Surveillance of Waterfowl in Nalabana Bird Sanctuary, Chilika Lake, Odisha

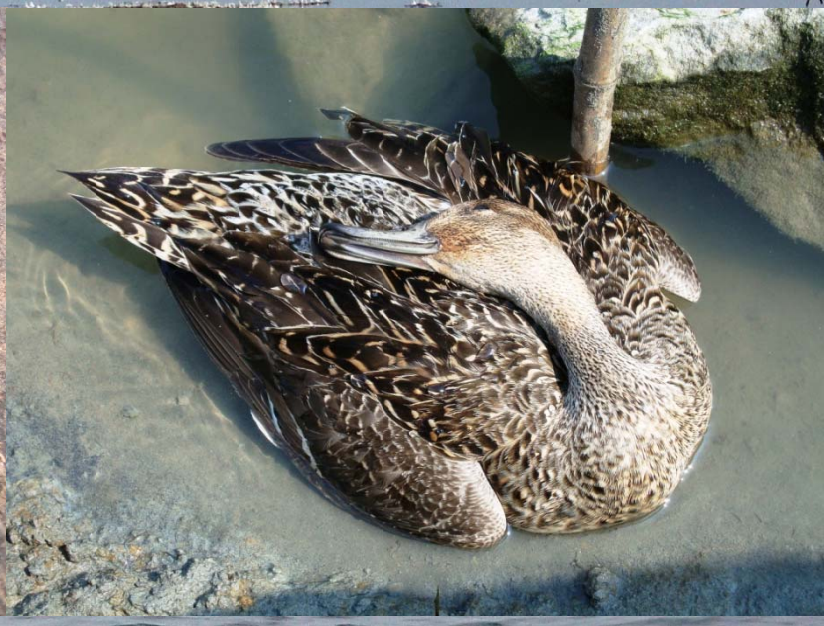
Final Technical Report

Submitted to

Ministry of Environment, Forest & Climate Change, Govt. of India

Principal Investigator : Dr S Muralidharan

Co-investigators : Dr B Murali Manohar
Dr Chandra Sekar Kar



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Front cover : A view of Nalabana Bird Sanctuary, Insert: Tufted Duck: Unable to fly or swim and Northern Pintail with flaccid paralysis of head and neck
Back cover : Sunset at Chilika and Greater Flamingos
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Part II - Detailed Project Report

Part III

Executive summary

During 2006 winter, thousands of migratory birds died in Nalabana Bird Sanctuary, Chilika Lake, Odisha. Species, namely Northern Pintail, Northern Shoveler, Brown-headed Gull and Garganey Teal were notable among them. Pilot study was initiated to address the mass mortality in the Sanctuary. Preliminary investigations to find out the role of pesticides did not reveal any positive leads. Tests for New Castle Disease Virus (NDV) and a set of aflatoxins also turned out to be negative. Under these circumstances, since the mortality of birds continued, it was essential and imperative to look at the possible role of certain other diseases, apart from contaminants. Hence, a study was initiated in 2010.

Bird Survey: Direct count method was followed to estimate the birds in the study area. During the winter (Dec '09 - Mar '10), about 118 species of birds were observed; off which 83 were waterfowl while 35 were wetland dependant birds. Population was the maximum during February (2,80,870) and minimum during March (1,14,260). Among the birds, Gadwall (68,000) was found to be in maximum numbers followed by Northern Shoveler (60,000) and Northern Pintail (50,000). Between December 2009 and March 2010, overall mortality of waterfowl in Nalabana Bird Sanctuary was estimated at 1200 birds involving Northern Pintail, Northern Shoveler, Common Pochard, Eurasian Wigeon, Ruddy Shelduck, Gadwall, Tufted Duck and Garganey. Samples of dead birds were collected and after postmortem, tissues were preserved for various chemical and pathological investigations.

Pathological investigations: In the field, the sick birds showed clinical signs, such as inability to fly, swim, or walk, mucus discharge from the beak, flaccid paralysis of head and neck, and greenish diarrhea. During necropsy, while many birds revealed general hyperemia, a few showed lesions in liver with multi focal areas of necrosis. Heart blood smears and organs with lesions were collected and stored for microbiological investigations. Organs with lesions and part of brain were fixed in 10% formalin and stored at room temperature for histopathological studies. Based on the clinical signs and necropsy findings four diseases, namely Avian Vacuolar Myelinopathy, Botulism, Duck Viral Hepatitis and Fowl Cholera were suspected. Elimination approach was adopted to identify the disease.

Investigations on formalin-fixed brain tissues revealed no lesion in the white matter which indicated that Avian Vacuolar Myelinopathy may not be the cause for mass mortality.

The samples collected for microbiological investigations were inoculated into different media and incubated at 37°C both aerobically and anaerobically. Anaerobically maintained media revealed no growth of organisms which confirmed that botulism causing organisms were absent in the samples.

Further, to check for the presence of botulinum toxin in suspected tissues, the tissue homogenates were injected into mice through Intraperitoneal (IP) and Intravenous (IV) routes and observed for four days. No death of mice was noticed which confirmed the absence of toxin in tissue samples, and thereby proved that botulism was not the cause for mass mortality. Duck Viral Hepatitis suspected tissues were homogenized and injected into 8-10 day-old-chick embryonated eggs and observed upto eight days. No death of embryo was observed even after eight days. Thus it was clear that Duck Viral Hepatitis was not responsible for mass mortality of birds.

Aerobically maintained media revealed growth of organisms, and based on the colony morphology, staining characters, physical and biochemical properties, the organisms were identified. Investigations revealed non-hemolytic, non-motile and gram negative dew drop colonies of coccobacilli, namely *Pasteurella multocida*. This organism was indole, oxidase and catalase positive, whereas urease and citrate negative. Heart blood smears on examination under microscope, revealed presence of bipolar organisms and the liver sections showed multifocal areas of necrosis in liver parenchyma, which were highly suggestive of Fowl Cholera.

To assess the virulence of *Pasteurella multocida*, the isolated organisms were injected into mice. The (incubation) test confirmed that the isolated *Pasteurella multocida* was virulent. Although pathological study was suggestive of fowl chorea, to confirm the identity of *Pasteurella multocida*, Polymerase Chain Reaction (PCR), a molecular biology technique was carried out. PCR confirmed the presence of *Pasteurella multocida* by producing a molecular band at 460 bp. The gross and histopathological lesions observed in the present study were similar to the lesions observed during acute forms of fowl cholera in a few species of wild birds and poultry in many countries.

Based on the facts presented above, it was confirmed that the heavy mortality of ducks in Nalabana Bird Sanctuary was due to the outbreak of Fowl Cholera or Avian *Pasteurellosis* or Avian Hemorrhagic Septicemia caused by *Pasteurella multocida*. Although Fowl Cholera is predominantly a disease of poultry, it is a serious disease in waterfowl as well. *Pasteurella multocida* is a heterogeneous species with highly variable pathogenicity. Susceptibility to these bacterial strains varies considerably among avian species. Epizootics of avian cholera typically occur in wetlands with abundant waterfowl populations or at breeding colonies which have high densities of birds. Mortality often involves multiple species of birds. The bacterium has a worldwide distribution and produces septicemic and respiratory disease in a wide variety of domestic and wild birds. While there are treatment protocols available, it may not be possible in wild condition. However, if birds could be picked up, they could be isolated and treated. Carcass infected with *Pasteurella multocida* can spread the disease to other birds and also to adjacent areas. Hence, carcass collection and disposal by way of incineration is the best option. Control strategy for wetlands with epizootics would warrant regular surveillance within wetland areas where migratory birds are concentrated so as to take appropriate actions. Further, in the present context of increasing incidences of avian influenza, recognition of avian *pasteurellosis*, is of great importance for differential diagnosis with avian influenza. Further, normal avian health information sharing platform is also advised to be created. This could serve as a repository for sharing necessary information on mortality and disease incidences in birds in the country.

Pesticide contamination: Seven species of birds found dead were analyzed for pesticide residues in various tissues. All the samples were analysed for pesticides, namely alpha-hexachlorocyclohexane (α-HCH), β–HCH, δ-HCH, lindane, heptachlor epoxide (HE), dieldrin, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, α-endosulfan, β-endosulfan and endosulfan sulfate.

All the species of birds studied had detectable levels of residues of organochlorine pesticides (OCPs). Pattern of OCP concentration among various species of birds followed the order HCH > DDT > cyclodiene insecticides. The concentration of total organochlorine pesticide residues ranged between below detectable level (BDL) and 1561 ng/g. Among all the species, Gadwall

had the highest concentration of total OCPs (1165.1ng/g) followed by Eurasian Wigeon (886.9 ng/g), and Gargany had the lowest. It can be noted that the concentration of ΣHCH was much higher than other organochlorine residues. Residues of cyclodiene insecticide were comparatively low.

Among the isomers of HCH, β-HCH contributed the maximum to the total HCH and among the metabolites of DDT, *p,p'*-DDE contributed the maximum to the total DDT. Endosulfan sulfate contributed the maximum to the total endosulfan. Organochlorine pesticide residues detected in the present study are lower than most of the studies carried out in India and also elsewhere. Levels in none of the birds did indicate any pesticide poisoning.

Although, it was confirmed that the mortality of waterfowl was due to Fowl Cholera caused by *Pasteurella multocida,* organochlorine pesticide residues were estimated mainly to understand the situation after ban or restriction was imposed on many of the organochlorines. It is adequately clear from the findings of the present study that the levels of all the persistent pesticides are not high enough to press panic button, but indeed cause for concern. Of all the organochlorines, we ought to continue to monitor DDT, as we still use it for controlling malaria and *kala azar* in select pockets in India.

Heavy metal contamination: Even though we confirmed that mortality of waterfowl in Nalabana Bird Sanctuary was due to Fowl Cholera, we measured the levels of select heavy metals in dead birds as phase II of the project only to know the background level of contamination for future reference. Heavy metals, namely Cu, Cr, Cd, and Pb were estimated in the tissue of seven species of waterfowl. Data were compiled by the species, organ and sex of the birds. Of all the tissues, liver had significantly higher concentration of all the metals and muscles the least. Northern Pintail (0.82 μg/g) had highest load of all metals, while Common Pochard (0.08 μg/g) measured the least. Between sexes, females accumulated all the metals higher than males except Pb. Concentration of none of the metals studied was high enough to have killed any bird. In this present study we documented metal contamination only in birds which died because of disease. Although the present levels of contamination is not alarming, it is of concern as even low levels of exposure, if continuous in otherwise healthy birds, can pose serious problems in long-run. In order to evaluate any instance of metal poisoning in birds, "normal" levels of them for the species involved must be known and the abnormal levels which tend to produce either harm or no apparent harmful effects must also be recognized. Hence, organized surveillance and monitoring is recommended.