

**CIBA EHP Cura^{gro +} – A Phytobiotic
Intervention for the Effective Treatment of
Ecytonucleospora hepatopenaei (EHP) and
white feces syndrome (WFS).**



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CIBA EHP Cura^{gro} + – A Phytobiotic Intervention for the Effective Treatment of *Ecytonucleospora hepatopenaei* (EHP) and white feces syndrome (WFS).

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***Ecytonucleospora hepatopenaei* – prevalence, clinical signs, pathology, diagnostics and management (EHP)**

Ecytonucleospora hepatopenaei (EHP), the shrimp microsporidian causing the disease *Hepatopancreatic microsporidiosis* (HPM). The microsporidian parasite was reported to affect the black tiger shrimp *Penaeus monodon* in Thailand in the year 2009. Since then, EHP is widespread in most of the Southeast Asian countries, including India. It is referred to as hepatopancreatic microsporidiosis (HPM) since the parasite is confined to the shrimp hepatopancreas (HP). EHP infection did not cause mass mortality but was reported to be associated with stunted growth and WFS. EHP inflicts reduced feed consumption, stunted growth, and severe production losses.

Host range, distribution and prevalence

The epizootics and spread of EHP were reported in different shrimp farming nations, including Taiwan, Malaysia, Brunei, Vietnam, Venezuela, Korea, Australia, and India. In India the spread of EHP has been recorded in both east and west coast. Co-infection of EHP has been recorded with other viral diseases such as WSSV, IMNV, TSV, HPV and with bacterial diseases such as AHPND and *Vibrio* spp. This microsporidian reported to infect *Penaeus monodon*, *P. vannamei*, *P. indicus* and *P. merguensis* are known to get affected. Many aquatic invertebrates organisms such as polychaetes, artemia, wild crabs, small aquatic crustaceans, insects, molluscs etc. also act as carriers for cultured shrimp.

Clinical signs

Shrimp infected with EHP do not have any specific signs when they are small. The main clinical signs of EHP at the farm level is slow growth/growth retardation leading to size variation (Fig. 1). EHP infection in shrimp HP affect physiology, metabolism and inturn the shrimp growth. Also, EHP infected shrimp display reduced feed intake, increase food conversion ratio (FCR), empty gut, lethargy and display soft shells. EHP infection did not cause mass mortality but severe infection result in daily mortalities. It is also associated with occurrence of WFS as the infection progress. EHP-WFS affected shrimp exhibit gold brown/white intestine, loose shell, and excreting white fecal strings. Severe infections by EHP can increase the susceptibility to other bacterial infections due to *Vibrio* spp. in shrimp farms and can result in mortality.



Fig. 1 : EHP affected shrimp show size variation and stunted growth

EHP transmission

EHP can infect shrimp at all stages of development and at various salinity levels of many penaeid species. Transmission of the disease mainly occurs by oral route. Shrimp may get infected by consuming feed contaminated with faeces and through cannibalism of infected shrimp or even by consuming spores present in the pond water and sediment. However, the vertical transmission of EHP from female brooders to the offspring is unlikely. Many invertebrates such as polychaetes, artemia, wild crabs, small aquatic crustaceans, insects, bivalves, etc. also act as carriers for cultured shrimp. In hatcheries, polychaete worms are considered as main carriers for shrimp broodstock.

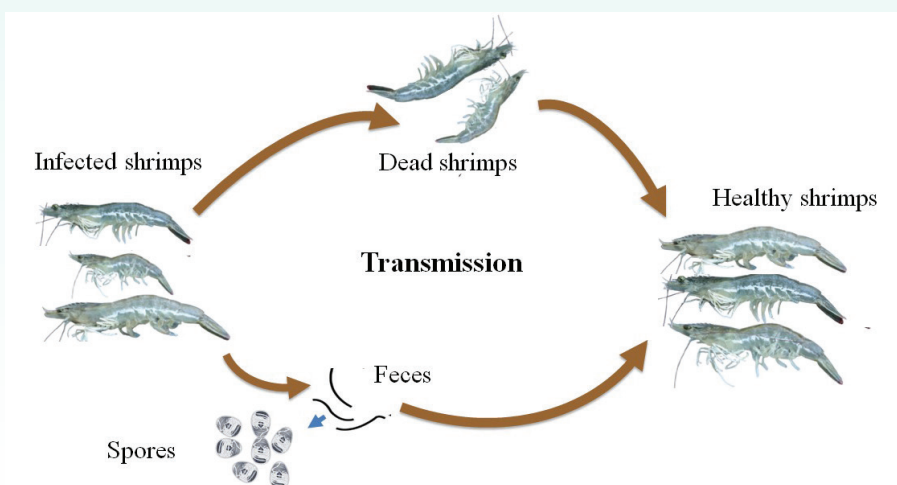


Fig. 2 : Transmission of EHP

Pathology

EHP is an intracellular spore-forming parasite. It replicates within the cytoplasm of the tubular epithelial cells of the hepatopancreas. EHP infection causes severe necrosis and dilation of HP epithelial tubules, increase in the hemal sinus, and sloughing of epithelial cells. Characteristic histopathological features of EHP include the presence of any life stages or free and mature EHP spores in HP cells. Early stages of EHP infection stained with hematoxylin and eosin are observed as eosinophilic to basophilic inclusions and mature spores are refractile.

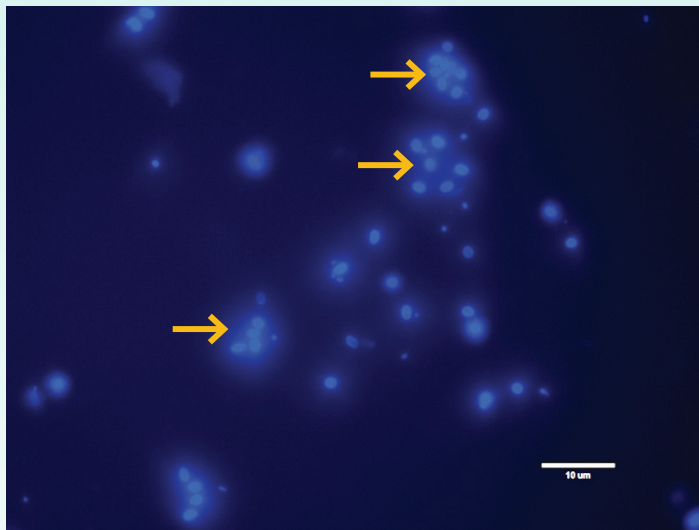


Fig. 3 : Infected hepatopancreas wet mount stained with calcofluor stain shows EHP spores

EHP diagnosis

The EHP can be diagnosed by demonstration of spores in fecal sample and in hepatopancreas by microscopic examination (Fig. 2). Spores can also be demonstrated in the affected animals by different stains such as Giemsa, phloxin, trichrome, calcofluor white, hematoxylin and eosin. Histology of infected tissues reveals several developmental stages, including plasmodium and spore stages. But microscopic demonstration remains successful only in severely affected cases and often undetectable in the early infection. However, many molecular methods such as PCR, qPCR, Loop mediated isothermal amplification (LAMP), are available for routine use for field applications. These techniques are faster, easier and far more accurate to detect the EHP in feces, post-larvae and hepatopancreatic tissue.

Prevention and control of EHP

EHP - When to diagnose?

Brooders should be checked for EHP before stocking in hatcheries. PL should be tested for EHP if any growth retardation, reduced feeding activity, slow moulting, and enlarged hepatopancreas tubules were observed. Shrimp seeds should be checked for EHP before stocking in ponds. Similarly, the grow-out shrimp should be checked for EHP if any growth retardation, white/golden shrimp gut, or floating white faecal threads are observed in shrimp ponds.

Management of EHP in hatcheries

For larval production, only SPF brooders/ EHP negative brooders should be used. The live feeds and other feed inputs should be tested for EHP by PCR. Also, the live feeds should be pasteurized at 70°C for 15 min or frozen at -20°C for at least 48 h before feeding brooders instead of feeding in live conditions. Effective disinfection should be followed to inactivate EHP spores. The hatchery facilities such as tanks, pipelines, and other implements can be disinfected with 2.5% sodium hydroxide for 3 hrs followed by drying for a week.

Management of EHP in grow-out ponds

In shrimp ponds, only EHP negative seeds should be stocked. Once spores are in ponds, it is very difficult to eradicate the disease. Hence farmers should adhere to strict biosecurity protocols and adopt better management practices (BMPs). Pond preparation should be carried out by proper drying and disinfection after every harvest to ensure that the EHP spores along with the carriers are destroyed. It is recommended to use >15 ppm KMnO₄ or >40 ppm chlorine to inactivate spores in the soil. The pond should be left for one week for drying before filling. Pond bottoms should be kept clean; accumulating organic matter can act as a spore reservoir. Poly aluminium chloride can be used to coagulate, flocculate, and sediment the suspended organic material including spores which can then be removed/pumped away. Any new water should be treated before entering the ponds to avoid reinfection. Also, nursery rearing can be implemented in the shrimp culture system. The nursery phase of culture and subsequent PCR testing may be useful in intersecting disease transmission and massive contamination into the grow-out ponds. Strict biosecurity measures and better management practices should be implemented to prevent EHP.

White feces syndrome – pathology, causative agent and management measures (WFS)

White feces syndrome (WFS) has emerged as a serious concern for global shrimp aquaculture in recent years. Affected shrimp excrete white feces and affected ponds were observed with floating white fecal threads. The syndrome has been reported both in cultured *P. vannamei* and *P. monodon*. In India, since 2015, the occurrences of WFS were very severe in *P. vannamei* grow-out farms. The disease can cause moderate to severe economic loss by reducing the shrimp survival by 20–30% compared to the normal ponds.

Clinical signs of WFS

The shrimp affected with WFS can exhibit clinical signs as early as 20-30 days of culture. The WFS affected shrimp exhibit white/golden brown intestine, excrete white fecal threads and show reduced feeding and retarded growth. Ponds affected with WFS shrimp show floating white fecal threads on the surface of the pond for 10 to 45 days or more and will have increased FCR, size variation/growth retardation, loose shells and daily mortalities (Fig. 3). The animal affected with loose shell show loose exoskeleton and sluggish swimming activity at the pond surface.

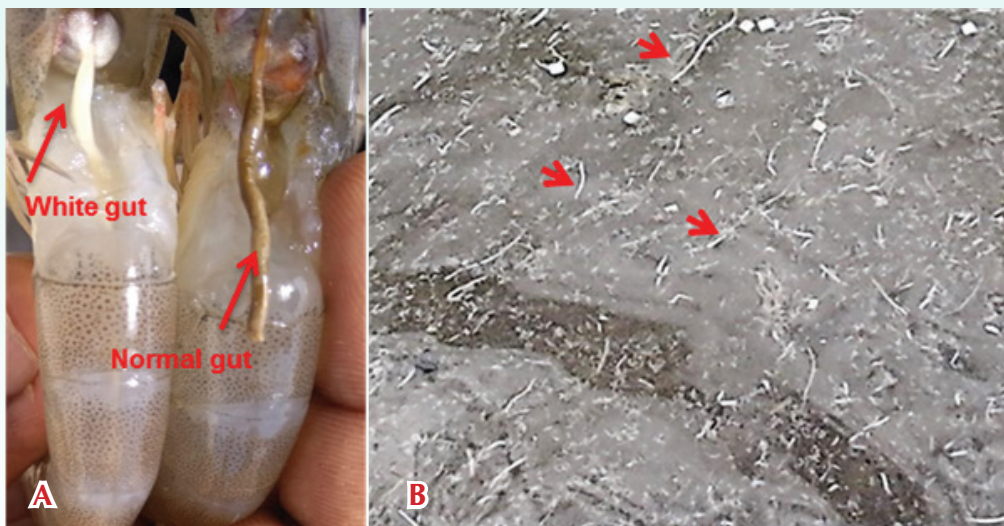


Fig. 4: A- Shrimp with normal gut and WFS shrimp with white gut. B – White fecal threads floating on the WFS affected shrimp pond surface.

Pathology

Severe damage of WFS-affected shrimp hepatopancreas result in vermiform bodies. Vermiform bodies are ATM structures that are formed due to aggregated transformed microvilli (ATM) from HP cells. Severe accumulation of vermiform bodies in affected shrimp result in white feces. Squash, smear preparation, and histology section of WFS affected HP can show the vermiform bodies. EHP is an intracellular parasite that proliferates within the cytoplasm of the affected tubule epithelial cells in the hepatopancreas. Hence, the squash and smear preparations of hepatopancreas at higher magnification (100x) reveal sporoplasms and dense spores. Histology of infected tissues reveals several developmental stages, including plasmodium and spore stages (Fig. 4).

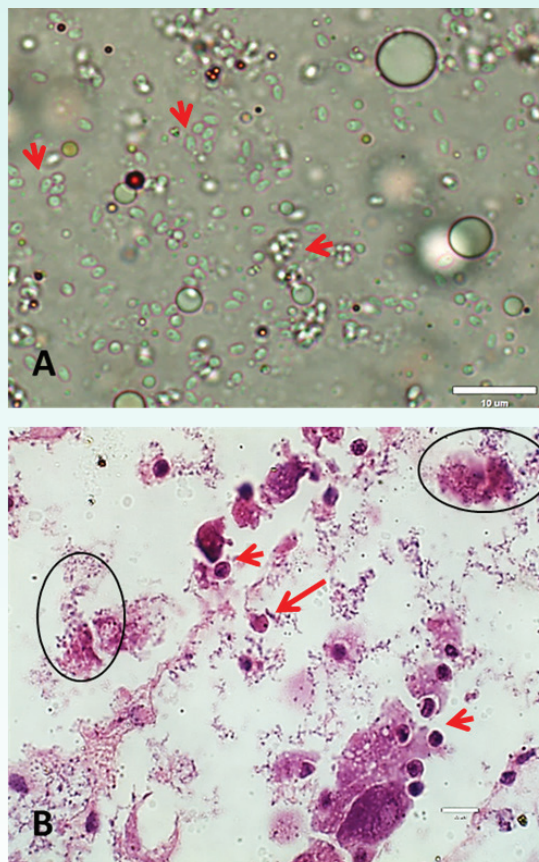


Fig. 5: A- Squash preparation of WFS HP show dense mature EHP spores, B- Histology section of WFS HP stained with H&E show severe necrosis, sloughed off epithelial cell (long arrow), spores (circle), and plasmodial stages (short arrow).

Causative agent

The etiology of WFS is more complex and is reported to be caused by more than one pathogen. So far the incidences of WFS have been associated with gregarine worms, *Vibrios*, EHP, bacteria, fungi, and algae. However, white fecal strings are primarily composed of dense mature EHP spores, and vermiform bodies, sloughed-off epithelial cells, gut mucus, and rod-shaped bacterium observed in white feces. Also, WFS was reproduced under laboratory experimental conditions by challenging EHP infected tissues, EHP and *Vibrio parahaemolyticus* and by specific *Propionigenium* and specific *Vibrio* in combination with EHP. Furthermore, the gut microbiome of EHP-infected shrimp is significantly different from that of healthy shrimp. When EHP infiltrates, the abundance of the hepatopancreatic and gut microbiome decreases significantly. Along with this, there is an increase in disease-causing bacterial groups replacing the beneficial bacterial groups from the gut. This change in gut bacteria is a precursor to the progression of WFS. The clinical manifestation of WFS is more complicated and similar to a clinical condition such as diarrhoea. Thus EHP-WFS is a clinical condition caused by EHP in combination with *Vibrio* sp./other pathogens.

Management

Occurrence of WFS can be reduced by good management practices and proactive biosecurity measures in the farm. Feed can be reduced during the WFS affected period in shrimp ponds. Floating white fecal strings can be frequently removed from the shrimp pond on day to day basis. Neutraceuticals/feed additives which improve HP regeneration and epithelial cell proliferation might help the shrimp to recover from WFS condition in the affected ponds. Overfeeding should be avoided; energy spent in digestion will only weaken the shrimp. Also, a high protein diet can be used during the infection to help the shrimp's digestive capacity and recovery of the HP. Since the role of gregarine is negligible in WFS, anti-gregarine treatment may be avoided. Since EHP is significantly associated with the occurrences of WFS and is considered one of the causes of WFS. Hence the management measures recommended against EHP can be followed in WFS affected farms. In grow-out ponds, EHP-free PL should be stocked in the ponds. Pond preparatory measures should be followed properly by drying and disinfection after every harvest. Potassium permanganate > 15 ppm and Chlorine > 40 ppm can be used to inactivate the spores in the soil. Better management practices should be strictly followed.

CIBA EHP Cura^{gro+} - A Phyto biotic formulation for EHP and WFS treatment

ICAR-CIBA developed a natural therapeutic formulation 'CIBA EHP Cura^{gro+}' for the treatment and control of *Ecytonucleospora hepatopenaei* (EHP). EHP is the causative agent of the disease Hepatopancreatic microsporidiosis (HPM) in shrimp. The microsporidian parasite was reported to affect the black tiger shrimp *Penaeus monodon* in Thailand in the year 2009. Since then, EHP is widespread in most of the Southeast Asian countries, including India. EHP cause severe morbidity, stunted growth and white feces syndrome (WFS) and results in severe production and economic losses in shrimp farming.



CIBA EHP Cura^{gro+}

Product composition and Mode of action

CIBA EHP Cura^{gro+} is a unique formulation of bioactive phytochemical, hepatoprotective nutraceutical, and nutritional supplements exhibiting anti-

microsporidian, anti-microbial, immune modulating and anti-inflammatory properties. The formulation targets the EHP pathogen and support shrimp by:

Inhibits spore germination: The phytochemical component in CIBA EHP Cura ^{gro +} can penetrate the spore wall and can directly inhibit the spore germination and prevent the EHP form further proliferation within the shrimp hepatopancreas.

Hepatoprotective effects: The nutraceutical component and nutritional supplements acts as anti-oxidants, supports the cellular integrity, reduces the oxidative stress and inflammation and protect the hepatopancreatic cells affected by EHP.

Boosting immune defence: The components in CIBA EHP Cura ^{gro +} all can stimulate the phagocytosis, immune response and can resist the EHP infection.

Improving digestive health: By reducing the pathogenic load and exhibiting the hepatoprotective function, CIBA EHP Cura ^{gro +} facilitates the digestion, nutrient absorption, enhancing better growth, survival and feed conversion ratios.

Control white feces syndrome: The formulation also exhibit anti-bacterial activity. By reducing the both EHP and vibrio load, this product can control the manifestation of white feces syndrome.

Benefits of CIBA EHP cura I:

Reduction in EHP infection: By targeting the EHP pathogen directly, the product protects the shrimp population from EHP infection spread and severity.

Enhanced growth and survival: By reducing the pathogen load, improving the digestion, supporting the metabolic function, increasing the immune response and ultimately enhancing the growth and survival.

Sustainable and Eco-friendly: As a natural formulation, CIBA EHP Cura ^{gro +} is very safe to the shrimp and environment and can be an apt alternative measure to antibiotic usage in targeting the sustainable aquaculture practices.

Improved Farm profitability: By improving the health, growth and reduces the disease severity, CIBA EHP Cura ^{gro +} increases survival rate, better feed conversion and ultimately the profitability of shrimp culture.

Application and dosage:

Dose: Administer 50ml per kg of feed for 1-2 weeks

Mode of application: By top coating in the feed

Feeding Schedule: The product can be given twice two meals per day preferably in the morning and late evening. If the infection is severe, administer in all the four meals. The product can be used as a prophylactic measure as one meal per day.



Field Evaluation:

The product has been extensively field validated in the shrimp farms of Tamil Nadu, Andhra Pradesh, Gujarat, West Bengal and Punjab.



Field Evaluation of CIBA EHP Cura^{gro+} in 5 States of India



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