial sac, but most frequently it results from perforation or rupture of the left ventricle or aorta, and due to severe idiopathic/viral, uremic or neoplastic pericarditis. Treatment of pericarditis and reduction of inflammatory process in pericardium may reduce death rates due to pericardial fluids.

Post mortem lesions in hydropericardium

A build-up of clear or yellowish brown, thin fluid in the pericardium is the major post-mortem finding of Hydropericardium syndrome. Changes observed in other body organs include discolored and enlarged liver with zones of focal necrosis and hemorrhage, edematous and congested lungs, and pale kidneys with enlarged tubules due to urate deposits.

Gizzard erosions

There have been several reports describing outbreaks with gizzard erosions in broilers infected with FAdV-1 and FAdV-8 strains. The striking feature of the disease is that affected birds die with no evident clinical signs. At necropsy, the gizzard shows several black areas, and is filled with blood stained fluid.



IBH. In some cases, gizzard erosions can be seen

Diagnosis

1. In young, growing flocks a sudden increase in mortality is suggestive of IBH. Typical gross lesions and a history of prior outbreaks from the same parental flock(s) or on the premises are helpful.

2. Histopathology – Demonstration of typical microscopic lesions in the liver, including the characteristic intranuclear inclusions, is required for a diagnosis of IBH.

3. Virus isolation – Isolation of FAdV from the liver of affected chickens

4. PCR – detection of FAdV DNA in the liver of affected chickens

5. Serology – Seroconversion to IBH-associated serotypes (FAdV2, FAdV8, FAdV11) can be detected by a micro-neutralization assay. Group antigens can be detected by agar gel immunodiffusion or ELISA, but these tests are of limited value because adenovirus infection is widespread and they do not differentiate among non-pathogenic and pathogenic serotypes/ strains.

6. Genotyping - Analysis of the nucleotide sequences encoding adenovirus hexon protein, the most abundant viral surface protein that contains major antigenic determinants, has been used for genotyping of fowl adenoviruses.

Immunity

Following infection, birds rapidly developed neutralizing (type-specific) antibodies that were detectable after 1 week and reached peak titers after 3 weeks. It has been found that birds were resistant to reinfection with the same serotype 45 days after primary infection, whereas birds were successfully reinfected with the same strain after 8 weeks, eliciting a secondary response of both neutralizing and precipitating antibodies.

Virus excretion also occurred, despite the presence of humoral antibodies. Peaks of virus excretion were found when birds were 2.5, 4.5, and 7.5 months of age, consistent with the theory that local immunity lasts about 8 weeks. Therefore, it is possible that the resistance to challenge found after infection is due to short-lived local immunity while circulating antibodies protect mainly against invasion of the internal organs. The apparent correlation between appearance of circulating antibodies and cessation of virus excretion is more likely due to concurrent development of both local immunity, which is more transient, and humoral immunity, which is more persistent. Support for this hypothesis is provided by the finding that maternal antibody do not protect against natural routes of infection but does protect against intra-abdominal infection.

Importance of immune enhancement theory against adenovirus: With regard to protection, neutralizing antibodies are obviously not solely responsible for protection, determined in a severe challenge model.

Pathology

The main lesions of IBH are pale, friable, swollen livers. Small white foci can be seen on the liver, and petechial or ecchymotic hemorrhages may be present in the liver and skeletal muscles. Inclusion bodies are seen in the hepatocytes. These can be eosinophilic, large, round, or irregularly shaped with a clear pale halo or occasionally basophilic. Virus particles were detected only in cells with basophilic inclusions, and the eosinophilic inclusions were composed of a fibrillar, granular material. Lesions including atrophy of the cloacal bursa and thymus, aplastic bone marrow, and hepatitis were described in natural outbreaks and experimental studies. Glomerulonephritis determined by histomorphometric evaluation was noticed during an outbreak of IBH.

Concept of PHYTOVIR

It has been elucidated in above discussion that adenovirus infection outcome is sudden and severe in which primary immune response overwhelm and inflammatory processes certainly overcome the normal working of vital organ such as liver and heart. Secondary immune response is time taking and short lived but that is part of overall prevention plan against adenovirus infection. Reducing primary immune response and interfering viral replication could be prime target areas which can reduce losses due to sudden mortality.

PHYTOVIR is therefore includes various herbal extracts of different researched herbs that found working against adenovirus in recent researches. It also includes compounds which lower downs primary immune responses and enhance secondary immune responses.

Contents

Herbal antioxidants, phytobiotics, crude extracts of capsicum, garlic and shallots, anti- histaminic & anti-inflammatory compounds, anti-oxidant vitamins and other supportive compounds.

Usage Guideline

PHYTOVIR is helpful as supportive care in IBH, IBD, and CIA.

Use in morning hours in fresh water (this medicated water should be finished within 3 to 4 hours)

Dose:

Prevention:- 1g per 5Kg bodyweight of water after 3rd week of life (after 21st day vaccine) 3 time in a week (on alternate days).

Treatment:- 1g per 3 to 4kg bodyweight as per severity of disease and mortality suspected of IBH.

In Feed:- 750 gm to 1 Kg/ MT of Feed.