



Rajasthan University of Veterinary and Animal Sciences, Bikaner

Directorate of Human Resource Development

# *E-Compendium*

Online Training for Veterinary Officers  
on

*Management Practices for Combating Avian Health  
Emergencies in Wetland Ecosystem in Rajasthan*

5<sup>th</sup> to 9<sup>th</sup> January, 2021



**Organized by**

**Centre for Studies on Wildlife Management and Health  
Rajasthan University of Veterinary and Animal Sciences,  
Bikaner-334001**



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**Edited by**

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**Centre for Studies on Wildlife Management and Health**  
**Rajasthan University of Veterinary and Animal Sciences,**  
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## **STRATEGIC PREPAREDNESS AND MANAGEMENT PRACTICES FOR AVIAN HEALTH EMERGENCIES**

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### **Introduction**

Avian health emergencies are serious conditions that affect, the avifauna and not just an individual bird. Avian health emergencies may manifest in one of several categories, such as

- Sudden increase in mortality rates.
- Poor avian traffic flow.
- Financial loss.
- Environmental-related issues.
- As a threats to biodiversity.
- True or perceived outbreaks of infectious disease.

Avian disease emergencies are of significant environmental and biodiversity importance when it related to wetland ecosystem. Since these diseases can spread very rapidly, their control requires advance planning, otherwise they become widespread and their eradication can be extremely difficult, costly and hazardous to environment and biodiversity. Animal disease emergency preparedness and particularly contingency planning should be regarded as an essential tool for the control of emergency diseases.

### **Transboundary Animal Diseases (TADs)**

Transboundary Animal Diseases (TADs) may be defined as those epidemic diseases which are highly contagious or transmissible and have the potential for very rapid spread, irrespective of national borders, causing serious socio-economic and possibly public health consequences.

The control and eradication of animal diseases are primarily the responsibility of individual countries and due to the transboundary nature of many important animal diseases, regional and global cooperation and some international organizations dealing with control of these such as -

- The Office International des Epizooties (OIE),
- Food and Agriculture Organization of the United Nations (FAO)
- World Health Organization (WHO)
- Pan American Health Organization (PAHO) and
- Inter-African Bureau for Animal Resources of the African Union (AU/IBAR)

## Importance of Migratory Birds

- Migratory birds serve key functions in the interconnected systems that keep nature healthy, including pollination and seed dispersal of crops for human and livestock consumption, pest regulation and as an aesthetic source of pride for cultures across the globe.
- Birds generally reflect the status and trends affecting wider biodiversity.
- They are important for monitoring changes in the environment, since they are an integral part of almost all ecosystems in the world.
- Throughout the year, migratory birds cross countries and continents, some of them from the tundra to the tropics, covering hundreds or even thousands of miles linking different ecosystems.
- By conserving these birds and their environment, we ensure the conservation of biodiversity on a wider scale.

## Strategic Preparedness in Emergency

Strategic preparedness in emergency is a continuous process in which action, funding, partnerships and political commitment at all levels must be sustained. It relies on all stakeholders working together effectively to plan, invest in and implement priority actions.

## The Need for Health Emergency Preparedness Planning

- As mentioned earlier, an outbreak of a transboundary animal disease can have serious socio-economic, ecosystem disturbance consequences.
- Therefore, it is crucial to recognise a new disease quickly while it is still localized and take necessary measures promptly to contain the disease and then progressively eliminate it.
- The aim of an emergency planning should always be to eliminate a disease progressively and finally eradicate it.

## Components of Avian Health Emergency Preparedness Planning

There are two component of preparedness planning

- 1 Early Warning of Diseases
- 2 Early Reaction To Disease Outbreaks

### Early Warning of Diseases

- Early warning is the rapid detection of the introduction or sudden increase in, any disease of livestock, which has the potential of developing to epidemic proportions and causing serious socio-economic consequences or public health concerns.

The basis of early warning of diseases are

- 1) Disease Surveillance
- 2) Disease Reporting and

### 3) Epidemiological Analysis

#### **Importance of early warnings of diseases**

- These improved awareness and knowledge of the distribution and behaviour of disease outbreaks and infection.
- Allow forecasting of the source and evolution of the disease outbreaks and the monitoring of the effectiveness of disease control campaigns.

#### **The success is depends on..**

- Public awareness programmes for animals and birds diseases.
- Training of field veterinarians and veterinary auxiliary staff on recognition of important epidemic diseases.
- Sustained active disease surveillance.
- Efficient emergency disease reporting mechanisms.
- Implementation of an emergency disease information system.
- Availability of laboratory diagnostic capacity.
- Presence of links between national and international reference laboratories.
- Prompt and comprehensive international disease reporting to the OIE and neighbouring countries.

#### **Early Reaction to Disease Outbreaks**

Early reaction is to carry out without delay the disease control activities needed to contain the outbreak and then to eliminate the disease and infection in the shortest possible time-frame.

To achieve this, the following elements need to be in place-

- Installation of diagnostic capabilities for all high threat diseases. These should be fully developed and tested in national and regional reference laboratories.
- Ensured arrangements for involvement of the private sector.
- Arrangement for epidemic livestock diseases to be included in national disaster plans so that the police, army and other services can be involved as and when necessary.
- Preparation of legislative and administrative frameworks to permit all necessary disease control actions to be implemented without delay.
- Arrangements whereby funding for disease control campaigns can be rapidly provided.
- Ensuring that Veterinary Services are structured in such a way as to facilitate disease reporting and implementation of a nationally coordinated disease control/eradication campaign without delay during an emergency.

#### **Risk Analysis as a Component of Animal Disease Emergency Preparedness Planning**

- Risk analysis involves examining how project outcomes and objectives might change due to the impact of the risk event. Once the risks are identified, they are analysed to

identify the qualitative and quantitative impact of the risk on the project so that appropriate steps can be taken to mitigate them.

- Risk analysis is a tool that can also be used to good advantage for animal disease emergency preparedness planning. In this context, it is most readily applied to preparedness planning for exotic diseases

### **Component of risk analysis**

- 1) Risk assessment
- 2) Risk management
- 3) Risk communication

Risk assessment - In this component the risks of an event occurring or of taking a particular course of action are first identified and described. The likelihood of these risks occurring is then estimated, their potential consequences evaluated and the assessment of the risk modified accordingly.

Risk management - This is the process of identifying, documenting and implementing measures to reduce risks and their consequences. Risks can never be completely eliminated. The aim is to adopt procedures to reduce the level of risk to an acceptable level.

Risk communication - This is the process of exchange of information and opinions on risk between risk analysts and stakeholders. Stakeholders in this context include all those who could be affected by the consequences of risks

### **The Value of Risk analysis for Animal Disease Emergency Preparedness Planning**

- Determining those emergency diseases for which there is the greatest need and urgency to prepare specific contingency plans.
- Determining where and how quarantine procedures and border controls need to be strengthened.
- Determining how laboratory diagnostic capabilities need to be strengthened.
- Planning training courses for veterinary staff and publicity campaigns;
- Determining needs for vaccine banks or preparedness;
- Determining how and where active disease surveillance needs to be strengthened.

### **Contingency Plans**

A contingency plan for a specific disease should include a disease strategy, which is the authoritative reference to the control/eradication policies for that emergency animal disease in that particular country. It should provide information about

- The nature of the disease
- The principles of its control
- Control policies



Each strategy should provide sufficient information to allow authorities to make informed decisions on what policies and procedures should be used to control an outbreak of that disease in that country

## **Component of the Contingency Plan**

### **1. Legal powers**

The most important component of animal disease emergency preparedness is the availability of legal powers to carry out all necessary disease control actions. This legislation may include regulations on:

- Compulsory notification of animal diseases.
- Obligation of animal owners to cooperate in control of infectious animal diseases.
- Establishment of restrictions (quarantine, surveillance zone, control of movements).
- Destruction of infected or suspected products and materials.
- Compensation for damage caused by the disease and Sanitary measures.

### **2 Financial provisions**

- Emergency disease control is a costly operation and therefore financial planning is an essential part of any contingency plan.
- Without available funds, rapid response to emergency disease outbreaks may be delayed.
- The availability of special funds, which are accessible during emergencies, will help to save major expenditure.
- Financial plans thus need to be developed that provide for the immediate provision of contingency funds to respond to disease emergencies.

### **3 Chain of command**

- The success of any contingency plan for emergency disease control depends on the organized management of all operations. In order to achieve this, the chain of command from the Chief Veterinary Officer (CVO) to field staff should be clearly mentioned in the contingency plan.

### **4 National Disease Control Centre (NDCC)**

- The structures, responsibilities and powers of NDCC and local disease control centres and the list of equipment that should be available within these centres should be listed clearly. The NDCC is responsible for
  - Maintaining disease preparedness and awareness.
  - Direction of local disease control centres
  - Liaison with diagnostic laboratories
  - Liaison with agricultural and trading bodies, and the media
  - Arranging financial provisions for the contingency plans
  - Arranging training programmes, and arranging disease awareness campaigns

- Directing the national strategy in the event of an outbreak of disease
- Deployment of staff and other resources to local disease control centres and liaison with other employers for the release of staff
- Determination of protection and surveillance zones
- Provision of information to and liaison with the media and national agricultural and trading bodies

## **5 Control at local level**

- The responsibilities of the local centres include
- Maintaining disease awareness and preparedness within its territory
- Directing and implementing the local control strategy in the event of a disease outbreak.

## **6 Expert groups**

- In the event of an outbreak report, the group(s) will be alerted by the NDCC and mobilised in the field as soon as the disease is confirmed. The primary task of the expert group is to provide the national and local disease control centres with a report. The team will also advise on sanitation and carcass disposal.

## **7 Required resources**

- Contingency plans prepared for animal disease emergency preparedness should include detailed information on personnel, equipment and other physical resources.
- A list of all personnel, who are likely to be involved in emergency disease control, should be maintained.
- The qualifications and expertise of these personnel should be recorded and regularly updated.
- Arrangements should be made for the temporary employment of veterinarians and other staff.

## **8 Diagnostic laboratories**

- Information on the diagnostic laboratories, such as trained laboratory staff, laboratory equipment, diagnostic reagents and the capability of the laboratories is essential.
- It is worth mentioning that for the overall success of any contingency plan the time period between the introduction of the disease and its detection at the laboratory is crucial.

## **9 Emergency vaccination**

- Vaccination may be used as an option for the control of emergency animal diseases. If so, arrangements should be made for the emergency vaccine stocks, vaccination teams, equipment for vaccination and equipment to maintain cold chain.
- If there is no vaccine production in the country, special agreements should be made with vaccine producers to obtain the vaccine.

## 10 Training Programmes

- The personnel to be involved in an emergency situation should be trained in their roles, duties and responsibilities.
- The key personnel should be given more intensive training. Back-up staff should also be trained for each position.

## 11 Disease awareness

- The success of even the best contingency plan depends on the support and cooperation of farmers, public and the media. Therefore, these interest groups should be very well informed on the consequences of specific animal diseases.

## Management Aspect during Avian Health Emergencies

### General Principles

- A list of all local centres capable of managing outbreak diseases should be compiled
- The local control centre must be able to contact quickly those people and organisations which must be directly or indirectly involved if an outbreak occurs
- Suitable equipment and the materials necessary to conduct the proper procedures for disease eradication must be readily available at all times
- Detailed instructions on the measures to be implemented in case of suspected or confirmed outbreak of disease, including instructions on the disposal of carcasses, should be provided
- Training programmes to develop and maintain the necessary professional skills should be conducted
- Diagnostic laboratories must be provided with the necessary equipment for post-mortem examinations, serological and histology tests, etc. Personnel should have their skills continuously updated to provide efficient diagnosis. A rapid means of transporting samples must also be provided
- Susceptible animals are slaughtered
- Contaminated foodstuffs and materials are destroyed
- Protection and surveillance areas are created
- Proper animal health control measures to avoid the spread of infection are implemented

### Management Procedure

#### 1) Collecting an Accurate History

- The mechanics of collecting a thorough medical history and performing a good physical examination of a flock are probably the two most challenging but very important components to understand.
- An accurate history should include questions that pertain to the overall homeostasis of the flock and the sick or dead bird if present.

## 2) If affected area is aviary then close it

- A conceptually closed aviary is one that has effective control over human, animal, and fomite traffic flow into and within an aviary. In a closed aviary, the birds must earn their way into an aviary by passing a predetermined set of criteria. Movements of people and potential fomites are similarly controlled.

## 3) Physical Evaluation

- Assuming a sick bird represent the whole flock and it is essential to evaluation of both patients, the individual bird and the location. Use those findings plus their relevant histories to help set a plan. There are some basic components to the site examination.

### I. Aviary or site Map

### II. Traffic Flow Map

#### Traffic Flow Map

- Ideally, traffic flow maps should reveal concise, orderly movements throughout the aviary or location.
- In emergency situations, evaluating traffic flow is key in allowing all parties to understand how potential pathogens and noninfectious disease processes are being or have been introduced into and within the area.
- In reality, most epidemic proportion losses to infectious disease outbreaks in aviaries are usually the result of diseased traffic flow.
- A common situation involves placing susceptible birds, such as weaning babies, in or near areas of high traffic flow. An infectious pathogen introduced into an environment with poorly controlled traffic flow almost predictably results in a clinically diseased aviary.

#### Primary Versus Secondary Avian Disease

- Pathogens create disease based on their interaction with host and environmental factors and not necessarily by themselves.
- As a result, testing and treating for disease-associated agents may well be incomplete or inappropriate flock management in some settings.
- Primary avian diseases, when present, are responsible for the bulk of flock morbidity or mortality.
- When multiple diseases are involved in an avian emergency, the organisms involved are best viewed as secondary to an unhealthy affected area with other underlying problems.
- Poor traffic flow, nutrition, aviary design, extreme environmental conditions are underlying disease issues that can weaken the aviary's health. Even organisms that would be considered primary invaders in a healthy aviary can be one of many secondary pathogens in an overall improperly managed or unhealthy aviary.

- Distinguishing between primary and secondary diseases becomes important in managing the disease. Primary disease processes may actually be easier to control because usually one deals with only the causative organism or process.
- Secondary diseases point to underlying aviary imbalances and require that the present pathogens or disease processes, as well as the aviary's overall health and homeostasis, must be managed.

### **Treating the Aviary or affected area.**

- Until such time that the contagious and the infectious character or population effect of the flock emergency is understood, containment policies are key to protection of the flock at risk.
- Moving birds within the aviary should be very specific and planned carefully so as not to further disseminate the disease process or expose at-risk birds.
- Updated aviary maps, with the disease and its locations depicted, may reveal where the problems lie.
- Traffic flow maps may help define potential routes of the pathogen, toxin, and so forth as well as the transmission and the dissemination. These two maps are used together to set up functional roadblocks. Isolate areas with affected individuals and establish an orderly traffic flow in a manner that does not allow additional subpopulations of birds within the aviary to be placed at risk.

### **Treatment of injured birds**

- Veterinarians and qualified veterinary para-professionals require supplies of the basic medicines and equipment to allow them to do their work. Care must be taken to ensure that the correct medicines are used for each species as some medicines can be dangerous to certain species

### **Provision of Feed and Water in Emergency Situations**

- During emergencies, the type of supplementary feeding required varies depending on the type of animal involved and the nature of the emergency.
- The types of nutrients and the quantities required by birds depend on factors such as species, size, age and physiological status of the bird.
- Protein and energy content of feed are the most important components regarding avian health.
- Determining an appropriate and cost-effective combination of the different types of available feeds for the different classes of affected animal is a key aspect in designing an effective supplementary feeding programme. As in feed supplementation, water provision programmes can involve either by moving animals to where water is available or by increasing water availability.



## **Disposal of Carcasses**

- Carcasses be moved away from water sources or human settlements immediately and protected from scavengers
- Keeping in mind the common method of carcass disposal in the region
- Proposed disposal method must be permitted by the authorities and accepted by the community
- Means of transport should be available to roll out disposal in reasonable time
- Monitoring in place to assure all carcasses are removed, including if a new wave of mortality occurs during the intervention.
- The removal site should be properly guarded and the process of disposal should be supervised.

## **OVERVIEW OF VETERINARY SUPPORT PROVIDED DURING BIRDS/ANIMAL RELATED EMERGENCIES**

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### **Introduction**

Emergency is a serious event that needs immediate action, it is an unforeseen combination of circumstances that calls for immediate action. If the emergency is deemed critical, it is the most immediate level, meaning that it's a dire situation, such as the animal or bird is not breathing. A second priority level might include trauma, such as a broken bone or vomiting while a stable patient will be one who needs to be treated but is not in any danger. A veterinary critical care unit is one that treats critical, life-threatening emergencies. These units usually have specialized technologies and equipments, much like a human ICU, where the veterinarian can closely monitor the patient. Sometimes, too, a bird or an animal is moved to ICU when it is facing an illness that is highly contagious, and it must be quarantined for everyone's safety.

Most of the animals or birds will experience a veterinary emergency in their lifetime. These range from a sudden illness, to an injury, to more critical situations like poisoning or an animal attack. People may wonder what to do, and how they can help until getting it to a hospital.

Emergency patients present special challenges because their underlying disease processes can cause immediate life-threatening problems that require rapid and aggressive intervention. In addition, the full extent of the animal's illness, injuries, or toxicity may not be evident for some time after initial presentation. Problems can arise from an acute illness, toxicity, or injury; from a chronic illness that has decompensated or from an unexpected complication of a concurrent illness. The status of all postoperative patients should be considered critical until life-threatening anesthetic or surgical complications are excluded. The golden rule of emergency medicine is to treat the most life-threatening problems first. When a patient is stable, it should be closely monitored for progression and possible complications. Therapy must be done at the right time, in the right amount, and in the right order. Therapeutic failures are generally a result of failing to act expeditiously at a crucial moment.

### **First Aid**

Emergency care often begins with the owner's initial telephone call. Instructing the owner on first aid and transport procedures can be life-saving for the animal. Owners can provide significant medical assistance at the scene of the injury. The clinic and staff must be in readiness, especially if more than one animal in critical condition has been reported at the same time. The primary survey, or triage, requires a quick and accurate assessment and decision regarding the stability of the animal.

At the time of the initial telephone call, the owner should be questioned about the level of consciousness, breathing pattern, type of injury or toxicity, and even some aspects of the animal's perfusion (i.e. gum color, level of responsiveness, heart rate). As life-threatening airway, breathing, and circulation problems are identified, immediate treatment is initiated. Once the animal has been stabilized, a more systematic and organized approach to the history and physical examination and more specific diagnostic and therapeutic procedures aimed at the underlying cause can be done. The first concern is for the safety of the owner. Placing a light cloth over the head of the animal can lessen external stimuli that may cause fearful and aggressive reactions. Owners can be instructed as to how to muzzle most dogs using a long strip of fabric if there are no facial injuries or respiratory distress. It is vital that the owner adequately restrain the pet before starting any first aid procedures to ensure the safety of the owner and pet. When moving the animal, motion of the head, neck, and spine should be minimized. A flat, firm board of wood, cardboard, or thick fabric can be used to provide support. Cats can be placed in boxes to minimize stress during transport; the box should have holes so that the cat can be observed and to allow adequate fresh air.

Owners should be asked whether hemorrhage is ongoing or whether bleeding was seen at the site of injury. Pulsating arterial bleeding should be controlled by direct digital pressure or a pressure bandage. If the bleeding from a limb is venous (dark, oozing), the limb can be elevated above the heart. Tourniquets should be used only on appendages (i.e. limbs, tail) when compression wraps have failed to control bleeding. Fractures below the elbow or stifle, support can be provided during transport. Once the animal has been adequately restrained, the owner can make a support splint from a rolled newspaper or magazine, which is secured in place by long pieces of fabric or duct tape and in large animals Niwar with wooden splint can be used very effectively. There should not be any jerking or thrashing motions, and manipulations of the neck or occlusion of the jugular veins should be avoided.

Analgesia is necessary in any patient with a painful condition. After a neurologic exam is complete, administration of analgesia medication is recommended. Opioids (e.g., fentanyl, morphine, hydromorphone etc.) are ideal first-line agents, providing effective analgesia with minimal impact on cardiac or respiratory systems. Butorphanol has minimal and short-acting analgesic effects. Addition of a benzodiazepine (Diazepam, Midazolam) will provide neuroleptanalgesia. NSAIDS are considered safe in most patients without shock, renal or GI disease, or need for steroids. Multimodal analgesia with adjunct infusions of ketamine, alpha-2 agonists (xylazine), or lidocaine will be helpful in many painful conditions. If a toxic ingestion is suspected, it is advisable to bring the animal immediately to a veterinarian. Administration of various substances by unskilled persons carries the risk of further injury such as a vagal response (resulting in collapse and bradycardia), choking on vomitus, aspiration pneumonia etc. If possible, the container of the toxin should be brought in for identification.



## **Large airway pathology**

Large airway of an animal can be obstructed by either of the following pathological conditions like foreign body, edema or hemorrhage laryngeal paralysis tracheal collapse or obstruction, including trauma brachycephalic airway syndrome (elongated soft palate, stenotic nares, hypoplastic trachea,) aspiration of saliva or stomach contents and neoplasia

Animals with severe small airway obstruction have labored breathing with an expiratory push of the diaphragm, cyanosis, and anxiety. Auscultation reveals high-pitched wheezes in the lung field. In severe life-threatening situations, the animal is cyanotic, open-mouth breathing, collapsed, and asphyxiating. Common causes include anaphylactic reactions, asthma (cats), and bronchial obstruction from edema, mucus, exudates, or foreign material

## **Establishment of an Airway in Complete Airway Obstruction**

Unconscious, apneic animals require immediate tracheal intubation. The clinician should be practiced in orotracheal intubation of animals in dorsal, lateral, and sternal recumbency. If an obstruction is present, it must be immediately relieved (with suction, manual removal, or the Heimlich maneuver) or bypassed via emergency tracheotomy. Once an airway is established, confirmed, and secured, ventilation is initiated with 100% oxygen via a bag-valve-mask. Heart sounds and pulse are checked and, when absent, cardiopulmonary resuscitation is initiated.

With partial large airway obstruction, flow-by oxygen is delivered through oxygen tubing at a high flow rate. Sedation may be used to relieve anxiety. When tracheal intubation is necessary, general anesthesia should be induced using rapid-acting IV anesthetics. A tracheotomy is necessary when pharyngeal, laryngeal, or tracheal pathology prevents orotracheal intubation or when prolonged intubation is anticipated. Once the airway is established, it should be secured with a tie and inflation of the cuff mechanism to a maximum of 20 mm Hg.

Once the airway is secured, intubation should be confirmed with at least two of the following methods: palpation of the tube within the trachea (only one "tube" palpated in cervical region) bilateral auscultation of the lungs, visualization of chest wall movement with manual ventilation, visualization of the tube entering the airway placement of end-tidal CO<sub>2</sub> monitor (normal is 35–45 mmHg) or radiographs.

## **Therapy for Small Airway Obstructive Disease**

Cyanosis from small airway obstructive disease is treated by providing oxygen. Epinephrine (0.01–0.02 mg/kg, IV/IM) as bronchodilator both in anaphylaxis and in life-threatening asthma. Corticosteroids (prednisone sodium succinate, 15 mg/kg, IV, or dexamethasone sodium phosphate, 0.1–0.2 mg/kg, IM or IV) are given for allergic bronchitis, asthma, or severe swelling of the larynx or pharyngeal tissues. Other bronchodilators, such as aminophylline or terbutaline, are given IM, or albuterol can be given

by nebulization in the case of an animal in crisis. Diphenhydramine (1–2 mg/kg, IM) is given for allergic reactions.

### **Emergency medicine and critical care in Birds**

Although the principles of emergency medicine and critical care are universal for all species, this approach must be balanced with an understanding of the unique aspects of avian medicine. Many companion birds are prey species, which tend to hide signs of illness until disease is advanced. A lethargic, “fluffed and ruffled” appearance is a very common non-specific sign of illness in the avian patient. To increase the likelihood that subtle signs of illness will be recognized during the visual examination, first provide prey species with a 5-10-minute acclimation period.

### **The Acclimation Period: Approach to Prey Species**

An acclimation period allows animals time to stabilize in a new environment or to adjust or adapt to a new temperature, climate, environment, or situation. Acclimation is the coordinated phenotypic response developed by the animal to a specific stressor in the environment while Acclimatization refers to the coordinated response to several individual stressors simultaneously. Many exotic animals seen in clinical practice are prey species. In the wild, individuals that appear sick or injured are easy prey for predators, and they may even be segregated or attacked by group members. When faced with the stress of a strange examination room, most prey species will attempt to appear alert and strong as an instinctive survival adaptation.

Signs of respiratory difficulty in the bird can include open-mouth breathing, increased sternal motion, and tail bobbing. Careful observation is essential. Is the patient strong enough to handle manual restraint and a complete physical examination? Or is the bird so ill that only a cursory examination can be performed? In the debilitated or dyspneic patient, it may be prudent to first place the patient in an incubator or oxygen cage in a dark, quiet room before evaluation. Even after the bird has had time to gather its strength and calm down, it may only be strong enough to handle diagnostics and treatment in stages.

Lethargy is a common non-specific sign of illness. The affected bird often displays closed or partially open eyes, frequent blinking, and/or unfocused eyes. The normal prey species will appear as wide-eyed and alert as it possibly can for as long as it can. The lethargic bird will display inactivity, weakness, and possibly an inability to perch, which means the bird may present on the bottom of the cage. There may be a history of partial or complete anorexia and weight loss. Birds with non-specific signs of illness frequently display fluffed and ruffled feathers as well as decreased preening and unkempt feathers. There may be a report of change in the quality or quantity of droppings. Abnormal droppings can also be observed during the visual examination. Transient polyuria is commonly observed in birds under stress. The three components of the normal dropping are feces, urates and urine. If the urine component is abnormally large it is known as polyuria. It can be a normal physiologic response to stress or high fluid intake. Gastrointestinal signs of illness in the bird can include

gagging or retching, stretching of the neck, regurgitation, or vomiting. Signs of illness involving the upper respiratory tract can include periocular swelling, oculo-nasal discharge, sneezing, scratching around the eyes, and frequent yawning. Soiling or matting of the feathers on the head or around the nares is observed when oculo-nasal discharge or regurgitated material is flicked back by the bird.

## **History**

The avian history must be detailed and includes signalment and recent medical history, but also source of the bird, whether migratory or native, caging history if any exposure to other birds/ animals, as well as recent illnesses or deaths of other birds in the area.

## **Restraint & handling**

Proper restraint of birds, that does not lead to patient or veterinary staff injury, requires training and practice. Prey species or wild birds will undergo a stress response that can cause catecholamine release and even death due to handling and treatment alone. Never restrain the avian patient for a prolonged period. Always plan a procedure that requires restraint and gather all equipment that may possibly be needed beforehand.

## **Housing**

House avian patients in a quiet area away from the sight and sound of predator species like cats, dogs, and ferrets. The cage setup should also physically block the view of one animal from another. Many birds will also benefit from some form of visual security. Drape a towel over part of the incubator or tape newspaper or some other opaque material over part of a treatment cage door. Dim light levels as needed to calm the nervous patient.

A rapid metabolic rate means that small birds have a greater susceptibility to hypothermia. Debilitated birds should be kept warm. Target incubator temperatures range between 80-90°F (26-32°C) for most avian patients. Carefully observe the patient for signs of overheating, such as flat, sleek feathers, outstretched wings, and open-mouth breathing. Use particular caution in overweight birds. All but the weakest perching birds will be much more comfortable if provided with perch material. Place perches on the cage floor or elevate perches only slightly to minimize the risk of falls. Passeriformes are also known as perching birds. Perching birds have three unwebbed toes in the front and one strong, flexible toe in the back called the hallux, that lets them perch on tree branches.

## **Vascular access**

Even loss of small volumes of blood can leave a tiny animal critically hypovolemic. For these small patients, use small-volume fluid resuscitation with frequent reassessment rather than large fluid boluses. Vascular access sites are limited in the bird. Peripheral veins can be difficult to access, especially during shock, and the vessels are also prone to hematoma formation. Intraosseous catheter placement is generally faster and easier in birds and should be used as a first choice in an emergency situation. Subcutaneous fluids are an excellent way to

provide maintenance fluids to stable avian patients and to correct mild dehydration. Subcutaneous fluids may also be the safest route initially for extremely debilitated patients as well as those with respiratory distress or coelomic distension. Fluids administered should always be warmed. An isotonic crystalloid, like lactated Ringer's solution, is a good choice for many critical patients.

### **Medical therapy**

Antimicrobial choice is more limited when treating birds, antibiotics selected in the critically ill patient are generally bactericidal and broad-spectrum, such as cefotaxime 75-100 mg/kg IM, IV or piperacillin-tazobactam 100 mg/kg IM (Jenkins 2016, Stout 2016, Hawkins *et al* 2013). When oral medications are indicated, commercially available or compounded suspensions are preferable. The specific Drugs can also be administered by the intramuscular or subcutaneous routes. Insulin or tuberculin syringes provide more accurate dosing than larger syringes.

### **Analgesia**

Clinical signs of pain are often more subtle in birds when compared to those seen in mammals. Behavioral signs of pain in birds can include reduced vocalization, decreased activity, anorexia, isolation from the group, and increased aggression. Physical signs of pain can include tachycardia, hypertension, arrhythmias, tachypnea, hypoxemia, hypercapnia, acidosis, abnormal posture and/or lameness, and weight loss. As in all veterinary patients, provide pre-emptive analgesia and multimodal analgesic agents whenever possible. Non-steroidal anti-inflammatory drugs (NSAIDS), such as meloxicam, and opioids are frequently used. Kappa-agonists opioids, like butorphanol, are commonly used in birds however growing evidence suggest mu-agonists, such as hydromorphone and fentanyl, are more effective in some avian species.

### **Corticosteroid**

Corticosteroid use is controversial in birds, and there are many contraindications for its use. Aspergillosis can develop in avian patients during times of stress or immunosuppression, and corticosteroid use is an important predisposing condition. Therefore, corticosteroid use is not recommended as a standard treatment for most clinical conditions. Select another drug with the potential for fewer adverse effects whenever possible.

### **Nutritional support**

The rapid metabolic rate of small avian patients leads to rapid depletion of glucose reserves. Fasting should be avoided and if required, should be < 6 hours. Nutritional support is essential in these patients. Provide familiar food items ad libitum. After the patient has been warmed and hydrated, tube or gavage feeding is often an essential part of avian supportive care. Tube feeding is a relatively straightforward technique in the bird, however there are serious potential complications, including aspiration, laceration of the oropharynx, cellulitis,

and even death. Therefore, this technique should be practiced beforehand so tube feeding can be performed efficiently, safely, and gently in the clinical patient. Closely monitor patient body weight and droppings.

### **Variables that contribute to the overall success of emergency treatment**

- Severity of the primary illness or injury
- Amount of fluid or blood lost
- Age of the animal
- Previous health problems
- Number and extent of associated conditions
- Time delay in instituting therapy
- Volume and rate of fluid administration
- Choice of fluids (eg, crystalloid, blood components, or synthetic colloids)
- Possible complications that can occur either from therapy (eg, side effects of drugs) or the underlying disease

### **As per AVMA**

Animal emergencies that require immediate veterinary care

1. Severe bleeding or bleeding that doesn't stop within five minutes
2. Choking, difficulty breathing or nonstop coughing and gagging
3. Bleeding from nose, mouth, rectum, coughing up blood, or blood in urine
4. Inability to urinate or pass feces (stool), or associated pain
5. Injuries to eyes and or face
6. When we suspect ingestion of poison by the animal or bird in any form (such as pesticides, rodent poison, etc.)
7. Seizures and/or staggering
8. Fractured bones, severe lameness or inability to move limbs
9. Obvious signs of pain or extreme anxiety
10. Heat stress or heatstroke, hypothermia
11. Severe vomiting or diarrhea – more than two episodes in a 24-hour period, or either of these combined with obvious illness
12. When animal/bird is in a state of 'Shock'
13. Unconsciousness
14. Distended or bloated abdomen
15. Animal attack/bite

## WET LAND ECOSYSTEM AND AVIFAUNA BIODIVERSITY IN PERSPECTIVE TO RAJASTHAN

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### **Introduction:**

Wetlands are amongst the most productive ecosystems on the Earth, comparable to rain forests and coral reefs. They provide many important services to human. However, they are also ecologically sensitive and adaptive systems. They also are a source of substantial biodiversity in supporting numerous species from all of the major groups of organisms –from microbes to mammals. Numerous species of birds and mammals rely on wetlands for food, water, and shelter, especially while migrating and breeding. Wetlands are considered to have unique ecological features which provide numerous products and services to humanity. Ecosystem goods provided by the wetlands mainly include: water for irrigation; fisheries; non-timber forest products; water supply; and recreation. Major services include: carbon sequestration, flood control, groundwater recharge, nutrient removal, toxics retention and biodiversity maintenance “Wetlands are often described as “Kidneys of the landscape”

### **Definition:**

The Ramsar Convention takes a broad approach in determining the wetlands which come under its aegis. Under the text of the Convention (Article 1.1), wetlands are defined as: “areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters”. Wetlands will have a relative abundance of obligate hydrophytes in the vegetation community and soils featuring “hydric” characteristics.

**Types of Wetland:** There are four main types of Wetland

1. **Swamps:** Depends on nutrient-rich ground water derived from mineral soils.
2. **Bogs:** Made by peat accumulation, usually dominated by moss. Bogs appear where the water at the ground surface is acidic. Bogs are generally formed by rain water.
3. **Marshes:** Permanently or periodically flooded sites characterized by nutrient-rich water.
4. **Fens:** are made by peat accumulation. It may be dominated by sedge, reeds, shrub or forest. Fens are mostly fed by ground water or surface water.

### **Characteristics of Wetlands:**

Wetlands must have one or more of the following four attributes:

1. Occupy a transitional zone.

2. Diversity varies according to origin, geographical location, water regime and chemistry, dominant flora and soil or sediment characteristics.
3. Sustainability of wetlands depends largely on the dynamics of water supply and loss.
4. The ecosystem function of a wetland is dependent on its biogeochemical processes.

### Wetland classification:

Wetlands vary greatly in their genesis, geographical location, water regime, chemistry, and plant communities. One of the first widely used wetland classifications systems (devised by Cowardin *et al.*, 1979) categorized wetlands into marine (coastal wetlands), estuarine (including deltas, tidal marshes, and mangrove swamps), lacustrine (lakes), riverine (along rivers and streams), and palustrine ('marshy' – marshes, swamps and bogs) based on their hydrological, ecological and geological characteristics. Wetland types based upon vegetation morphology.

Sr. No.	Major Categories	General Location	Wetland types
<b>01.</b>	<b>Coastal Wetlands:-</b>		
I	Marine (undiluted salt water)	Open coast	Shrub wetland, salt marsh, mangrove swamp
II	Estuarine (salt/freshwater mix)	Estuaries (deltas, lagoons)	Brackish marsh, shrub wetland, salt marsh, mangrove swamp
<b>02.</b>	<b>Inland Wetlands</b>		
I	Riverine (associated with rivers and streams)	River channels and floodplains	Bottomlands, freshwater marsh, delta marsh
II	Lacustrine (associated with lakes)	Lakes and deltas	Freshwater marsh, shrub and forest wetlands
III	Palustrine (shallow ponds, misc. freshwater wetlands)	Ponds, peatlands, uplands, ground water seeps	Ephemeral ponds, tundra peatland, ground water spring oasis, bogs

### Ecology of wetland:

Wetlands are amongst the most productive ecosystems on the Earth (Ghermandiet *et al.*, 2008).

1. The most important factor producing wetlands is flooding. The duration of flooding determines whether the resulting wetland has aquatic, marsh or swamp vegetation.
2. Other important factors include Fertility, Natural disturbance, Competition, Herbivory, Burial, Salinity
3. When peat accumulates, bogs and fens arise

### **Distribution and extent of wetlands in India:**

Wetlands exhibit enormous diversity according to their genesis, geographical location, water regime and chemistry, dominant species, and soil and sediment characteristics (Space Applications Centre, 2011).

1. India, with its varying topography and climatic regimes, supports diverse and unique wetland habitats.
2. Wetlands are distributed in different geographical regions ranging from Himalayas to Deccan plateau.
3. Wetlands in India cover an area of around 58.2 m ha.

### **Wetlands in India**

1. There are 19 different types of wetland in India
2. It includes mangroves high altitude lakes marshes and ponds.
3. It covers an estimated 3% of India's land area
4. Wetlands in India occupy 58.2 million ha including areas under paddy cultivation

### **Ramsar Convention**

1. Inter-governmental treaty which provides framework for the conservation and wise use of wetlands and their resources.
2. There are at present 154 Contracting Parties to the Convention, with 1634 wetland sites.
3. It is the oldest global nature conservation treaty
4. Started in 1971, came into force 1975
5. India is a signatory to Ramsar Convention and plays an important role in conservation and wise use of wetlands.
6. Twenty-five sites from India have been designated as Ramsar sites of International Importance

### **Ramsar criteria for identifying wetlands of International importance:**

Wetlands should be selected for the List of Wetlands of International Importance on account of their international significance in terms of ecology, botany, zoology, limnology or hydrology and indicates that in the first instance, wetlands of international importance to waterfowl at any season should be included.

### **Criteria:**

Group A: Sites containing representative, rare or unique wetland types

Criteria for unique, rare or representative wetlands, which are natural or near natural

Group B: Sites for conserving biological diversity –criteria based on species and ecological communities

Criteria specific to water birds

Criteria based on fish

Criteria specific to other taxa



### **Wetlands of Rajasthan:**

Wetlands are important breeding areas for wildlife and provide a refuge for migratory birds. In many such wetland areas of India, like Keoladeo Bharatpur wild life sanctuary and Sambhar lake in Rajasthan.

### **The Keoladeo National Park:**

The Keoladeo National Park or Keoladeo Ghana National Park formerly known as the Bharatpur Bird Sanctuary in Bharatpur, Rajasthan, India. The Keoladeo National Park is a famous avifauna sanctuary that plays host to thousands of birds especially during the summer season. Over 230 species of birds are known to have made the National Park their home. It is also a major tourist centre with scores of ornithologists arriving here in the hibernal season. Keoladeo Ghana National Park is a man-made and man-managed wetland and one of the national parks of India.

One third of the Keoladeo National Park habitat is wetland systems with varying types of microhabitats having trees, mounds, dykes and open water with or without submerged or emergent plants. The uplands have grasslands (savannas) of tall species of grass together with scattered trees and shrubs present in varying density. Woodlands with thickets of huge Kadam trees (*Neolamarckiacadamba*) are distributed in scattered pockets.

### **Avifauna of Keoladeo National Park:**

The park's location in the Gangetic Plain makes it an unrivalled breeding site for herons, storks and cormorants, and an important wintering ground for large numbers of migrant ducks. The most common waterfowl are gadwall, shoveler, common teal, cotton teal, tufted duck, knob-billed duck, little cormorant, great cormorant, Indian shag, ruff, painted stork, white spoonbill, Asian open-billed stork, oriental ibis, darter, common sandpiper, wood sandpiper and green sandpiper. The sarus crane, with its spectacular courtship dance, also lives here.

Keoladeo National Park is known as a “bird paradise”, since more than 370 bird species have been recorded in the park. Ornithologically, the park assumes significance in two respects: One because of its strategic location as a staging ground for migratory waterfowl arriving in the Indian subcontinent before dispersing to various regions. Further waterfowl converge here before departing to breeding grounds in the western Palearctic region. In addition, the wetland is a wintering area for massive congregations of waterfowl. It is also the only regular wintering area in India for the Siberian crane. Birds present include warblers, babblers, bee-eaters, bulbuls, buntings, chats, painted francolins and quails, Indian grey hornbill and Marshall's iora. Raptors include osprey, peregrine falcon, Pallas' sea eagle, short-toed eagle, tawny eagle, imperial eagle, spotted eagle and crested serpent eagle. The greater spotted eagle has recently been recorded breeding here, a new breeding record for the species in India.

### **Sambhar lake:**

The Sambhar Lake, India's largest inland salt lake, a bowl shape lake encircles historical Sambhar Lake Town located 96 km south west of the city of Jaipur (Northwest India) and 64 km north east of Ajmer. Geography The lakes receives water from an endorheic basin with 5700 square km catchment area. The water is fed to the lake from streams from the rivers Mendha, Runpangarh, Khandel and Karian. The Mendha and Rupangarh are main streams.

### **Avifauna of Sambhar lake:**

Sambhar Lake Wetland is a shallow wetland, the depth of which ranges between 0.5 and 2.0 metres. Four main streams feed the lake from a drainage area of about 2,688 sq. metres. The vegetation present in the catchment area is mostly xerophytic type.

Ecological importance Sambhar has been designated as a Ramsar site (recognized wetland of international importance) because the wetland is a key wintering area for tens of thousands of flamingos and other birds that migrate from northern Asia. The specialized algae and bacteria growing in the lake provide striking water colors and support the lake ecology that, in turn, sustains the migrating waterfowl. There is other wildlife in the nearby forests, where Nilgai move freely along with deer and foxes.

The following migratory avian fauna were recorded. 1. Greater flamingo 2. Common shelduck 3. Red shank 4. Common sand piper 5. Black wing stilt 6. Kentish plover 7. White cheeked Bulbul 8. Ruff 9. Ringed plover 10. Sociable Lapwing from Siberia.

In November 2019 the deaths of over 18,000 birds in Sambhar Lake have been attributed to Avian botulism. Avian botulism is the cause of the death of domestic and migratory birds in the Sambhar Lake. This has been confirmed by the Indian Veterinary Research Institute of Bareilly.

### **Conclusion:**

Wetlands provide tremendous economic benefits, for example: water supply (quantity and quality); fisheries (over two thirds of the world's fish harvest is linked to the health of coastal and inland wetland areas); agriculture, through the maintenance of water tables and nutrient retention in floodplains; timber production; energy resources, such as peat and plant matter; wildlife resources; transport; and recreation and tourism opportunities.

In addition, wetlands have special attributes as part of the cultural heritage of humanity: they are related to religious and cosmological beliefs, constitute a source of aesthetic inspiration, provide wildlife sanctuaries, and form the basis of important local traditions.

These functions, values and attributes can only be maintained if the ecological processes of wetlands are allowed to continue functioning. Unfortunately, and in spite of important progress made in recent decades, wetlands continue to be among the world's most threatened ecosystems, owing mainly to ongoing drainage, conversion, pollution, and over-exploitation of their resources.

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## **AVIAN INFLUENZA**

**(Synonym: Bird Flu, Avian Flu or Fowl Plague)**

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### **Introduction**

Avian influenza refers to infection of birds with avian influenza Type A viruses. These viruses occur naturally among wild aquatic birds worldwide and can infect domestic poultry and other bird and animal species. Wild aquatic birds can be infected with avian influenza A viruses in their intestines and respiratory tract, but usually do not get sick. However, avian influenza A viruses are very contagious among birds and some of these viruses can sicken and even kill certain domesticated bird species including chickens, ducks, and turkeys. Infected birds can shed avian influenza A viruses in their saliva, nasal secretions, and feces. Susceptible birds become infected when they have contact with the virus as it is shed by infected birds. They also can become infected through contact with surfaces that are contaminated with virus from infected birds. Avian influenza A viruses are classified into the following two categories: low pathogenic avian influenza (LPAI) A viruses, and highly pathogenic avian influenza (HPAI) A viruses.

### **Avian Influenza in Wild birds**

Avian influenza A viruses have been isolated from more than 100 different species of wild birds. Most of these viruses have been LPAI viruses. The majority of the wild birds from which these viruses have been recovered represent gulls, terns and shorebirds or waterfowl such as ducks, geese and swans. These wild birds are often viewed as reservoirs (hosts) for avian influenza A viruses.

### **Avian Influenza in Poultry (Domesticated birds)**

Domesticated birds (chickens, turkeys, etc.) may become infected with avian influenza A viruses through direct contact with infected waterfowl or other infected poultry, or through contact with surfaces that have been contaminated with the viruses. Infection of poultry with LPAI viruses may cause no disease or mild illness and may only cause mild signs (such as ruffled feathers and a drop in egg production) and may not be detected. Infection of poultry with HPAI viruses can cause severe disease with high mortality. Both HPAI and LPAI viruses can spread rapidly through flocks of poultry. HPAI virus infection in poultry (such as with HPAI H5 or HPAI H7 viruses) can cause disease that affects multiple internal organs with mortality up to 90% to 100%, often within 48 hours. Some ducks can be infected without any signs of illness.

## Etiology

Avian influenza viruses are type A orthomyxoviruses (*Alphainfluenza virus* or *Influenza virus A*) characterized by antigenically homologous nucleoprotein and matrix internal proteins, which are identified by serology in agar gel immunodiffusion (AGID) tests. AI viruses are further divided into 16 haemagglutinin (H1-16) and 9 neuraminidase (N1-9) subtypes based on haemagglutination inhibition test and neuraminidase test.

## Where is the disease found?

AI occurs worldwide and different strains are more prevalent in certain areas of the world than others. Of particular interest are outbreaks of highly pathogenic H5N1 AI that began in south-east Asia in late 2003. Over the past years, several other Asian countries have reported outbreaks and in some, the disease is now considered to be endemic (always present). Outbreaks of HPAI H5N1 have also been reported in Africa and Europe.

## Disease transmission and spread

Several factors can contribute to the spread of AI viruses including globalization and international trade (legally and illegally), marketing practices (live bird markets), farming practices and the presence of the viruses in wild birds. Wild birds normally can carry avian influenza viruses in their respiratory or intestinal tracts and usually do not get sick. Wild birds have historically been known as reservoirs for AI viruses, mostly of low pathogenicity.

Around the world, surveillance measures have been put in place to monitor occurrence and characteristics of AI viruses in wild birds. In wild birds, it is common during routine testing to find some influenza viruses. The vast majority of these viruses do not cause disease. In studying current HPAI H5N1 outbreaks, the exact role of wild birds in spreading the virus over long distances is still not fully understood in all situations.

Generally, there are many uncertainties about the wild species involved, the migratory routes used and, above all, the possibility that some species could become permanent reservoirs of the H5N1 virus, with carriers showing no clinical signs of the disease.

The incubation period is highly variable and ranges from a few days in individual birds to 2 weeks in the flock. AI viruses can be spread through direct contact with secretions from infected birds, especially faeces or through contaminated feed, water, equipment and clothing. Apart from being highly contagious among poultry, avian influenza viruses are readily transmitted from farm to farm by the movement of domestic live birds, people (especially when shoes and other clothing are contaminated), and contaminated vehicles, equipment, feed, and cages.

Highly pathogenic viruses can survive for long periods in the environment, especially when temperatures are low. For example, the highly pathogenic H5N1 virus can survive in bird faeces for at least 35 days at low temperature (4°C). At a much higher temperature (37°C), H5N1 viruses have been shown to survive, in faecal samples, for six days.

Other species including cats and pigs can exceptionally become infected with the HPAI

H5N1 virus. Infections of pigs are of concern because the species is susceptible to infections of both avian and human influenza viruses and this may provide the H5N1 AI virus the opportunity to reassort or mutate. While sporadic infection with HPAI H5N1 virus in both species have been reported over the past three years there is no scientific evidence to suggest that either species plays a significant role in the epidemiology of the disease or as a source of virus to other species.

### **Pathogenesis**

Most HPAI virus strains from domestic poultry have been isolated from turkeys and chickens, but infection, clinical signs and high mortality have been reported in diverse genera and species within the order Galliformes, family Phasianidae, including Japanese quail, helmeted guinea-fowl (*Numida meleagris*), chukar partridges (*Alectoris chukar*), northern bobwhite quail (*Colinus virginianus*) and common pheasants (*Phasianus colchicus*). Highly pathogenic avian influenza is the result of systemic replication of the virus and cell death in a variety of visceral organs, brain and skin. Poultry flocks infected with HPAI virus have high morbidity and mortality rates with birds developing severe clinical signs, often with rapid death. In contrast, the majority of AI virus strains are non-HP under natural or experimental conditions, and produce either subclinical infections or mild-to-moderate disease syndromes affecting the respiratory, urinary and reproductive systems. These viruses replicate locally, predominantly in the respiratory and alimentary tracts. Generally, the mortality rates due to LPAI are low compared to HPAI, but mortality rates can be high when LPAI is accompanied by secondary viral or bacterial pathogens.

### **Clinical findings**

#### **Low Pathogenicity Avian Influenza Viruses (LPAI)**

Low pathogenicity avian influenza viruses produce respiratory signs mostly such as sneezing, coughing, ocular and nasal discharge and swollen infraorbital sinuses in poultry. Sinusitis is common in domestic ducks, quail, and turkeys.

#### **High Pathogenicity Avian Influenza Viruses (HPAI)**

Even in the absence of secondary pathogens, HPAI viruses cause severe, systemic disease with high mortality in chickens, turkeys, and other gallinaceous poultry; mortality can be as high as 100% in a few days. In peracute cases, clinical signs or gross lesions may be lacking before death. Birds that survive the peracute infection may develop CNS involvement evident as torticollis, opisthotonos, incoordination, paralysis, and drooping wings.

#### **Lesions**

The appearance of gross lesions is variable depending on the virus strain, the length of time from infection to death, and the age and species of poultry affected. Histological lesions usually vary in severity and location, but typically include necrosis, haemorrhage and/or inflammation within multiple visceral organs; especially the heart, brain, adrenal and pancreas

and skin. Variation in the distribution and severity of lesions is the result of differences between strains of HPAI virus and species of bird.

### **Lesions of Low Pathogenicity Avian Influenza Viruses**

Lesions in the respiratory tract typically include congestion and inflammation of the trachea and lungs. In layers and breeders, there may be decreased egg production or infertility, ova rupture (evident as yolk in the abdominal cavity) or involution, or mucosal edema and inflammatory exudates in the lumen of the oviduct. A few layer and breeder chickens may have acute renal failure and visceral urate deposition (visceral gout).

### **Lesions of High Pathogenicity Avian Influenza Viruses**

In acute cases, lesions may include cyanosis and edema of the head, comb, wattle, and snood (turkey); ischemic necrosis of comb, wattles, or snood; edema and red discoloration of the shanks and feet due to subcutaneous ecchymotic hemorrhages; petechial hemorrhages on visceral organs and in muscles; and blood-tinged oral and nasal discharges. In severely affected birds, greenish diarrhea is common. The location and severity of microscopic lesions are highly variable and may consist of edema, hemorrhage, and necrosis in parenchymal cells of multiple visceral organs, skin, and CNS.

### **Morbidity and mortality**

The morbidity and mortality is usually low in Low Pathogenicity Avian Influenza Viruses unless accompanied by secondary bacterial or viral infections or aggravated by environmental stressors. Sporadic infections by any subtype of LPAI viruses can occur, but H9N2 LPAI is common in commercial and live bird market poultry in Asia, the Middle East, and North Africa. The morbidity and mortality is very high in High Pathogenicity Avian Influenza Virus infection.

### **Diagnosis**

Avian Influenza virus diagnosis resources available in India

#### **Two National Reference Laboratories**

- For animals - HSADL, Bhopal
- For humans- NIV, Pune

#### **Diagnosis of Avian Influenza include**

- Avian influenza virus isolation
- Detection of AI viral RNA
- Detection of AI-specific antibodies

A disease outbreak is usually the first indication of an infection with AI virus however the presence of clinical disease alone is not diagnostic. A combination of virus isolation, serological tests, and direct antigen detection is often used to detect infected flocks. Low pathogenicity and high pathogenicity avian influenza viruses can be readily isolated from oropharyngeal and cloacal swabs, and HPAI viruses from many internal organs.

Isolation of influenza viruses has been achieved by direct inoculation of nine- to eleven-day-old embryonating chicken eggs with homogenates from the lung, trachea, faeces and internal organs. Alternative methods of virus isolation have been used, primarily cell culture. Influenza viruses have been routinely isolated from clinical samples, however failures have occurred due to bacterial or viral contamination, incorrect storage of samples, inadequate samples or sample numbers, or because the infected birds were sampled after viral shedding had ceased. AI viruses agglutinate RBCs and such hemagglutination is not inhibited by Newcastle disease or other paramyxoviral antiserum.

**AI viruses are identified by demonstrating the presence of:**

- Influenza A matrix or nucleoprotein antigens using AGID or other suitable immunoassays
- Viral RNA using influenza A-specific reverse transcriptase PCR
- Reaction with antibodies specific for AI virus

**Laboratory Tests for AI Antibodies**

Birds that have recovered from clinical disease can be confirmed as AI infections based on serologic testing for influenza virus A (AGID or ELISA) and further subclassified as to hemagglutinin and neuraminidase subtype based on hemagglutinin inhibition and neuraminidase inhibition tests, respectively.

**Differential diagnosis**

LPAI must be differentiated from other respiratory diseases or causes of decreased egg production, including:

- Acute to subacute viral diseases such as infectious bronchitis, infectious laryngotracheitis, low virulent Newcastle disease, and infections by other paramyxoviruses
- Bacterial diseases such as Mycoplasmosis, Infectious coryza, Ornithobacteriosis, Turkey coryza, and the respiratory form of Fowl cholera
- Fungal diseases such as Aspergillosis

HPAI must be differentiated from other causes of high mortality such as virulent Newcastle disease, the peracute septicemic form of fowl cholera, heat exhaustion, and severe water deprivation.

**Treatment and supportive care**

Treating LPAI-affected flocks with broad-spectrum antibiotics to control secondary pathogens and increasing house temperatures may reduce morbidity and mortality. Treatment with antiviral compounds is not approved or recommended.



## Prevention and control

It is extremely important to have early detection and warning systems and prevention measures in place as part of an effective strategy for AI. This needs to be coupled with similar efforts placed on preparing for a potential outbreak. Around the world, surveillance measures have been put in place to detect the presence of infection in poultry according to OIE Guidelines for the Surveillance of Avian Influenza. Additionally, surveillance programmes monitor the occurrence, prevalence and characterisation of AI viruses found in wild birds. Wild bird surveillance considers different migratory flyways and particularly at mingling points for migrating birds from different continents. It is essential for poultry producers to maintain biosecurity practices to prevent introduction of the virus in their flock.

Antigenically matched and properly administered vaccines can prevent clinical signs and death and greatly reduce virus replication and shedding from the respiratory and GI tracts. Specific protection is achieved through autogenous virus vaccines or from vaccines prepared from AI virus of the same haemagglutinin subtype. Antibodies to the homologous viral neuraminidase antigens may provide partial protection. Currently, only inactivated whole AI virus, DNA of H5 haemagglutinin, RNA particle (defective eastern equine encephalitis virus) with H5 hemagglutinin insert, recombinant fowlpox-AI-H5 and recombinant herpesvirus-turkey-AI-H5 (rHVT-AI-H5) vaccines are licensed in the USA.

## Zoonotic Risk of Avian Influenza

Human infections have occurred, usually as isolated, rare individual cases, most human case have originated from infection with Eurasian H5 HPAI virus and most recently, H7N9 LPAI virus (Eurasian lineage). This lineage of H5N1 HPAI virus has total accumulated human cases in Asia and Africa from 2003 to July 2020 of 861, of which 455 were fatal.

Primary risk factor of human infection has been direct contact with live or dead infected poultry, but a few cases have resulted from consumption of uncooked poultry products, defeathering of infected wild swans or close contact with human case.

Respiratory infection has been the most frequent presentation of human H5 case. This virus has very limited human to human transmission. Other HPAI viruses and all LPAI viruses have produced very rare or no human infections.

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## AVIAN BOTULISM

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### Introduction

Botulism is a paralytic intoxication that results from ingestion of preformed exotoxin (Botulinum neurotoxin) of *Clostridium botulinum*. Avian Botulism occurs all over the world and its understanding is important for wildlife managers, hunters, bird watchers, and anyone who owns wetland property as this disease can account for over 1,000,000 waterbird deaths in a year. This disease has been identified in at least 117 species of wild birds representing 22 families. There is concern that endangered avian species may be at risk of extinction because of the disease. Mammalian species affected by the toxin include people, mink, ferrets, cattle, pigs, dogs, horses, laboratory rodents, and various zoo animals. Intoxications are sporadic in poultry, but massive mortality has occurred in waterfowl.

**Key Points:** Birds, *Clostridium botulinum*, Botulinum neurotoxin and Neurological signs

### Etiology of Botulism in Poultry

*Clostridium botulinum* is a gram-positive, spore-forming, anaerobic bacterium. There are four genotypically distinct groups, designated by the Roman numerals I to IV. Group IV is also referred to as *Clostridium argentinense*. Strains of *C. botulinum* can be proteolytic or nonproteolytic. All strains can produce neurotoxins that cause flaccid paralysis. *C. botulinum* produces BoNT types A-F and H.

*C. baratii* produces type F, *C. butyricum* BoNT type E and *C. argentinense* BoNT type G (Koluman *et al.*, 2013 and Woudstra *et al.*, 2012). BoNT types A, B, E, F and H cause disease in humans, types B and D cause botulism in herbivorous mammals (equine and cattle) and type C mainly affects birds (Elad *et al.*, 2004). Also, recombinant toxins (C/D) have been described in some cases of avian botulism (Woudstra *et al.*, 2012).

Outbreaks in poultry and waterfowl are predominantly caused by type C toxin. The Type E strain is also commonly associated with avian outbreaks and is frequently found in fish species which is why most outbreaks occur in piscivorous (fish-eating birds) birds such as gulls.

*C. botulinum* is ubiquitous, inhabiting soils and freshwater wetland (lakes, marshes) sediments. It is commonly found in the intestine of wild birds, but its presence in the intestine of healthy poultry is questionable. Spore germination and cell multiplication with toxin production require an anaerobic environment that must also provide the necessary substrates and conditions required for multiplication. For toxinotype C, optimal growth occurs between 25°C and 40°C, which may explain why most outbreaks of botulism in waterfowl occur during the summer and fall.

## Epizootiology of Botulism

Wetlands are home to numerous invertebrates that can accumulate high levels of botulinum toxin. Carcasses and organic matter provide suitable substrates for multiplication of *C. botulinum* and toxin production. Invertebrates, especially fly larvae (maggots), feed on carcasses that have died from botulism and thus consume botulinum toxin. They are resistant to the toxins and concentrate them in their bodies. Outbreaks in waterfowl and other water birds result from consumption of toxin-laden invertebrates. Birds that die from botulism become infested with maggots, which perpetuate the outbreak in what is known as a "carcass-maggot cycle." Evidence suggests that botulism may also result from direct ingestion of decaying organic matter that contains toxin.

Environmental factors that contribute to the occurrence of outbreaks in wild birds, particularly waterfowl, include low and fluctuating water levels, the presence of vertebrate carcasses and rotting vegetation, and high ambient temperatures. The decomposition of the rotting fish, birds, and other carcasses produce an environment suitable for toxin production.

## Outbreaks in commercial poultry

It is rare and generally tend to recur on the same farm, often affecting the same houses or pens. The disease tends to be most severe in pen-reared pheasants, with mortality as high as 100%. Often, the source of the toxin in these outbreaks is difficult to find, leading to speculation that botulism in poultry is a "toxico-infection." Toxico-infection, also called "gut toxigenesis," results from proliferation of *C. botulinum* in the intestine with subsequent production and absorption of the toxin.

It is unclear what factors cause *C. botulinum* to proliferate in the intestine and produce toxin. Studies have shown that healthy broilers do not harbor *C. botulinum* type C in the ceca, and *C. botulinum* was not recovered from the environment of poultry houses that did not have a history of botulism. These findings suggest that *C. botulinum* is neither frequent nor widespread in broiler populations and that outbreaks result from infrequent and sporadic colonization of flocks with *C. botulinum* introduced into a poultry house.

## Pathogenesis

*C. botulinum* spores can survive in soils and water sediments and produce toxins in anaerobic microenvironments including stagnant waters, carcasses of dead animals, and decaying organic matter. Floods, pesticides and other agricultural pollutants can lead to changes in the ecosystem that promote BoNT production () [5]. Insect larvae and other flying invertebrates may concentrate BoNTs in their body (Woudstra *et al.*, 2012 and Takeda *et al.*, 2005) then vertebrate animals may ingest these invertebrates along with the toxin. In vertebrates, BoNT alters the neuromuscular junction and prevents the release of acetylcholine in nerve synapses, hindering muscle contraction (Woudstra *et al.*, 2012 and Caya *et al.*, 2006). Finally, progressive flaccid paralysis occurs in intoxicated animals resulting in respiratory failure and death (Caya *et al.*, 2006).

## Cycle of Avian Botulism

A wide variety of bird species may inhabit preferred nesting and resting locations. This commonly results in healthy, sick, and dead birds being found together during a botulism outbreak. As the dead birds decompose they become hosts for maggots that carry the toxin. New birds arriving to the area feed on the maggots that have developed on the dead birds. The new birds are then affected with the toxin, die, and a new botulism cycle begins. This method of transfer affects a wide variety of bird species.

## Clinical Findings of Botulism

The time between consuming the botulinum toxin and the appearance of the first clinical sign of botulism is dose dependent and varies from a few hours to several days. Clinical signs in poultry and wild birds are similar. Leg weakness and paresis that progress to flaccid paralysis of the legs, wings, neck, and eyelids are characteristic clinical signs. “Limberneck,” the common name for botulism in birds, comes from the neck paralysis typically seen in affected birds. Signs in broiler chickens may also include ruffled or quivering feathers, feathers that are easily pulled out, labored breathing, and sometimes diarrhea with excess urates in loose droppings. Severely affected birds are in ventral recumbency on the floor with their eyes partially or completely closed and neck outstretched. They are unable to lift or hold their neck up and cannot raise their eyelids because of the flaccid paralysis that develops. Affected birds may have their legs extended behind them, because they are unable to pull them into a normal sitting position. Weakness is the earliest sign in waterfowl. Birds are initially reluctant to fly when approached and have weak wing-beats and difficulty taking flight. As the disease progresses, they lose their ability to fly and exhibit stumbling gaits and eventually paralysis. Birds in water can drown, because they cannot keep their heads above the water. The incubation period and time to onset of clinical signs is determined by the amount of toxin absorbed.

## Lesions

*C. botulinum* toxins do not cause lesions in affected birds, but maggots or other invertebrates may be found in the crop. Carcasses are usually in good condition and do not show evidence of a chronic or debilitating disease.

## Diagnosis of Botulism

### Detection of toxin in serum or GI contents

Preliminary diagnosis of botulism is based on clinical signs and the absence of gross or microscopic lesions that can explain the neurologic signs.

Definitive diagnosis requires detection of the toxin in serum from sick birds. Crop, gizzard, and/or intestinal contents from moribund or very freshly dead birds may also be collected for toxin detection. Samples from birds that have been dead for several hours are unreliable, because *C. botulinum* toxin can result from postmortem multiplication of the

organism and toxin production. It is preferred to collect serum from birds with clinical signs of varying severity.

A bioassay test in mice (mouse-lethality assay) used to be the standard method to detect *C. botulinum* toxins, but molecular tests based on ELISA can be used. A PCR test can be used on a variety of samples to detect the genes responsible for toxin production and differentiate the different toxinotypes, but it does not detect biologically active neurotoxin and should not be used alone. Because botulinum toxins are heat labile and can be destroyed at 80°C for  $\geq 10$  minutes, sera must be refrigerated or frozen as soon as possible after collection and shipped with ice packs to the testing laboratory.

### Differential Diagnosis

Leg weakness or paralysis may be the only sign in mild intoxications, which must be differentiated from Marek disease, drug and chemical toxicosis (especially ionophore toxicity), or appendicular skeletal problems.

In waterfowl, botulism must be differentiated from chemical toxicosis, especially lead poisoning, and acute, virulent infectious diseases (fowl cholera, duck viral enteritis, highly pathogenic avian influenza, etc).

### Control and Prevention of Botulism in Poultry

- i. Birds with botulism may recover without treatment. Antibiotics effective against clostridia may be useful if the disease is toxico-infectious.
- ii. Collection and disposal of dead birds is critical to prevent and limit outbreaks, especially in pheasant and broiler chicken flocks.
- iii. Fly control may reduce the risk of toxic maggots in the environment. Some, but not all, recurrent outbreaks in broiler flocks may be prevented by cleaning and disinfecting with products effective against spore-forming bacteria.
- iv. Disinfection around poultry houses is suggested, because spores can be found outside of the houses and be reintroduced.
- v. In waterfowl outbreaks, ducks should be dispersed from affected areas and water levels stabilized. Elimination of large, shallow areas may prevent conditions favorable for decay of vegetation and die-off of invertebrates.
- vi. Active immunization with inactivated type C bacterin-toxoids has been successful in pheasant operations but is not cost-effective or feasible in commercial chickens and wild ducks. Treatment with type-specific antitoxin is effective but not practical.

### Zoonotic Risk of Botulism in Poultry

The zoonotic potential of type C botulism is minimal. Botulism in people is caused mostly by toxin types A, B, and E. Furthermore, pathogenicity of toxin type C for people is questionable. However, botulism caused by type C toxin has been confirmed in several nonhuman primates.

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## INFECTIOUS DISEASES OF WILD AND MIGRATORY BIRDS

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### Some important infectious diseases of wild and migratory birds:-

Viral Diseases	Bacterial Diseases	Fungal Diseases	Parasitic Diseases
1. Duck Plague	1. Avian Cholera	1. Aspergillosis	1. Hemosporidiosis
2. Avian Herpesviruses	2. Tuberculosis	2. Candidiasis	2. Trichomoniasis
3. Avian Pox	3. Salmonellosis		3. Sarcocystis
4. Newcastle Disease	4. Chlamydiosis		4. Eustrongylidosis
	5. Mycoplasmosis		
	6. Necrotic enteritis		

### Viral Diseases

#### 1. Duck Plague

**Synonyms:** Duck Virus Enteritis, DVE, anatid herpesvirus

#### Introduction

Duck plague is an acute, contagious disease of waterfowl caused by a herpesvirus classified as Anatid herpesvirus 1 of the subfamily alphaherpesvirinae. Waterfowl surviving virus infection can become lifelong asymptomatic virus carriers and shedders (Burgess et al. 1979) providing virus reservoirs for future disease outbreaks. Infection often results in an acute, contagious, and fatal disease. Duck plague outbreaks are thought to be caused when birds that carry the virus shed it through fecal or oral discharge, thus releasing the virus into food and water with which susceptible birds may have contact.

#### Distribution and Host range

Only ducks, geese, and swans are susceptible to duck plague. Other aquatic birds do not become infected, and the absence of mortality of American coot, appears to be the peak season for duck plague outbreaks shorebirds, and other water birds that may be present during a waterfowl die-off can be an important indication that duck plague may be involved. Late spring

#### Transmission

The primary method for natural duck plague virus transmission is direct contact between virus-shedding and susceptible waterfowl or contact with a virus contaminated environment, especially water.

## Clinical signs

There is no prolonged illness associated with duck plague; therefore, sick birds are seldom seen in the field, and birds that are healthy one day may be found dead the next. The incubation period between virus exposure and death is generally 3–7 days in domestic ducks. Sick birds may show clinical signs including depressed activity, ruffled unpreened feathers, labored breathing, ataxia (lack of coordination), photophobia, extreme thirst, anorexia (lack of appetite), ocular and nasal (sometimes bloody) discharge, watery diarrhea with bloody vent, prolapsed penis in males, and drop in egg production hypersensitive to light, causing them to seek dense cover or other darkened areas. An ulcerative “cold sore” lesion under the tongue from which virus can be shed has been seen in some infected waterfowl.

## Treatment and Control

Minimize exposure of the population-at-risk at the outbreak site and to minimize the amount of virus present in the environment. The only prevention for duck plague is vaccination. Disease control efforts in captive and wild waterfowl have utilized depopulation of all exposed or potentially exposed birds from a die-off site.

### 2. Avian Herpes viruses

**Synonyms:** Inclusion body disease of falcons, owl herpesvirus, pigeon herpes encephalomyelitis virus, psittacine herpesvirus

## Introduction

Avian Herpes viruses (HV) cause a variety of disease conditions in birds. Herpesviruses other than duck plague and inclusion body disease of cranes have been isolated from many groups of wild birds.

## Distribution and Host range

Avian herpesviruses have been reported from North America, Europe, the Middle East (Iraq), Asia, Russia, Africa, and Australia and they are probably distributed worldwide. It affects many domestic wild and migratory birds including, Cranes, Black Stork, Bald Eagle, Penguin, Japanese quail, Domestic Turkey, fowl etc.

## Transmission

Breeding season probably provides the best time of the year for bird-to-bird virus transmission in solitary species. Transmission of herpesviruses via the egg has been shown for some species.

Inhalation of virus-containing dust derived from feathers, nasal excretions, saliva, nasal discharge, urine, and feces is the predominant way of transmission from bird to bird. Adults that feed their offspring with crop-milk experience an activation of their latent infection in the oropharyngeal region during egg incubation and transmit the HV via crop-milk to their newly hatched offspring. Vectors are not required for transmission.



## Clinical Signs

The general signs of disease include depression of normal activity and sudden mortality in a group of birds. Respiratory distress may also be seen. Captive pigeons may show pronounced neurological signs such as extremity paralysis, head-shaking, and twisting of the neck. Birds dying from infection with this group of viruses can have tumors (chicken and pigeon), hemorrhagic lesions (chicken, pheasants, ducks, cranes, peafowl, and guinea fowl), or, more commonly, hepatitis, and disseminated focal necrosis or visual areas of localized tissue death that appear as spots within the normal tissue in the liver, spleen, and bone marrow along with occasional intestinal necrosis.

## Control

Control actions warranted for outbreaks of herpesvirus infections are dependent upon the type of herpesvirus infection and the prevalence of disease in the species or populations involved. Euthanasia of infected flocks should be considered for exotic viruses and viruses that are likely to cause high mortality within the population at risk. Dead birds should be removed immediately and submitted for disease evaluations. Standard bagging and decontamination procedures should be used to avoid off-site transfer of the virus.

### 3. Avian Pox

**Synonyms:** Fowl pox, avian diphtheria, contagious epithelioma, and poxvirus infection

## Introduction

Avian pox is a mild-to-severe, slow developing disease of birds that is caused by a large virus belonging to the avipoxvirus group, a subgroup of poxviruses. This disease is characterized in birds by discrete, proliferative lesions on the skin of the toes, legs, or head, and/or mucous membranes of the mouth and upper respiratory tract. Systemic infections may also occur.

## Distribution and Host range

The geographic distribution of avian poxviruses is worldwide. There are now recognized approximately 183 families and 9,800 species of birds

## Transmission

Avian pox can occur at any time of the year in wild birds. The most common method of transmission is by means of biting insects such as mosquitoes, mites, midges, and/or flies (mechanical vectors). Poxviruses can be transmitted in a number of different ways. Even though they are unable to penetrate unbroken skin, small abrasions are sufficient to permit infection.

## Clinical signs

Avian pox has two disease forms. Birds with wart-like nodules on one or more of the featherless areas of the body, including the feet, legs, base of the beak, and eye margin should

be considered suspect cases of avian pox. The birds may appear weak and emaciated if the lesions are extensive enough to interfere with their feeding. This form of the disease is usually self-limiting; the lesions regress and leave minor scars. The diphtheritic form of disease is referred to as wet pox and it is primarily a problem of young chickens and turkeys. This appears as moist, necrotic lesions on the mucus membranes of the mouth and upper digestive and respiratory tracts.

### **Control**

The fundamental principle for controlling avian pox is to interrupt virus transmission and can be achieved by preventing vector access to birds. The difficulty in applying control procedures is related to the type of transmission taking place, the mobility of the infected birds, and the size of the affected area. Special vigilance of captive birds is needed, especially when threatened and endangered species are involved. Because poxvirus is resistant to drying, disease transmission by contaminated dust, food, perches, cages, and clothing can pose a continuing source of problems.

### **4. Newcastle Disease**

**Synonyms:** ND, paramyxovirus-1, NDV, VVND, NVND

### **Introduction**

Newcastle Disease is caused by highly contagious RNA virus, Newcastle Disease virus or Avian Paramyxovirus Type 1 (APMV-1). It is widespread among several different groups of wild birds, and appears capable of infecting all species of birds and some other vertebrates, including humans. Newcastle Disease (ND) in domestic poultry is a focus for concern throughout much of the world's agricultural community because of severe economic losses that have occurred from illness, death, and reduced egg production following infection with pathogenic or disease causing strains.

### **Distribution and Host range**

ND viruses are worldwide in distribution. NDV is capable of infecting a wide variety of avian species. In addition to poultry, more than 230 species from more than one-half of the 50 orders of birds have been found to be susceptible to natural or experimental infections with avian paramyxoviruses. Most infections are asymptomatic and do not result in disease.

### **Clinical signs**

Clinical signs, observed only in sick juvenile double crested cormorants, include torticollis or twisting of the head and neck, ataxia or lack of muscular coordination, tremors, paresis or incomplete paralysis including unilateral or bilateral weakness of the legs and wings, and clenched toes. Paralysis of one wing is commonly observed in birds surviving NVD infection at the Salton Sea in southern California.

## Control

Vaccination with both live and killed vaccines is used in domestic birds as an adjunct to biosecurity procedures, which are the primary means of prevention of ND. As soon as ND is suspected, strict biosecurity procedures should be followed to contain the outbreak as much as possible and to prevent disease. Wildlife managers should work with local poultry enterprises to reduce the risk of having ND enter these premises from wild bird sources and the risk of wild birds becoming exposed to ND virus strains present in poultry.

## Bacterial Diseases

### 1 Avian Cholera

**Synonyms:** Fowl cholera, avian pasteurellosis, avian hemorrhagic septicemia

## Introduction

Avian cholera is a contagious disease resulting from infection by the bacterium *Pasteurella multocida* an encapsulated Gram-negative bacterium whose shape may vary from a rod to a coccobacillus. Epizootics of avian cholera typically occur in wetlands with abundant waterfowl populations or at breeding colonies with high densities of birds. Mortality often involves multiple species of birds.

## Distribution and Host range

In domestic birds, avian cholera probably occurs worldwide. Recent reports of avian cholera in wild birds are considerably more limited in global distribution. More than 190 species of birds, from at least 44 families, have been reported as naturally infected with *P. multocida*. Bird groups most frequently affected by avian cholera are waterfowl and coots, followed by scavengers (gulls, raptors, and crows), and to a lesser extent other water birds (waders, shorebirds, and cranes) and upland species.

## Transmission

Transmission of *P. multocida* to susceptible animals has been hypothesized to occur in several ways, including inhalation, ingestion, cat bites, and arthropod bites. Transmission to susceptible birds from contaminated wetlands or from direct bird-to-bird contact are the most likely routes of transmission during epizootics.

## Clinical signs

Sick birds often appear lethargic or drowsy and they can be approached quite closely before they attempt to escape. When captured, these birds often die quickly, sometimes within a few seconds or minutes after being handled. Other birds have convulsions swim in circles, or throw their heads back between their wings and die. These signs are similar to those seen in duck plague and in some types of pesticide poisoning. Other signs include erratic flight, such as flying upside down before plunging into the water or onto the ground, and attempting to land a foot or more above the surface of the water; mucous discharge from the mouth; soiling and matting of the feathers around the vent, eyes, and bill; pasty, fawn-colored, or yellow

droppings; and blood-stained droppings or nasal discharges, which also are signs of duck plague.

## Treatment and Control

Antibacterial chemotherapy (sulfonamides and antibiotics) has been used extensively in the treatment of avian cholera in domestic birds and could be used in captive birds. Vaccination is commonly used in domestic fowl to reduce the potential for disease epizootics and related mortality. Ponds can be treated with copper sulfate mixed in hydrochloric acid, small puddles and water holes have been disinfected with a cresylic compound, and small drinking ponds have been filled in attempts to eradicate the disease.

### 2. Tuberculosis

**Synonyms:** Mycobacteriosis, tuberculosis, TB

## Introduction

Tuberculosis occurs worldwide in birds as a contagious, chronic, bacterial disease caused principally by *Mycobacterium avium* and less frequently by *M. genavense*. Less commonly, tuberculosis in birds is caused by *M. intracellulare*, *M. fortuitum*, *M. tuberculosis*, *M. gordonae*, and *M. nonchromogenicum*.

## Distribution and Host range

Avian tuberculosis occurs in captive and free-flying wild birds worldwide including reports from North America, Europe, Australia and India. A wide range of free-living and captive birds in at least 15 orders have been diagnosed with avian tuberculosis. Tuberculosis occurs in many orders of birds and susceptibility varies; incidence in captive collections appears to be highest in Anseriformes (ducks, geese, and swans), Gruiformes (cranes), and Galliformes (pheasants, chickens, and turkeys)

## Transmission

Avian tuberculosis generally is transmitted by direct contact with infected birds, ingestion of contaminated feed and water, or contact with a contaminated environment. Inhalation of the bacterium can cause respiratory tract infections.

## Clinical signs

Birds with avian tuberculosis usually are sick for a few weeks to several months with anorexia, progressive weight loss, dull and ruffled feathers, weakness, Infected birds are often emaciated, weak, and lethargic, and they exhibit wasting of the muscles. These signs are similar to those of lead poisoning and other debilitating conditions. Other signs depend on which body system is affected and signs may include diarrhea, lameness, and unthrifty appearance. Darkening and dulling of plumage have been reported in the United Kingdom for wood pigeons infected with tuberculosis, but not for other species.

## Treatment and control

Tuberculosis rarely causes a major die-off, and there are no practical nonlethal testing procedures for mobile wild birds. Therefore, there is no focal point and, hence, no method developed for disease control in wild bird populations. By contrast, tuberculosis can cause die-offs in captive flocks, and mortality has been reported in sea ducks and other birds, including chukar partridge and pheasants. Some captive flocks of wild birds have experienced losses of nearly 30 percent or more from tuberculosis. Treatment for avian mycobacterial infections with isoniazid, rifampin, ethambutol, streptomycin, clofazimine, and cycloserine is infrequent because drug treatment must be continued for 18 months or more, is very expensive, and involves constant exposure of other birds and humans to a very infectious organism.

### 3. Salmonellosis

**Synonyms:** Salmonellosis; paratyphoid; bacillary white diarrhea (a synonym for pullorum disease); pullorum disease<sup>1</sup>, fowl typhoid

#### Introduction

Avian salmonellosis is caused by a group of bacteria of the genus salmonella. Approximately 2,300 different strains of salmonellae have been identified, and these are placed into groupings called “serovars” on the basis of their antigens or substances that induce immune response by the host, such as the production of specific antibody to the antigen.

Current taxonomic nomenclature considers the 2,300 different serovars to be variants of two species, *Salmonella enterica* and *S. bongori*. *S. enterica* is further subdivided into six subspecies on the basis of biochemical characteristics. The outcome of salmonella infections is reported to be highly dependent upon the age of the birds, concurrent stress, serovar and strain virulence, and susceptibility of the host species.

#### Distribution and Host range

The potential avian host range of the genus *Salmonella* appears unlimited. Many serotypes do not have a specific target host, and wild birds that are closely associated with people and livestock are more likely than others to show a relatively high prevalence of intestinal carriers of these serotypes because of increased levels of environmental contamination by these bacteria.

#### Transmission

*Salmonellae* are primarily enteric bacteria, and the fecal-to-oral route of exposure is the main means of transmission. In confined captive environments with high humidity, it is possible for salmonellae to be transmitted by inhalation or conjunctival inoculation, although, in either case, some of the bacteria probably reach the pharynx and are swallowed. Egg transmission through direct infection of the ova is also reported.

## Clinical signs

Different species and ages of birds may have different signs even if they are infected with the same serovar; young birds typically exhibit more pronounced signs of disease. Infection may result in acute disease with sudden onset of death, or it may result in a more prolonged course of infection that may become septicemic or be characterized by the presence and persistence of bacteria in the blood, or result in localized infection within the body. These birds huddle, are unsteady, shiver, and breathe more rapidly than normal; their eyes begin to close shortly before death; and they exhibit nervous signs including incoordination, staggering, tremors, and convulsions. Blindness has also been reported in some birds. The rapid death of songbirds at feeding stations has often caused observers to believe the birds had been poisoned. Neurological signs, such as those described above for poultry, have also been reported in infected songbirds. In contrast, young domestic ducklings are reported to die slowly, exhibiting tremors and gasping for air. Their wings often droop and they sometimes stagger and fall over just before death. Like infected chickens, these birds often have pasted vents and eyelids that are swollen and stuck together by a fluid discharge.

## Treatment and control

Landfills and waters where sewage effluent is discharged are common feeding areas for gulls, the wild bird species group with the highest prevalence of salmonella infections. The potential point sources of infection include garbage, sewage wastewater, and wastewater discharges from livestock and poultry operations. The only practical approach to control of outbreaks and prevention of clinical salmonellosis in songbirds depends on reducing the risk of transmission by a combination of hygienic measures and attempts to reduce densities at feeding stations, where most transmission seems to occur.

### 4. Chlamydiosis

**Synonyms:** Parrot fever, psittacosis, ornithosis, parrot disease, Louisiana pneumonitis

#### Introduction

Chlamydiosis refers to an infection with organisms of the genus *Chlamydia* sp., which are bacteria that live within animal cells. *Chlamydia psittaci* is the species generally associated with this disease in birds.

#### Distribution and Host range

Among free-living birds, avian chlamydiosis has been found worldwide in the pet birds, domestic and feral pigeons, and domestic poultry (turkeys, ducks, and geese) fulmars on islands of coastal Great Britain, in waterfowl and shorebirds in the Caspian Sea, and in herons, waterfowl, gulls, and doves in the United States. Infected parrots and parakeets have been found throughout the tropics and Australia.

## Transmission

*Chlamydia* sp. are present in the tissues, feces, discharges from the eyes and nares, and may also be present on plumage of infected birds. When the excreta and discharges dry, the resulting material can become airborne. Infection may be transmitted by direct contact with affected birds, or by inhaling dried bird fecal material or respiratory exudates that contain *Chlamydia* sp. organisms.

## Clinical Signs

*C. psittaci* can cause severe, acute disease that may be rapidly fatal in highly susceptible species. Birds often become weak, stop eating, and develop purulent (fluid containing pus) discharges of the eyes and nares. Birds tend to become motionless, remain in a fixed position, huddled up with ruffled feathers. Birds may have diarrhea, sometimes rust-colored because of the presence of blood, and respiratory distress is common. Feces from birds that stop eating are often dark green. In an outbreak of chlamydiosis in wild gulls, primarily fledglings died and the birds that were found dead were typically thin. Captive snowy and American egrets with chlamydiosis exhibited weakness, abnormal gait, ruffled feathers, diarrhea, and rapid weight loss; the birds generally died 1–2 days after the onset of signs. In other species of egrets, the infection may be inapparent even though the organism can be isolated from swabs of the cloaca or respiratory tract.

## Treatment and control

Chlamydiosis can be treated with oxytetracycline (50 mg/kg) injected into food items. Sick birds should be collected and euthanized and carcasses should be picked up. The removal and incineration of carcasses will help reduce the amount of infective material in the area.

## 5. Mycoplasmosis

**Synonyms:** Chronic respiratory disease, infectious sinusitis, house finch conjunctivitis

### Introduction

Twenty-three species of *Mycoplasma* have been described in avian hosts but five of these species have been isolated from wild birds only: *M. buteonis*, *M. corogypsi*, *M. falconis*, *M. gypis*, and *M. sturni*. *Mycoplasma gallisepticum* (MG) is of known importance for wild birds.

### Clinical Signs

The prominent field signs are puffy or swollen eyes and crusty appearing eyelids. A clear to somewhat cloudy fluid drainage from the eyes has been reported for some birds. Birds rubbing their eyes on branches and birdfeeder surfaces have also been reported. Other observations of infected birds include dried nasal discharge, severely affected birds sitting on the ground and remaining at feeders after other birds have departed, and birds colliding with stationary objects due to impaired vision.

## Treatment and control

Routine cleaning and disinfection of birdfeeders with household bleach is recommended to prevent mycoplasmosis and other diseases that can be transmitted at birdfeeders. A 10 percent solution of household bleach applied weekly for feeders with high bird use will reduce the potential for contaminated surfaces to transmit disease. Tylosin, enrofloxacin, gentamicin and tetracycline have been used successfully for treating clinical disease.

## 6. Necrotic Enteritis

### Introduction

Necrotizing enteritis is caused by an enterotoxemia or toxins in the blood produced in the intestine resulting from infections with *Clostridium perfringens*.

### Distribution and Host

This disease is found throughout much of the world where poultry are produced, and it is often an important cause of mortality for adult domestic breeder ducks. Sporadic cases have been diagnosed in waterfowl collections and in wild mallards, black ducks, and Canada geese. A die-off in Florida involved mallards and other wild ducks along with several species of shorebirds and wading birds. Wild ducks are also reported to have died from this disease in Germany.

### Clinical signs

The toxins produced by these bacteria are the cause of death. The onset of death is generally rapid and without obvious clinical signs. Severe depression is sometimes observed in chickens along with reluctance to move, diarrhea, and ruffled feathers. Lesions generally appear as a mixture of dead cellular materials and plasma debris, tan-yellow in color, that covers much of the lower region of the intestine of affected waterfowl.

### Fungal Diseases

#### 1 Aspergillosis

**Synonyms:** Brooder pneumonia, pseudotuberculosis, “asper” mycosis, mycotic pneumonia

### Introduction

Aspergillosis is a respiratory tract infection caused by fungi of the genus *Aspergillus*, of which *A. fumigatus* is the primary species responsible for infections in wild birds.

### Distribution and Host range

Aspergillosis is distributed worldwide and has wide range of host. Waterbirds including penguins (Spheniscidae), pelicans (Pelecanidae), flamingos (Phoenicopteridae), loons (Gaviidae), petrels (Hydrobatidae), and shorebirds (Chradadriiformes) are especially susceptible to aspergillosis.



## Clinical signs

The typical aspergillosis-affected bird is emaciated, and it frequently exhibits severe and progressive difficulty in breathing by gaping or rapid opening and closing of the bill. Birds often appear to be unthrifty, and their wings may droop. Infected birds are usually weak and may fail to try to escape. With the exception of visible evidence of breathing difficulties, these signs are similar to those for lead poisoning. Infection that reaches the brain can result in obvious loss of muscular coordination and twisting of the head and neck so that the head is held in unnatural positions. Inflammation of the covering of the brain or meningoencephalitis with associated areas of brain tissue death has been reported for eider ducklings dying from aspergillosis.

## Treatment and control

Treatments for birds with the antifungal agent amphotericin B (usually in combination with other drugs such as 5-fluorocystocine, itraconazole, fluconazole, or clotrimazole). Avoid using moldy or dusty straw, silage, or feed, and dumping moldy waste grain in areas where waterfowl and other birds feed. Birds should be denied the use of fields where moldy agricultural waste products such as waste corn, peanuts, straw, or hay have accumulated.

## 2 Candidiasis

**Synonyms:** Moniliasis, candidiasis, thrush, sour crop

### Introduction

*Candida albicans*, a yeast-like fungi, is the primary cause of candidiasis or candidosis. *C. albicans* is a normal inhabitant of the human alimentary canal, as well as that of many species of lower animals.

### Transmission

Ingestion in food or in water is the usual means for its transmission. Contaminated environments, such as litter from poultry and gamebird rearing facilities, refuse disposal areas, discharge sites for poultry operations, and areas contaminated with human waste have all been suggested as sources for *Candidia* exposure for birds.

## Clinical signs

There are no unique signs of disease. Affected poultry have retarded growth, stunted appearance, are listless, and have ruffled feathers. Lesions are generally confined to the upper areas of the digestive tract. The mouth, esophagus, and, primarily, the crop, may have grayish-white, loosely attached, plaque-like areas on their internal surfaces.

## Treatment and control

The infrequent reports of this disease in free-ranging wild birds do not warrant the need for disease control. This disease is more likely to be encountered in captive-rearing situations. Disease prevention should be practiced to prevent infections. Cages, equipment, and other

materials in contact with infected birds should be disinfected because of the broad host range of species that can become infected.

## Parasitic Diseases

### 1 Hemosporidiosis

**Synonyms:** Avian malaria

#### Introduction

Hemosporidia are microscopic, intracellular parasitic protozoans found within the blood cells and tissues of their avian hosts. Three closely related genera, *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*, are commonly found in wild birds. Infections in highly susceptible species and age classes may result in death.

#### Distribution

The avian hemosporidia are cosmopolitan parasites of birds, and they have been found in 68 percent of the more than 3,800 species of birds that have been examined. Members of some avian families appear to be more susceptible than others.

#### Transmission

Hemosporidia are transmitted from infected to uninfected birds by a variety of biting flies that serve as vectors, including mosquitoes, black flies, ceratopogonid flies (biting midges or sandflies) and louse flies.

#### Clinical signs

These include emaciation, loss of appetite, listlessness, difficulty in breathing, and weakness and lameness in one or both legs. Survivors develop persistent, low-level infections in the blood and tissues that stimulate immunity to reinfection. These survivors do not exhibit any signs of disease, but they serve as reservoirs of infection, allowing the parasites to survive droughts and cold winter weather when vector populations have died off.

#### Control

Control of the avian hemosporidia is dependent on reducing transmission from infected birds to healthy birds through reduction or elimination of vector populations.

### 2 Trichomoniasis

**Synonyms:** Canker (doves and pigeons), frounce (raptors), avian trichomoniasis

#### Introduction

Avian trichomoniasis is caused by a single celled protozoan, *Trichomonas gallinae*. *Trichomonas gallinae* is a cosmopolitan parasite of birds including finches, pigeons, doves, turkeys, chickens, parrots, raptors (hawks, golden eagle, etc.).

Transmission of the parasite from one bird to another occurs in one of three ways: Infected parent feeding young, Contaminated drinking water, Infected bird is a prey meal for another bird.

### **Clinical signs**

Because oral lesions often affect the ability of the bird to feed, infected birds lose weight, appear listless, and stand grouped together. These birds often appear ruffled. Caseous or cheesy, yellowish lesions may be seen around the beak or eyes of mourning doves and the face may appear “puffy” and distended. Severely infected pigeons may fall over when they are forced to move.

### **Treatment and control**

For control the removal of infected birds is recommended for combating trichomoniasis in poultry and captive pigeons and in captive collections of wild birds. The focus in both instances is on birds that harbor virulent strains of the parasite. Elimination of infection from adult birds by drug treatment has also been recommended, but this is not a practical approach for wild birds.

## **3. Sarcocystis**

### **Introduction**

Sarcocystis is a nonfatal, usually asymptomatic infection that is caused by a parasitic protozoan. Various species of this parasite affect mammals, reptiles, and birds. The most commonly reported species of the parasite in North America is *Sarcocystis rileyi*, the species most commonly found in waterfowl.

### **Distribution and host range**

Infections have been reported from Africa, Europe (Germany, Spain and Poland), the United States (California), Central and South America, China, India, Tibet, Malaysia, and Southeast Asia. Sarcocystis is a common parasitic infection of some waterfowl species.

### **Clinical signs**

Usually, there is no externally visible sign of this disease nor is it recognized as a direct cause of migratory bird mortality. Severe infections can cause loss of muscle tissue and result in lameness, weakness, and even paralysis in rare cases. The debilitating effects of severe infections could increase bird susceptibility to predation and to other causes of mortality.

### **Control**

There are no known control methods for this disease, nor do any seem to be needed or are any being developed. Control of sarcocystis would require interruption of the life cycle of the parasite.

## **4. Eustrongylidosis**

**Synonyms:** Verminous peritonitis

## Introduction

Eustrongylidosis is caused by the nematodes or roundworms *Eustrongylides tubifex*, *E. ignotus*, and *E. excisus*. *Eustrongylides* spp. can cause large die-offs of nestlings in coastal rookeries, especially of egrets and other wading birds.

## Clinical signs

Disease results in a variety of clinical or apparent signs that are not specific to eustrongylidosis. However, consideration of the species affected, the age class of birds involved, and the full spectrum of signs may suggest that eustrongylidosis is the cause of mortality. Very early in the infection as the worm is penetrating the ventriculus, some birds will shake their heads, have difficulty swallowing, have dyspnea or difficult or labored breathing and, occasionally, regurgitate their food.

## Treatment and Control

Treatment for eustrongylidosis is limited in the wading-bird population due to the extensive amount of perforation in the stomach lining. Treatment of the infected birds (a large portion of wild populations) has not been found, nor will likely be practical. Control of eustrongylidosis depends on the difficult task of disrupting the parasite life cycle, which is further complicated by the length of time that the eggs can remain viable and that intermediate hosts can remain infective.

## **SAMPLE COLLECTION PROTOCOL FOR AVIAN DISEASE SURVEILLANCE**

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### **Importance of sample collection**

- Sample collection is one of the first and most important steps in any investigation. Laboratory results are extremely valuable, as they can help identify the causative agent, provide information about the mode of transmission and identify possible sources of disease.
- It is a foundational principle for any laboratory test procedure that the value of the test is compromised or even negated by using specimens that have not been properly collected, labelled, handled or stored prior to and during the testing process.

### **For correct diagnosis in the laboratory, the tissue materials must contain the following qualities**

- Correct labeling and identification
- Correct packing
- Correct transportation so that materials reach to laboratory in the shortest possible time.
- Identification of samples: In the covering letter, all particulars and a complete history including the following points should be submitted
- Owner's name and address
- Description of the animal/bird including species, age, sex, colour, number of ear tag/brand/tattooing etc.
- Duration of condition or outbreak
- Morbidity and mortality rate
- Clinical signs
- Clinical diagnosis
- Treatment History
- Time of bird/animal's death and that of necropsy
- Necropsy report
- Nature of feed including any recent change of feed
- Possibility of contact with the animal of neighboring farms
- Specific tests required
- Type of preservatives used
- Veterinarian name, address and telephone number.
- It is better to forward one copy of letter by post and to enclose another in the parcel containing specimen.

### **Protocol for sample collection and surveillance**

- As per FAO guidelines mortality investigation/ sample collection surveillance contains following steps (Special reference with Avian Influenza)
1. Clinical signs of infectious disease: triggers for disease investigation
  2. Live bird handling

3. Dead bird collection
4. Necropsy Sample collection
5. Sample collection list
6. Swabbing technique
7. Sample handling and transport

### **Clinical signs of infectious disease: triggers for disease investigation**

- For any disease investigation or surveillance reporting of clinical signs in animals affected is most important steps as it alarms us about the future outbreaks.
- Reports about sick wildlife from the general public are often the first indication that a larger mortality event is about to occur, and given the economic and political implications of the emergence of H5N1 AI in a new location, it is best to know at an early stage of disease emergence, that it is present. This will allow management steps to be taken, potentially prevent the spread of the disease to agricultural flocks and other wildlife, and ultimately, be more cost-effective than managing a large scale disease outbreak.
- Zoos, wildlife sanctuaries, rehabilitation centres or similar institutions that house birds in outdoor setting should also be briefed on clinical signs to watch for in their captive wild birds. If they observe any of these clinical signs, they should follow proper isolation procedures for the sick birds, have their staff veterinarian examine the bird immediately, follow appropriate procedures for collecting information and samples.
- If any of these clinical signs are observed in free ranging wildlife species, ranging wildlife species, either in an either in a few birds or many individuals, one should consider conducting a disease outbreak investigation.
- Inform a proper authority about the alarming situation.

### **Live Bird Handling - in the field or a facility**

- Always handle apparently healthy bird first followed by sick and at last dead bird
- Wear proper protecting clothes – latex gloves, face mask and protective eye cover.
- Do not use mobile, eat or drink in between handling of live or dead bird
- Be sure to wash your hands and disinfect /dispose of your instruments and clothes prior to leaving the field site
- Free-ranging birds may be captured by a number of methods including nets, live traps, and spotlighting.
- Note that surveillance for AI viruses and other infectious diseases, particularly in absence of a disease outbreak or dead birds in the area, can be performed by sampling healthy live birds.
- Once wild birds are captured, it is important to keep them in a well ventilated, cool, quiet environment to prevent them from overheating and to minimize stress-Capture myopathy

- Blood can be collected from the jugular vein (right side of the bird), basilic vein (wing vein), or medial metatarsal vein (leg vein) and immediately transferred to a serum or plasma separator tube.
- Oral and cloacal swabs which should be refrigerated if possible for AI surveillance.
- In certain instances, other samples may be requested to facilitate additional research such as feather samples for heavy metal analyses.
- Finally, when possible, it is best to place a stainless steel band on the bird's leg for individual identification purposes at a later time
- If there is need of euthanizing the affected bird to prevent further spread, sample should be collected before euthanasia.

### **1. Dead Bird Collection**

- When there is no facility for sample collection whole dead bird can be packed and shipped to the nearby pathological lab.
- Upon arrival to the site, evaluate the extent of the die off including how many birds, what species, are other wildlife or domestic animals involved, and over what geographic range has this event occurred. This information should all be recorded on the Sick or Dead Bird Mortality Event Sample Collection Log: Submitter's Cover Page
- In addition to preparing for animal sample collection, you may want to also consider collection of other environmental samples including water, soil, vegetation, or other samples that you think may have played some role in the mortalities.
- The best method to collect a dead bird is to invert a plastic bag around your gloved the hand and then surround the animal with the bag so that you do not directly touch the animal and have a double protective barrier.
- Seal the bag tightly (double bag if required for strength and cleanliness) and clearly and label the bag with the Animal Identification Number, species, date, time and location where the animal was found.
- If more than one species is affected, at a minimum, collect several specimens of each species for diagnostics.
- In general, carcasses that have been dead for less than 24 hours are best for diagnostic purposes. (fresh)
- For each affected species, select up to 3 birds that have recently died (less than 24 hours), up to 3 sick birds (suffering respiratory, neurologic or gastro-intestinal disease or moribund) and up to 3 apparently healthy birds in direct contact with the currently sick birds.
- If possible, also conduct a survey of other live birds that share the same habitat (cloacal swabs and/or tracheal swabs only). Priority should be given to birds that share wetlands with affected birds since the main mode of transmission of AI is probably fecal contamination of water, shores, or banks.

## 1. Necropsy Sample Collection

- A necropsy is performed to determine the cause of death and it involves careful examination of the carcass both externally and internally.
- The ability of the lab to diagnose the cause of death is dependent on how well the necropsy is performed, how carefully samples are collected, labelled, stored and delivered to the lab.
- If the field necropsy is done well, it will increase the likelihood of diagnosing the cause of death
- Each sample collected must be properly labelled with the Animal ID, species, location, date, and organ or sample type.
- Always label the jar or vial rather than the lid to ensure that the identification is not lost when lids are removed during sample handling.
- Use only one Animal ID per bird, even if you are collecting several samples from the animal.
- Ensure that labels are made with pencil or permanent ink, which will not dissolve in the fixative that you are using (alcohol-based fixatives will dissolve the ink of many “permanent/indelible” markers).

### 1. Sample Collection List

- Sample should be collected as your priority of, sample which is most specific for the disease should be collected followed by less specific-
- Oropharyngeal swab–
- Cloacal swab–
- Serum or Plasma - from a live animals or centrifuge Serum or Plasma heart blood from a dead animal –
- Fresh tissue samples collected into sterile vials a Fresh tissue and frozen: Liver, kidney, trachea and lung, air sacs, brain, spleen, pancreas, intestine, proventriculus, cecae and heart
- Plus: Half of any lesion Cecae and intestine if the animals exhibit diarrhoea.
- **Formalin fixed tissues:** -Brain, trachea, lung, heart, liver, kidney, spleen, pancreas, bursa of Fabricius if present, proventriculus/ventriculus, duodenum, cecae, skin including feather follicles, half of any lesion.
- Place samples into polypropylene screw-top, gasketed cryovials with liquid nitrogen safe labels only.

### Samples to collect for to collect for H5N1 AI

- All birds cloacal swabs ± tracheal swab in viral transport media (always in duplicate- one for RT-PCR and one for virus isolation)
- All necropsied birds Piece (at least 2cm x 2cm but larger is acceptable) of spleen and lung, and any obviously abnormal tissue



### Samples from Sick and healthy in-contact birds

- Blood into red or green top tubes, refrigerated, serum or plasma placed into a cryovial and frozen.

#### 1. Swabbing Techniques

- Swabs taken from the cloaca (vent) and oropharynx (back of the mouth/throat) then stored in viral transport medium can be used for viral culture or RT-PCR to detect the presence of a variety of viral pathogens.
- Viral transport media can either be prepared locally at a lab (2.5% veal infusion broth, 0.5% BSA, 100 µg/ml gentamicin sulfate, 2 µg/ml amphotericin B in distilled water) or commercial kits may be purchased.
- When viral transport medium is not available, nasal turbinates or trachea may be a suitable substitute for a tracheal swab and feces may be a suitable substitute for a cloacal swab.
- Use either a sterile plain polyester swab on a plastic applicator, don't use cotton swab as it hinders the PCR results.
- Cut or break off the part of the applicator that you touched while depositing the swab into the vial of viral transport medium, or use commercially available sterile swabs that come with a tube containing transport medium.

### COLLECTION OF MATERIALS FOR LABORATORY EXAMINATION

S. NO.	DISEASE	MATERIAL TO BE COLLECTED
1.	New Castle Disease	cloacal swabs or faeces should always be sampled, virus can also be isolated from homogenised organs from dead birds- Brain, Bone marrow, lung, Liver, spleen and serum samples.
2.	Infectious bursal disease	Bursa of Fabricius, Spleen, Kidney and Liver.
3.	Mark's disease	Brain, Nerve, Ovary, Testis, Liver, Spleen and Feather follicle (for virology).
4.	Lymphoid leucosis	Liver, Spleen, Kidney and Gonads.
5.	Infectious bronchitis	Trachea, Lungs, Air sac, Kidney (for virology).
6.	Infectious laryngotracheitis	Tracheal exudates for serology and virology. Trachea for histopathology.
7.	Avian Encephalomyelitis	Brain for virology, serum samples, Brain, Pancreas, Gizzard and Proventriculus for histopathology.
8.	Avian influenza	Tracheal plug, Brain and Pancreas for virology, Brain, Pancreas, Liver, Spleen and lungs for histopathology.
9.	Inclusion body Hepatitis	Liver for virology and histopathology.
10.	Egg drop Syndrome	Affected eggs for virology and serum samples.
11.	Fowl Pox	Piece from skin and other tissue lesions, serum samples.

12.	Leechi disease	Liver for virology and histopathology.
13.	Chicken infectious anaemia	Liver impression smears, Thymus, bone marrow, and liver for virology.
14.	Colibacillosis	Heart, Liver and lungs for bacteriology.
15.	Infectious coryza	Swabs from infra orbital sinus.
16.	Fowl Cholera	Impression smear from liver and lungs, heart blood smear, Liver, Bone marrow, Heart blood for bacteriology.
17.	Fowl Typhoid	Liver, Spleen and Caeca for bacteriology, whole blood and serum for serology.
18.	Pullorum disease	Liver, spleen and caeca for bacteriology, whole blood and serum for serology.
19.	Necrotic Enteritis	Intestinal contents, Scraping of intestinal wall, haemorrhagic lymphoid nodules for bacteriology.
20.	Gangrenous Dermatitis	Exudates of skin and subcutaneous tissue or underlying muscle for bacteriology.

### Preservation and Dispatch of samples

#### 1. Refrigeration:

- **By natural ice:** Natural ice is effective for properly packed samples that will be sent to the laboratory of a less distance. Tissue can be preserved for 18-24 hrs in winter and 8-12 hrs in summer. For this purpose, thermo flask is generally used.
- **By dry ice:** The tissue specimen should be placed in a plastic or other water proof container without direct contact with the dry ice. Don't send dry ice in an airproof metal container as the ice will volatilize and pressure may result to an explosion.

#### 1. Chemical preservatives

- For histopathological examination: For this purpose, fixation is done by killing and hardening the tissue with the use of fixatives. The aim of fixation is the preservation of cells and tissue constituents in as life-like manner and to allow preparation to cut thin section. eg- 10% Formalin
- For virological examination: As all the known viruses have a tendency to selective tissue localization, it is important to select appropriate specimen as free as possible from bacteriological examination.
- Tissue specimen may be placed in a sterile, wide mouthed, cork- stoppered bottles and forwarded frozen in dry ice or in 5-10 volumes of sterile 50% glycerol saline or preferably in a medium containing equal parts of pure buffered glycerine. The material should reach the laboratory in the shortest possible time after collection. Preferably over ice in a thermo flask.

**For bacteriological examination:** material must be collected in aseptic condition and displaced in sterile container.

- Sterile swab
- Sterile syringe
- If blood smear should be taken in case of Pasteurella (**Specimen for immunological examination**) –Serum sample ,tissue samples should be refrigerated in transportation. Serum for agglutination and complement-fixation test may be preserved with 0.5% phenol or methionine 1:10,000.
- Parasitological samples - During postmortem examination ectoparasites and endoparasites should be collected for identification. Ectoparasites should be collected as soon as before or after death of animal because these parasites leave the dead animal as it begins to cool.
- Ticks, fleas, lice and mites should be carefully removed from the fur/hair using a brush and preserved in 70% ethyl alcohol or methanol.
- In case of mange lesions, deep skin scrapings are preserved in a small amount of 5% formalin solution.
- Thin blood smears are prepared for the demonstration of protozoa and thick smears for microfilariae.
- Samples of feces for helminthic ova are preserved by the addition of 10% formalin. For preservation of most helminthes, 5% formol saline is used.

**Chemical or toxicological examination:**

- The viscera are to be preserved separately for the chemical analysis in clean, wide mouthed glass bottles of 1 liter capacity with stoppers. Samples are shipped under refrigerated conditions.
- If required, depending upon the situation use either saturated salt solution of salt or use rectified spirit.

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## AVIAN NECROPSY PROTOCOLS

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### Post-Mortem examination

Post-mortem examination is the examination of an individual after death by systematic dissection.

### Importance of post-mortem examination

- To establish the cause of death
- To find out whether it is contagious or zoonotic in nature.

### Types of post-mortem Examination

Complete post mortem examination: When the whole body is available to perform Post-mortem examination.

Incomplete post mortem examination: When whole body is not available to perform post mortem examination.

### Pre-requisites of PM Examination

Written requisition from competent authority- Farm manager, owner, forest authorities.

- For Vetero- legal cases: Requisition of S.H.O.
- Time of PM Examination- Immediately after death
- If not possible- overrule PM changes
- Conduct the PM in day light
- Post-mortem Examination:

External Examination

Internal Examination

### External Examination of Carcass

1. Record the details such as sex, age, wing band or leg band number etc.
2. Record the condition of body as fair (well-fed), poor, emaciated or cachectic
3. Record rigor mortis as present or absent.
4. Natural orifices-nature of discharge, if any
5. Visible mucous membrane –pink, pale (anaemia), icteric (jaundice) congested, haemorrhagic, cyanotic
6. Feather loss (patchy or complete); look of the presence of wounds, abrasions, lacerations, perforations, swelling, abscess and tumours.
7. Look for ectoparasites-ticks, lice, fleas *etc.*

## **Internal Examination of Carcass**

1. Keep the bird on its back with wings and legs extended. Abduct the legs by cutting the skin between the leg and the abdomen on each side and breaking open the hip joint and dislocating the head of each femur from acetabulum.
2. Cut the skin between the keel and the vent transversely and peel off the skin over the abdomen and legs. Free the crop from the clavicular space by blunt dissection.
3. Cut open the abdomen by incising the abdominal wall transversely between the keel and the vent and remove the sternum by cutting through the ribs and clipping the corocoid and clavicles with bone cutter.
4. Examine the muscles of the thigh and sternum.
5. Examine the joint of the limbs.
6. Part the thigh and examine the sciatic nerve with ventral side up.
7. Remove the sternum and expose the internal organs to study them in situ.
8. Remove the stomach and intestine in one piece after cutting through the esophagus just anterior to the proventriculus and at cloaca and examine the organs beneath. Examine bursa.
9. Remove the liver separately with gall bladder and then the spleen.
10. In mature females remove the ovary from the base.
11. Cut through the dorsal and ventral attachment of oviduct with mesentery and remove.
12. Remove the kidneys by blunt dissection and examine the sciatic plexus beneath.
13. Incise the pericardial sac and observe for increased pericardial fluid.
14. Remove the heart by cutting the large blood vessels arising from the heart.
15. By means of blunt dissection, free the lungs from the thoracic wall and cut through the dorsal attachment. Remove it by cutting through the trachea just anterior to the syrinx
16. Examine the brachial plexuses on either side near the thoracic inlet along with the vagus nerve.
17. Expose the nasal cavity by cutting the upper beak transversely near its attachment with the skin.
18. With the pointed end of the scissor, incise through the openings on either side to expose the paranasal sinuses and examine the abnormalities therein.
19. Open the mouth and cut from one angle of the jaw (preferably right side) with the blunt point of scissors inside the esophagus through the pharynx down the esophagus and open the crop.
20. Similarly open the trachea along its whole length from the larynx and examine.
21. Examine the condition of the ribs for softness/hardness while cutting and assess the degree of bend and snap in the long bones.
22. Section the proximal tribiotarsal and trasometratarasal epiphyses with a knife and examine for evidence of rachitic changes or abnormal structures.
23. Open and examine major joint.

24. Examine the surface of each visceral organ and incise for examination of cut surfaces.
25. Open hollow organs and examine its contents and inner surface.
26. Examine the brain by cutting and reflecting the skin over the skull and the upper mandible carefully cut the skull and lift it to expose the brain.
27. Strip off the skin from the carcass and examine skeleton, bones, joint and muscles.
28. Record the lesions in the prescribed proforma.
29. After necropsy give a pathological diagnosis' (Tentative/ confirmative)

### **Necropsy observations**

- While describing the gross lesions, weight, colour, size, shape, consistency and appearance of cut surface are to be included. Nature of exudates may be serous, mucus, fibrinous, purulent, haemorrhagic, serofibrinous, mucopurulent.
- Record the kind of bird and who had sent the carcass (owner/local police in vetero-legal cases.)
- Examine the peripheral blood smear and also the smear from oedema fluid (pigs, horse) to rule out anthrax since no PM is conducted in case of anthrax. Besides, blood parasites, other bacteria and PM invaders can be detected.

### **Post- mortem findings in Vetero- legal cases**

#### **Burns**

Burns are injuries produced by the contact of the surface of the body with flame, radiant heat or some heated solid substance like metal or grass. Injuries caused by friction lightning, electricity, X-rays and corrosive chemical substances are all classified as burns for vetero-legal proposes.

#### **Causes of death in burns**

Shock –

Severe pain due to extensive burns causes shock to the nervous system and result in a feeble pulse, pale and cold skin and collapse, leading to death, instantaneously or within 24- 48 hours. If death does not occur from shock, it may occur subsequently from toxaemia due to the absorption of toxic products from the injured tissues in the burned area.

Suffocation-

Birds removed from areas destroyed by fire are often found dead from suffocation due to the inhalation of smoke, carbon dioxide and carbon monoxide. In such cases, burns found on the body are usually after death.

#### **Lightening**

During thunderstorms, birds are sometimes struck down by lightening or discharge of atmospheric electricity in the open field or in their sheds.

Internal findings -

There may be extensive haemorrhages in the brain which is occasionally lacerated. The pericardium and surfaces of all vital organs shows petechial haemorrhages. A track is often formed indicating passage of electric current affecting the organs present in line of it.

**Electrocution**

Cases of injury or death from electric shocks may occur in birds. The wire and poles of the electricity are mainly responsible for such instances and burning marks are found on the affected areas of skin.

Internal findings -

There may be extensive haemorrhages in all vital organs and their surfaces shows petechial haemorrhages.

## GROSS AND MICROSCOPIC PATHOLOGICAL FEATURES OF IMPORTANT AVIAN DISEASES

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### Gross and microscopic pathological features of important avian diseases

All birds are categorized as the biological class “Aves” under Latin taxonomy. Out of all avians Poultry farming business is a fastly growing profitable business in Agricultural sector. The Indian poultry market, comprising of broilers and eggs was having a very good growth from year 2018 to 2019. The market is further projected to reach INR 4,340 Billion by 2024, growing at a CAGR of 16.2% during 2019-2024. Growth rate of this market very much dependent on certain factors. Disease outbreaks are one of the major threatening agents to the poultry sector so for Correct diagnosis of Poultry disease, Flock History, Clinical Signs/Symptoms Post mortem lesions, Histopathology should be known. Poultry diseases are divided into bacterial, viral, fungal and nutritional. We will discuss the gross and microscopic features of important avian (poultry) diseases.

- **Marek’s Disease (MD)**

Marek’s disease is a neoplastic disease caused by an oncogenic herpesvirus. The virus infects lymphocyte cells that, in some cases, will become tumour cells and infiltrated in different organs and tissues of the animal.

**Gross lesions:-**

- 1) The presence of tumours in liver, spleen, kidneys, lungs, ovary, muscles, or other tissues is indicative of MD.
- 2) However, nerve involvement, either grossly (swelling of leg, wing or other nerves) or microscopically, is typical of MD.
- 3) Eye involvement can be visible as an irregular constriction of the iris (ocular lymphomatosis).
- 4) Skin involvement often consists of tumours of feather follicles or in between follicles it is a reason for broiler condemnation in certain parts of the world. A proper diagnosis to differentiate MD from LL requires histological examination.
- 5) The paralysis is caused by lesions and enlargements of the affected nerves.

### Microscopic appearance –

#### In Liver

- a) The normal hepatocellular architecture is disrupted by an accumulation of large numbers of medium to large sized lymphocytes and lymphoblasts that moderately expand the sinusoidal spaces.



- b) The pleomorphic lymphoid cells are more concentrated around the central veins (centrilobular area).
- c) Multifocally, the hepatocytes are mildly compressed by the infiltrating lymphoid cells and occasionally few hepatocytes show single cell necrosis with hypereosinophilic cytoplasm, pyknotic nuclei, and a clear space around them.
- d) Similar lymphoid infiltrates were present in the kidneys, proventriculus, ventriculus, small intestine, ceca, spleen, ovary, and globes.

### **Newcastle Disease (ND)/ Ranikhet Disease**

It is a highly contagious disease, in which the usual form is a respiratory condition, but other main clinical signs may include nervous manifestations (depression, drooping wings, twisted head or paralysis), diarrhea and swelling of the eyes and the neck.

This disease also known as Ranikhet disease in India .

#### **Gross lesions:--**

- 1) Shows diphtheritic hemorrhagic lesions of the alimentary tract, mainly in the ceca and cloaca,. Haemorrhagic lesions are also observed in the proventriculus and the intestines.
- 2) It is also common to find edema and petechiae in the gizzard and hemorrhagic ovaries.
- 3) Inflamed tracheas, pneumonia, and/or froth in the airsacs are the main lesions.

#### **Microscopic appearance:-**

- 1) The lesions consisted of severe lymphoid depletion and necrosis, resulting in the accumulation of prominent macrophages, necrotic debris, and scattered heterophils. In the intestines, destruction of the MALT(mucosa-associated lymphoid tissues) resulted in necrosis and ulceration of the overlying epithelium.
- 2) The respiratory tract had severe lesions in the laryngeal tonsils and a certain degree of lymphocyte depletion and necrosis was observed within the bronchus-associated lymphoid tissue (BALT) of the secondary and tertiary bronchi, occasionally resulting in a loss of overlaying respiratory epithelium.
- 3) The myocardium showed areas of multifocal necrosis associated with the accumulation of macrophages.

### **Infectious Laryngotracheitis**

It is a respiratory disease that could appear in different presentations: peracute, subacute or chronic. Due to this, clinical signs vary from an extreme severity, with deaths by asphyxia, to a very mild symptomatology, that is not possible to differentiate from other respiratory diseases.

This virus causes fibrinous and hemorrhagic infiltration of the respiratory tract, so the presence of blood in the trachea, sinuses and oral cavity makes breathing difficult. Clinical picture with birds showing respiratory distress and expectoration of bloody mucus are indicative for ILT.

**Gross lesions:**

- 1) In the postmortem examination, fibrinous, necrotic, caseous and/or diphtheritic plaques and plugs are found in the trachea, larynx and mouth.
- 2) Lesion are found throughout the respiratory tract but most pronounced in the larynx and trachea. Depending on the severity of the infection you can find tracheitis with haemorrhagic and/or diphtheric changes.

Histopathology showing intranuclear inclusion bodies in tracheal epithelial cells.

**Gumboro Disease (IBD)**

Gumboro disease, also known as infectious bursal disease (IBD), as its name indicates, it mainly affects the Bursa of Fabricius, destroying the immature B lymphocytes of young animals.

In cases showing clinical signs of the disease, diffuse hemorrhagic lesions are often observed in pectoral muscles and thighs. Hemorrhages and erosions may also appear at the level of the proventriculus-gizzard junction, as well as different degrees of nephritis or nephrosis.

Lesions at the level of the Bursa of Fabricius are variable and depend on the evolution of the disease.

**Gross lesions:-**

- Post mortem lesions; in acute cases the bursa of Fabricius is enlarged and gelatinous, sometimes even bloody.
- Muscle haemorrhages and pale kidneys can be seen.
- Infection by variant strains is usually accompanied by a fast bursal atrophy (in 24-48 hours) without the typical signs of Gumboro disease.
- In chronic cases the bursa is smaller than normal (atrophy). The bursa destruction is apparent on histologic examination. The lack of white blood cells (lymphocytes) results in a reduction in the development of immunity and decreased resistance of the birds to other infections.

**Histopathology:-**

- The histopathological changes in the bursa of Fabricius mainly showed the fibrotic and atrophic type of changes, which were in the consonance with the gross observations, in which the bursa were found atrophic.

- Histopathologically, there was rarefaction of the bursal follicles along with the mixed cellular infiltration in the bursal follicles. The cystic changes in the bursal follicles were also evident in few cases.
- The desquamation and sloughing of bursal epithelium was a frequent finding, which led to erosion of the bursal epithelium.

### **Infectious Bronchitis (IB)**

This viral disease, exclusive of chickens, is confined to the respiratory system and the urogenital tract.

#### **Gross lesions:-**

- 1) The most frequent lesions caused by this disease are: serous, hemorrhagic, catarrhal or caseous exudate in trachea; pneumonia and/or opaque air sacs with possible caseous material;
- 2) Atrophied ovary and inflamed oviduct and interstitial nephritis, where kidneys are enlarged and pale.
- 3) Pulmonary lesions were generally not severe and the air sacs were only slightly oedematous for 4 days following exposure.

#### **Histopathology:-**

- 1) Desquamation of the ciliated and glandular epithelium followed by rapid proliferation of residual basal cells with the production of a stratified undifferentiated epithelial covering.
- 2) Small areas of the tracheal submucosa showed lymphocytic infiltration .
- 3) Necrosis of a few tubules , cystic tubules containing epithelial debris and polymorphonuclear leukocytes were prominent in both cortex and medulla .

### **Infectious Coryza (IC)**

Infectious coryza is a highly contagious bacterial disease , shows mainly rhinitis and infraorbital sinusitis, seen as facial edema in this area.

Conjunctivitis and abundant nasal and ocular secretion that may cause eyelids adherence could also appear. Usually, this state is accompanied by decreased consumption of food and water, and in laying birds, by variable reduction of egg production. Low respiratory tract infection rarely occurs.

#### **Gross lesions:-**

- 1) There is an acute catarrhal inflammation of mucous membranes of nasal passages and sinuses.
- 2) Frequently a catarrhal conjunctivitis and subcutaneous oedema of face and wattles.

- 3) Pneumonia and air sacculitis are rarely present.

### Histopathology

- 1) The nasal cavity, infra orbital sinuses, and trachea shows sloughing, disintegration and hyperplasia of mucosal and glandular epithelia, oedema, and hyperaemia with heterophil infiltration in the tunica propria of the mucous membranes.
- 2) In birds with involvement of the lower respiratory tract, acute catarrhal bronchopneumonia was observed, with heterophils and cell debris filling the lumen of secondary and tertiary bronchi.
- 3) Epithelial cells of air capillaries were swollen and showed hyperplasia, catarrhal inflammation of air sacs was characterized by swelling and hyperplasia of the cells, with abundant heterophil infiltration.
- 4) A dissecting fibrinopurulent cellulitis reported in broiler and layer chickens. In infectious coryza, lesions were confined to the upper respiratory tract.

### Chronic Respiratory Disease (CRD)

It is a contagious disease, caused by bacteria of the genus *Mycoplasma spp.* Affected animals develop respiratory symptoms such as sneezing, nasal discharge or dyspnea.

### Gross Lesions:-

- 1) Sinusitis, tracheitis, air sacculitis, thickening and turbidity of the alveoli, exudative accumulations, fibrinopurulent pericarditis and perihepatitis observed.
- 2) Uncomplicated *M. gallisepticum* infections in chickens result in relatively mild catarrhal sinusitis, tracheitis, and air sacculitis
- 3) Turkeys develop severe mucopurulent sinusitis and varying degrees of tracheitis and airsacculitis.

### Microscopically

Involved mucous membranes are thickened, hyperplastic, necrotic, and infiltrated with inflammatory cells. The mucosal lamina propria contains focal areas of lymphoid hypoplasia and germinal center formations.

### Fowl Cholera or Avian Cholera

It is a contagious bacterial disease, caused by *Pasteurella multocida* which affects adult birds or birds in the period of sexual development.

### **Gross Lesions:-**

- 1) Lesions observed in peracute and acute forms of the disease are primarily vascular disturbances. These include general passive hyperemia and congestion throughout the carcass, accompanied by enlargement of the liver and spleen. Petechial and ecchymotichemorrhages are common, particularly in subepicardial and subserosal locations.
- 2) Increased amounts of peritoneal and pericardial fluids are frequently seen. In addition, acute oophoritis with hyperemic follicles may be observed. In subacute cases, multiple, small, necrotic foci may be disseminated throughout the liver and spleen.
- 3) In chronic forms of fowl cholera, suppurative lesions may be widely distributed, often involving the respiratory tract, the conjunctiva, and adjacent tissues of the head. Caseous arthritis and productive inflammation of the peritoneal cavity and the oviduct are common in chronic infections. A fibrinonecrotic dermatitis that includes caudal parts of the dorsum, abdomen, and breast and involves the cutis, subcutis, and underlying muscle has been observed in turkeys and broilers. Sequestered necrotic lung lesions in poultry should always raise suspicion of cholera.

### **Histopathology:-**

Acute form of disease shows Coagulative areactive necrosis in the liver of a hen. Multiple nuclear debris among the necrotic tissue..

This protozoon causes enteritis of different severity, usually hemorrhagic, that is visible externally as petechiae. Species located in the cecum cause hemorrhagic typhlitis, with fresh or clotted blood mixed with fibrous exudate.

### **Coccidiosis**

Avian coccidiosis is a parasitic disease caused by protozoa of the genus *Eimeria*, originating a clinical or subclinical process characterized by bloody diarrhea and decreased production. There may also be a certain degree of dehydration, blood stained cloaca and anemia.

Destruction of the epithelial cells and the villi is what leads to a malabsorption syndrome that causes the loss of productivity of the infected animals. In addition, these lesions enable the action of other pathogens, such as *Clostridium*.

There are 7 species of *Eimeria* identified as pathogens of chickens, all located in the small intestine, except *E. tenella*, which is located in the caecum. Because of that, different lesions could be observed depending on the type of *Eimeria* and its virulence. Most of clinical outbreaks are due to mixed infections of several species of *Eimeria* and, therefore, lesions are observed in different areas of the intestine.

### **Gross Lesions.:**

This protozoon causes enteritis of different severity, usually hemorrhagic, that is visible externally as petechiae. Species located in the cecum cause hemorrhagic typhlitis, with fresh or clotted blood mixed with fibrous exudate.

### **Histologically:-**

Eimeria organisms at a various stage of development are detected in the epithelial intestinal cells. The diagnosis is made upon the results of the complex evaluation of the clinical picture, the macroscopic lesions, imprint preparations, histological study and flotation.

### **Fowl Pox or Avian Pox**

It is a moderate to severe viral disease, with a slow development, caused by a **poxvirus**.

### **Gross Lesions :-**

Lesions vary according to the stage of development: papules, vesicles, pustules, or crusts, mainly in the region of the head. Diphtheric lesions are yellowish or whitish plaques that grow on the mucous shells of the nasal and buccal cavities, sinuses, larynx, pharynx, trachea or esophagus.

### **Histologically:-**

Microscopic examination of affected tissues stained with H&E reveals eosinophilic cytoplasmic inclusion bodies.

## **BASIC HANDLING, RESCUE, REHABILITATION AND CRITICAL CARE OF SICK AND INJURED BIRDS DURING EMERGENCIES.**

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### **Introduction**

Veterinary Professional or Veterinarian should be able to recognize a bird is sick, debilitated or injured and require any medical or emergency care. The birds have an instinctive preservation reflex where they mask signs of sickness until the illness is very advanced. Birds also adopt survival strategy to avoid being "picked off" by predators and/or ostracized from the flock in emergencies. Subtle signs of "sick bird symptoms" (SBS) must be quickly recognized and acted upon, Since sick birds can quickly progress to dead birds without appropriate and timely intervention.

Many signs of illness are non-specific for any one disease problem, and can include any changes in behaviour

The birds with following signs should be seen immediately

- Bleeding from anywhere/trauma
- Breathing difficulties or abnormal respiratory noises
- Change or loss of voice
- Failure to empty crop (especially birds of prey)
- Fluffed-up appearance
- Regurgitation
- Having seizures
- Falling off perch
- Sitting on the bottom of the cage
- Sudden inappetence
- Sudden onset swellings or enlargement of any part of the body
- Sudden change in appearance of droppings
- Depressed behaviour or interaction
- Avian patients are usually presented or identified when these are either critically ill or requiring emergency treatment. The condition is more serious, where such illness, disease or any life threatening condition is seen in Migratory birds or native wild birds inhabiting in different ecosystems

### **Handling of injured Bird –**

- Do no harm to the bird and don't let the bird harm you
- Stress kills so don't handle a bird that is stressed
- Put it in a quiet, dark box and let it calm down for 10-15 minutes before handling the bird

- Have everything ready- catching net, towels, gloves, leg bands, medicine - to minimise handling time
- Use towels or gloves to pick up the bird
- Immobilize the head to reduce stress
- Cover the head with a dark cloth
- Hold the wings close to the bird's body to prevent further injury or Flapping cover the bird's eyes with the towel to minimize stress
- Caged Birds may need to be restrained for a variety of reasons such as while in the shelter, during transportation, while examination by the veterinarian, Medication and for safety reasons.
- Trained staff to properly handle and restrain a bird to avoid injury to the bird and to the handler and to prevent escape.
- Wild Birds may be restrained and handled during rescue from emergency situation, injured/ traumatized, found sick and critically ill
- Care must be taken while handling wild birds because following part of birds may be used as weapon.

### **Beaks**

- A raven's beak has enough striking force to punch a hole in your skull!
- Raptor beaks well adapted for tearing flesh and can inflict severe wounds.
- Heron beaks known to pierce eyeballs like plucking fish from the water

### **Talons**

- All raptors have large, sharp talons and very strong feet
- 2/3 persons may be required to open the toes of a strong eagle

### **Wings**

- Birds, such as swans and geese, use their wings as weapons and can inflict boxer-like blows to your face and body.
- Always use a blanket or sheet with such birds and keep their wings restrained at all times while holding them.

### **Important consideration during handling**

- Birds have no diaphragm - Free movement of the sternum is essential for respiration in birds.
- They breathe through expansion and contraction of their air sacs facilitated by their intercostal muscles not from lungs.
- Any undue pressure on their sternum would restrict breathing.
- When handling a bird, fingers should never be closed around the chest, rather cupped in the hand to allow for sternal movement.
- Careful monitoring the bird is essential for any signs of discomfort, stress or breathing difficulty due to struggling.



- Efforts to escape can lead to hyperthermia, especially if in a towel, Alert if the bird begins to pant heavily.
- If the bird is in extreme discomfort, release the bird and allow it to return to normal breathing before continuing, particularly as the bird may be compromised by a disease condition anyway.
- Overzealous restraint of a bird could lead to fracture or dislocation of a limb, fractures, bruises
- Small birds can be captured and restrained bare-handed, however, protection is required for larger birds.
- Cloth or paper towels are preferable as they expedite both capture and restraint. Hands are hidden behind the towel and protected and also the towel can be draped over the bird during capture so that the wings are protected.

### **Stress**

- During captive situations, wild birds are usually under significant stress, particularly, during phases of rehabilitation that require frequent capture and treatment.
- Experience of Veterinarian with individual birds will dictate one's approach to capture and restraint, but one should be aware that
- A slow, careful approach to capture followed by restriction of vision during restraint will generally yield best results.

### **Handling of Raptors**

- Although, injured birds may not have enough energy to put up a fight, but we must be prepared and careful, in case they find hidden energy and attack to the rescuer.
- Use a large towel or a blanket, coat, tarp, or similar sheet
- The bird is bundled in the towel with head covered
- Grasp the bird gently around the shoulders, keeping the wings folded in the natural position against the body.
- Horizontal Restraint of a Raptor
- Both legs firmly held in one hand, drapes the other arm across the neck, and cups the far wing with their forearm.
- For fractious birds, carefully wrap the feet with tape or self-adherent dressing
- Rescue of Injured Bird
- Rescue the animal in need. This process varies according to emergency situation and species of bird, sometime required to immobilisation by suitable method to capture it.
- On many instances, we find a sick or injured bird, we plan for the rescue and treat the inciting cause of suffering to save the life of the bird.

### **Rehabilitation**

The International Wildlife Rehabilitation Council (IWRC) defines wildlife rehabilitation as “treatment and temporary care of injured, diseased, and displaced indigenous animals/birds, and their subsequent release to the appropriate habitats in the wild”.

It is a process that involves safely capturing, examining, diagnosing, and treating the animals using proper veterinary and hospital care, feeding, physical therapy, exercise, medicating, and a pre-release conditioning program.

#### Importance of Wildlife Rehabilitation

- Promoting Ecosystem Health

Every species plays a niche or role in its ecosystem that helps the whole ecosystem work.

- Disease Monitoring

Wildlife rehabilitation is good for human health too! Wildlife rehabilitators may be the first people to know when disease outbreaks happen because the animals with the disease are brought to them for care.

- Responsible Stewardship

Wildlife is everyone's responsibility, and deserves care from all people.

It is very rewarding to know that an animal survived and was able to be released because of something you did!

- Conserving biodiversities and wetland areas

wildlife rehabilitation is very important in emergencies during disease outbreaks which responsible for disturbing ecosystem and biodiversity by harming large number of avifauna

#### **Immediate Care of the Bird**

- Evaluate and examine the bird for critical conditions and administer emergency care as needed
- Provide quiet rest space in a quarantine area, separated from domestic animals human traffic and potential irritants.
- Domestic animals must not be in proximity to any wildlife under care where its presence will interfere with its care.

#### Temperature

- Ambient temperature to house most sick adult birds is 31 – 33° with a relative humidity of 50 – 70%.
- By providing a warm environment, the bird can preserve its energy for recovery and healing.
- If the environment is too dry, further risk dehydration

#### Hyperthermia

- This occurs when the birds has been exposed to heat or is overheated as a result of exertion or stress.
- Clinical signs include muscle twitching, seizures, panting, open mouth breathing, wings held away from body, bright red MM, trembling and collapse
- Rapid action must be taken if an bird is showing signs of heat stress. Remove any

source of heat directly affecting the animal.

### Hypothermia

- Clinical signs of hypothermia may include cold to touch, unresponsive, unconscious, trembling, slow heart rate, pale mucous membranes (MM).
- Animals that are hypothermic need to be warmed up as quickly and stress free as possible. A warm room is a good start.
- An infrared heat lamp can also be used if available.

### Transportation of birds

- The container must be large enough for the bird to fit comfortably but not large enough to fly or move around.
- Air holes are essential but should not be large enough for wings to be caught in
- Place a cloth inside the box for grip
- Cover with a dark cloth to reduce stress

### Shock and Dehydration

- Shock occurs when there is failure of the body's circulation to provide blood supply to the tissues.
- All animals that come into care should be assumed to be in shock and treated with supportive therapy.
- Signs of shock include: collapse, hypothermia, increased heart rate, increased respiration, head nodding, closed eyes, fluffed feathers.
- Supportive therapy that can be provided by the rehabilitator include providing heat, quiet stress free environment and providing oral fluids (if this is not too stressful).
- Do not overly disturb the bird
- Always assume that injured wildlife presented will have some degree of dehydration.

### Managing Haemorrhage

- Haemorrhage may internal or external. There is little that you can do for internal bleeding other than support and observe. Internal bleeding can be difficult to detect without the use of diagnostic tools.
- Alternatively stem the blood flow by placing a wad of gauze over the site and bandage this into place firmly. (Birds need to expand their chest to breathe - do not bandage too tightly).
- Potassium permanganate, ferric chloride works well to stop bleeding.
- Broken pin/blood feathers may bleed profusely. Options here include clamping the shard feather until the bleeding stops.
- Feather plucking is extremely painful and should be done only if essential.
- In some cases the bird requires an anaesthetic to do this.
- Always pull feathers in the direction of growth.

### Provision of food and shelter

- Shelter as per habitat helped in reducing the stress and early recovery.

- Level of the fear reduced to a great extent

#### Provision of Treatments/ Intensive Care

- Provide fluids
- Manage wounds and/or injuries
- Administer medications
- Provide nutrition
- House in appropriate facility
- Employ appropriate techniques to minimise imprinting as required

#### Acclimatisation

- Monitor weight
- Provide ongoing, appropriate nutrition
- Treat medical problems as needed (should be minimal)
- Climate acclimatisation
- Environmental acclimatisation
- Provide comfortable, appropriate housing and habitat with mental stimulation
- Minimise interaction with human activity
- Provide environmental physical therapy as needed

#### Nutrition/ Feeding

- A balanced diet appropriate to species and age of the animal is essential. Food must be of the good quality.
- Almost all birds, whatever their traditional diet, feed their young insects (protein). The exception being doves, pigeons, finches and parrots which obtain their protein needs from seed
- Specially formulated commercial food mixes are scientifically designed to provide a balanced diet
- If needed tube feeding/ forcep feeding and medication be used
- Forceful hand feeding helped in rapid recovery of the birds

#### Pre-Release Conditioning

- Provide larger, outdoor housing to develop natural behaviours
- Monitor weight and general condition
- Minimise interaction with human activity
- Provide ongoing, appropriate nutrition, introducing a more natural diet
- Exercise daily, as appropriate for that species

#### Release

- Appropriate habitat and within its natural range for that species.
- Choose appropriate season/time of year (migration, breeding season, etc.)
- Choose appropriate time of day
- Identify forecasted weather for suitability

- Provide food if appropriate
- Provide proper/safe transportation
- Monitor post-release if possible

#### Where to Release

- Rehabilitated animals and birds must, where possible, be released where the animal originated from, within the animal's normal home range and where such fauna is ordinarily found in the wild.
- If the site is no longer suitable due to habitat loss or other reasons, an alternative suitable site must be selected

#### **First aid and emergency care for the Avian casualty**

- Many birds, whether wild or domestic either sick or injured, require
- appropriate first aid and emergency care in order to stabilise their condition

#### **First aid**

- Skilled application of accepted principles of treatment following any injury or sudden illness using the facilities available at the time.
- It is the approved method of treating a casualty until it can be placed in the care of a medical professional.
- preserve life
- prevent worsening of the condition
- promote recovery

#### **Emergency care**

- Also designed to stabilise a patient's condition, but is provided by the medical professional.
- First aid is only provided until the casualty can be placed under veterinary care, at which point appropriate emergency care can be provided and the bird's condition further stabilised. The emergency medication and procedures are mainly-
  - Proper airway and breathing
  - Circulation.
  - Analgesia and Anti-Inflammatories
  - First aid for soft tissue and orthopaedic injuries
  - Antibiotic therapy
  - Warmth, quiet and darkness
  - Treatment of poisoning.

#### **Airway and breathing**

- The dyspnoeic patient should be placed in an oxygen cage.
- Induction chamber - 5 to 10 litre with plumbing attachments for connection to an anaesthetic circuit, is ideal.
- This can be covered with a towel to further calm the casualty. Alternatively, oxygen can be piped into the bird's airspace

- Cause of dyspnea must be established – whether Intrinsic or Extrinsic to the respiratory system, and whether it is inspiratory or expiratory in nature
- Severe dyspnoea results in more prominent extension of the tongue and glottis on inspiration and a greater degree of tail bob and ventral abdominal muscle excursion.
- Acute blockage of the upper airways due to, for example, inhalation of a foreign body or syringeal obstruction caused by a granuloma, causes inspiratory stridor and/or changed vocalisation.
- Obstruction of the intrathoracic airways by exudates or granulomas results in a prolongation of the expiratory phase and eventually expiratory stridor. The parabronchi of the avian lung do not collapse
- Consequently, moist or dry rales are heard in birds, as opposed to the wheezes usually heard in mammalian lungs.
- For gradual induction in critically ill patients, oxygen should be supplied first.
- Anaesthesia with isoflurane (0.25 per cent) and gradually increased slowly to 1.5 or 2.5 per cent over two to five minutes, with maintenance at 0.75 to 2 per cent
- The mouth and airway should be swiftly cleared using swabs and suction, if available.
- Tracheal intubation and/or air sac cannulation and/or mechanical ventilation can subsequently be used to maintain respiratory function.
- Uncuffed endotracheal tube (2.0 to 4.5 mm in diameter) used to avoid damaging the rigid tracheal walls.
- If the Crop is full, it has to be emptied by placement of crop tube

## **Circulation**

### **Bleeding**

- A damaged blood feather that is causing bleeding in a bird should be removed. The base of the feather should be identified and grasped firmly with a haemostat.
- The skin over the follicle is held between the finger and thumb and the feather gently pulled from the follicle.
- Any bleeding from the dermis should be controlled by applying pressure on the follicle or by packing it with surgical gel.
- Radiosurgical cautery should not be used as subsequent inflammation and tissue damage

## **Fluid therapy**

- The assessment of dehydration is especially important in birds as their high metabolic rate results in a rapid deficit in fluids (and nutrients) after a short period of anorexia.
- Avian skin is much thinner and less pliable than that of mammals and this makes assessment of skin tenting more difficult. The hydration status of birds can be evaluated

- Maintenance fluids should be provided at 50 ml/kg/ day (higher in passerines), in addition to which any fluid deficit should be corrected. Oral, subcutaneous, intravenous and intraosseous routes can be used.
- In all cases, the fluids to be administered should be warmed to 38 to 39°C in order to avoid the risk of life- threatening hypothermia.
- The use of a vacuum flask to keep oral fluids warm in the field is strongly advisable.

#### Fluid therapy regimen

- Fluid deficit = Percentage dehydration x Bodyweight
- DAY 1. Administer 50 per cent of the fluid deficit + maintenance (50 ml/kg)
- DAY 2. Administer 25 per cent of the fluid deficit + maintenance (50 ml/kg)
- DAY 3. Administer 25 per cent of the fluid deficit + maintenance (50 ml/kg)

#### Oral fluid therapy

- In the absence of digestive and/or neurological problems, oral fluid therapy is likely to be the route of choice.
- It is a quick, straightforward and effective way of administering fluids.
- The bird should be held upright by an assistant, and wrapped and restrained in a towel
- A stainless steel crop tube or urinary catheter can be introduced into the oral cavity. A dose of oral fluids can then be administered, while taking care to monitor the bird for signs of regurgitation.
- For birds > 1 kg bodyweight, the dose should not exceed 2 per cent of bodyweight, while for smaller birds, it should not exceed 3-5 per cent body- weight
- Oral fluid therapy can be repeated as often as four to five times daily in the initial stabilisation period, providing this is not too stressful for the bird.

#### Subcutaneous fluid therapy

- Effectively to provide large volumes of fluid on a maintenance basis, but is not effective for the rehydration of birds with poor peripheral circulation or those that are severely hypo- proteinaemic
- Suitable injection sites include the groin, thorax, interscapular area, thighs and tibiotarsus. The abdominal and cervical areas should be avoided to prevent injection into underlying air sacs.
- A volume of 8 to 10 ml/kg can be injected (2 to 15 ml per bird) at each site
- This procedure can be repeated a few hours later following absorption of the first dose of fluid.
- Where the casualty is too sick or stressed to keep oral fluids down, an intraosseous or intravenous catheter should be placed.

#### Intravenous fluid therapy

- Ideal means of treating dehydrated and shocked avian casualties.
- A soft narrow gauge catheter (25 to 27 gauge) should be used.

- Catheters can be placed in the brachial vein, proximal to where it branches into the ulnar and radial veins, immediately proximal to the elbow on the ventral aspect of the wing.
- An alternative site for venous catheterisation is the medial tarsometatarsal vein
- Intravenous catheters can be very difficult to maintain in birds, especially once the patient becomes more active and mobile.
- They are, however, ideal for intraoperative or short-term fluid therapy.
- Care should be taken to inject fluid slowly, to avoid disrupting the thin-walled blood vessels.
- A dose of 10 ml/kg can be injected over five to seven minutes every four hours for 48 hours, and then every eight hours for 48 hours
- Slightly larger volumes have also been suggested

#### Intraosseous fluid therapy

- The intraosseous route allows almost instantaneous access to the general circulation.
- It is an excellent means of replacing fluid deficits. Intraosseous catheters are more easily maintained than intravenous ones and are less likely to become displaced.
- The femur and humerus should be avoided as they are pneumatised and communicate with the bird's respiratory system.
- Site - Tibiotarsus, ulna or radius.
- An 18 gauge needle with a stylet is suitable for use in the tibiotarsus or ulna of a 0.75 to 1 kg bird.

#### Analgesia and Anti-Inflammatories

- Opioids - buprenorphine and butorphanol, can be used to provide emergency analgesia.
- NSAIDs - carprofen, meloxicam and ketoprofen provide analgesia and a reduction in tissue inflammation, but should be used with caution in the dehydrated patient due to their potential nephrotoxicity.
- Steroids are only indicated in the face of severe head trauma where relief of increased intracranial pressure or other central nervous system trauma is desirable.

#### First Aid for Soft Tissue and Orthopaedic Injuries

- Clinical examination should identify any soft tissue or orthopaedic injuries.
- Familiarity with typical wound presentations in certain avian species will ensure that wounds are looked for and detected.
- Avian skin is thin and easily torn, there is a significant risk of tissue desiccation.. Proper dressing of wounds with lavage and protective dressings applied.
- Once the patient has been stabilised, wounds can be reassessed and a decision made about their suitability for primary closure, delayed primary closure or secondary closure
- Orthopaedic injuries should be identified and steps taken to minimise any deterioration in the patient's condition. This usually requires immobilisation of the affected limb. The patient should be confined in a small box to restrict its movement.



- A towel can be wrapped around the bird to immobilise the limbs against its body.
- A bandage applied in a figure-of-eight formation is suitable for immobilisation of an orthopaedic injury to the wing distal to the elbow and the shoulder

### **Poisoning**

- Crop washing and flushing of the proventriculus and ventriculus allows removal of any poison from the upper gastrointestinal tract are best conducted under general anaesthesia with the airway secure and protected. Warm water is always used to minimise the risk of hypothermia.
- Crop washing is achieved by passing a wide-bore tube into the crop, introducing some warm water and massaging the crop contents gently. The bird's head is then lowered to allow the contents to flow out. This procedure can be repeated, as necessary.
- Flushing of the proventriculus and ventriculus is indicated for the removal of lead shot or galvanised bits of metal, thus preventing further absorption of heavy metals.
- Once a poison has entered the intestine, it may still be possible to limit further absorption into the body by using adsorbents and astringents.
- Mixtures containing activated charcoal, kaolin and bismuth salts, are indicated in oiled birds, for example, where oil has been ingested, concurrently with oral fluid therapy.
- A general adsorbent/astringent mixture, comprising 10 g activated charcoal, 5 g kaolin, 5 g light magnesium powder, 5 g tannic acid and 500 ml water, can be used in suspected cases of oral poisoning at a dose of 2 to 50 ml according to the size of the patient. If the poison is fat soluble, liquid paraffin can be administered.

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## **NUTRITIONAL CONSIDERATIONS AND FEEDING ACTIVITIES IN MIGRATORY BIRDS**

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### **Introduction**

When the seasons change, birds begin to migrate on account of reduced or non availability of food at the location. Food that was abundant in summer in a particular area may not be available in the autumn so the birds previously feeding on these supplies will fly toward the equator where food is more plentiful. They normally breed in north in the summer period and thereafter spend the non-breeding season in warmer climate. The birds only migrate if the quality and quantity of the food source at their target destination compensates for the energy it takes to get there with no risk of becoming prey. However, no matter where they fly or how far, it is always challenging for them to cope up with the earth's rapidly changing temperature behaviour.

Long-distance migrants rely virtually entirely on stored energy and nutrients to fuel each flight, and then must rapidly restore the necessary energy and nutrients at stopover sites along their migration route. The main fuel for migratory flights in many bird species is fat deposited in the bird's body in large amount prior to and during migration. The extent of migratory fat deposition correlates positively with distance. The birds adjust their digestive systems during migration to meet the changing energy demands of migration. The size of birds' bellies increase and the cells get larger so they can eat more and store more energy for their long flights. Such a period of exceptional energy demands requires appropriate nutritional strategies. The digestive systems of migratory birds essentially shut down during migration so most of their energy can be used in flight. When they stop to eat during migration, they eat less, until finally their systems re-adjust when they arrive at their destinations where food is sufficient again.

### **Food during migration**

Migrating birds need high protein and low fat food. They also need insects. Birds will seek out the insects in the mulch of leaves, so these travellers can have plenty of high protein seed to eat as well.

### **The process of fattening and the fatty acid composition of body fat**

Birds are like other vertebrates in that they get fatter by increasing energy intake, decreasing energy expenditure, and/or decreasing excreted energy (i.e. fecal and urinary energy). Migratory birds accumulate fat stores primarily by eating more (i.e., hyperphagia) and by selecting diets based in part on total lipid content (Blem 1990, Stiles 1993, Bairlein and Gwinner 1994, Biebach 1996).

Diet composition affects both rate of fattening and the fatty acid composition of the fat deposited. For example, diets with high protein-to-calorie ratios minimize fat storage and enhance muscle building in chickens because excess protein increases levels of insulin-like growth factor-I which inhibits fat deposition. In contrast, diets with low protein-to-calorie ratios maximize fat storage (Rosebrough and McMurtry 1993, Klasing 1998).

### **Behavioral preferences of birds for diets with specific fatty acids**

Migratory birds prefer diets with specific fatty acids and this may allow birds with quite different food habits to select those foods with certain fatty acid compositions. For example, red-eyed vireos preferred diets with long- chain unsaturated fatty acids (18:1) over diets with long- chain saturated fatty acids (18:0), and they ate more when offered diets with mostly unsaturated fat than when offered diets with more saturated fat (Pierce et al. 2004). Garden warblers (Bairlein 1991) and yellow-rumped warblers *Dendroica coronata* (McWilliams et al., 2002) also preferred diets with 18:1 over diets with 18:0. Although wood thrushes preferred diets with primarily 18:1 (olive oil) over diets with 18:2 and 18:3 (soy oil, Zurovchak 1997). Thus, migratory birds prefer diets with certain fatty acids. These diet preferences for specific fatty acids may ensure that a bird's fat stores are composed of certain fatty acids prior to some energetically demanding event i.e., migration, egg- laying, living in the cold.

Recent evidence highlighted that not only the amount of fat stored but also FA composition influence bird migratory performance (Klaassen et al., 2012). FA chain length, degree of unsaturation, and placement of double bonds can all affect the rate of mobilization, transport, and oxidation of lipid reserves during flight, thus influencing overall flight endurance (Price et al., 2009; Weber 2009). Furthermore, higher levels of free fatty acids (FFA) and glycerol in plasma results from the catabolism of fat stores in adipose tissue (Jenni-Eiermann et al. 2002). Although birds can alter the FA composition of their fat stores and the phospholipids in membranes through endogenous mechanisms or through diet, they are unable to synthesize several important polyunsaturated FA (PUFAs) such as omega-3 and omega-6, which must be strictly derived from dietary sources (Price et al., 2009). Studies based on migrating water birds performing long non-stop flights have proposed that omega-3 PUFAs play a significant role in migratory performance by enhancing the aerobic capacity of flight muscles. Migratory birds should therefore strive to include as much non-synthesizable PUFA as possible in their diet (the natural doping hypothesis) (Weber, 2009).

Long distance flights demand intense muscular activity during a period of fasting, which may enhance the need to quickly re-fuel. According to Ramenofsky (1990), after a fasting period, lipogenesis increases rapidly and occurs primarily in the liver (Leveille et al., 1968; Leveille et al., 1975) with several enzymes likely being crucial to this process both in the liver and in the adipose tissue. The acetyl-CoA carboxylase (*ACC*) and fatty acid synthetase (*FASN*) is the main enzyme complexes involved in lipogenesis. *ACC* is considered a key enzyme for lipogenesis as it promotes FA synthesis at high rates (Donaldson 1979; Goodridge 1975 and 1985) while *FASN* controls FA synthesis in lower lipogenesis rates. Lipoprotein lipase (*LPL*) activity is also important in lipid metabolism and this enzyme is known to be strictly regulated during the migratory cycle, mainly

during early stages of refuelling (Ramenofsky 1990). LPL's catalytic activity in the hydrolysis of triglycerides, on both muscle and adipose tissues, provides FFA resulting in fat storage.

### Feeding schedule of birds

Name of the bird	Diet prescribed	Quantity per day	Additional Supplements
House sparrow	Paddy, bajra, ragi	15 g	
Ducks (Anatidae)	Paddy	100 g	
Bengal goose	Wheat bran White bengal gram Carrot Cabbage	50g 25g 25g 25g	
Flamingo ( <i>Phoenicoptceridae</i> )	Thinai Wheat bran Mixed grains	150 g 50 g 50 g	
Black swan	Cabbage Wheat Paddy Cucumber White Bengal gram Bread slice Carrot Shell grit	25 g 50 g 50 g 100 g 25 g 1 No 25 g 5 g	
Brahmini kite/ pariah kite	Beef with bone Or rat	500 g 2 nos weekly once	Chick 2 Nos weekly once Fish 200 g weekly once
Bengal vulture	Beef with bone Or rat	1 kg 2 Nos weekly once	Chick 5 Nos weekly once Fish 500 g weekly once
Owl (Tytoninae)	Beef with bone Or rat	250 g 150 g 2 Nos per day	Chicks 10 g weekly once
Horn owl	Rat 100 to 150 g or chicks	2 Nos 2 Nos weekly once	
Stork (Painted stork Saurus crane) (Ciconiidae)	Fish	500 g	
Heron (Ardeidae)	Fish	20-500 g	
Pelican	Fish	0.75-1 kg	
Shuikra	Chopped beef	150 g	Rat of 100 to 150 g 2 Nos weekly once

Composition of mixed grain: Paddy 20%; Sorghum 20%; Bajra 20%; ragi 20 %; and wheat 20%.

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## **INTRODUCTION OF GEOSPATIAL TECHNIQUES FOR MONITORING OF MIGRATORY BIRDS**

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### **Introduction**

Geospatial technology is a rapidly growing and changing field. Technology relating to the collection or processing of data that is associated with location. Geographical information systems (GIS) allow researchers to visualize and investigate relationships between various geographic features, such as land, elevation, and vegetation cover. The term geospatial technology (GST), refer to geographical information system (GIS), global positioning system (GPS), and remote sensing (RS), all emerging technology that assist the user in the collection, analysis and interpretation of spatial data. Geospatial technology is used in various sectors in the public as well as private in India. The major sector using geospatial technologies in India are agriculture, telecommunication, oil and gas, environmental Management, Forestry, monitoring of migratory birds, public safety, infrastructure, logistics etc.

### **Importance of Migratory Birds:-**

Migratory birds play as a vital part of biodiversity in all ecosystems. Migratory birds serve key functions in the interconnected systems that keep nature healthy, including pollination and seed dispersal of crops for human and livestock consumption, pest regulation and as an aesthetic source of pride for cultures across the globe. Birds generally reflect the status and trends affecting wider biodiversity. They are important for monitoring changes in the environment since they are an integral part of almost all ecosystems in the world. The contribution of birds to maintain the ecological balance is immense. India has approximately 1250 species of birds among which about 300 species of birds migrate across Himalayas from Tibet, China, Europe and Siberia during winters. Some of these birds fly over 5000 km and above 8500 metres high to reach India. Greylag Goose, Pintail, Common teal, Red Crested pochard, Spotted sand piper, Black winged tail, Blue throat, Greater Flamingo, Brahmini duck, Gadwall, Shoveller, Wigeon, Coot and Mallard etc. are various migratory birds which come to our country in different seasons. Avian migration is a natural process, whereby different birds fly over distances of hundreds and thousands of kilometres in order to find the best ecological conditions and habitats for feeding, breeding and raising their young. Due to changing crop pattern and environmental pollution, the number of cranes coming to India over the years has reduced by as much as 75 per cent. As many as 4,000-5,000 bar-headed geese were sighted in

the past. But in the last two years, flocks of only 40-50 of these birds were seen at one time. By conserving these birds and their environment, we ensure the conservation of biodiversity on a wider scale. This effort must take into account breeding areas, wintering areas, and stopover sites along their migratory flyways. The loss of any of the sites used by migratory birds during their annual cycle can have a impact on their chances of survival.

### **Why Do Birds Migrate:-**

- For food.
- To disperse their own breed.
- To different regions.
- To protects their babies.
- To get away from predators.
- To avoid disease.
- Breeding.
- Warmer/colder climate.

### **How Do Migrating Birds Find Their Way**

- Inherit an ability to fly in a particular direction time of the year.
- The position of the sun in the sky.
- At night, recognise the position of the star.
- Landmark such as costal headlands, river valleys and ranges of hills.
- Magnetic sense.

### **Migration Pattern of birds**

#### **Latitudinal migration**

The latitudinal migration usually means the movement from north to south and vice versa.

#### **Longitudinal migration**

The longitudinal migration occurs when the birds migrate from east to west and vice versa.

#### **Altitude migration:-**

The altitude migration occurs in mountainous regions. Many birds in habituating the mountain peaks migrate to low lands during winters.

#### **Partial Migration:-**

Some members of a group take part in migration. Coots and spoon bills of our country are example of partial migration.



### **Total migration:-**

When all the members of a species take part in the migration.

### **Vagrant or irregular migration:-**

When some of the birds disperse to a short or long distance for safety and food, it is called vagrant or irregular migration.

### **Diurnal migration**

Many larger birds like crows, robins, swallows, hawks, jays, blue birds, pelicans, cranes, geese, etc. Migrate during daytime for food, called diurnal migration.

### **Nocturnal migration**

Some small-sized birds of passerine groups like sparrows, warblers, etc. Migrate in darkness, called nocturnal birds. The darkness of the night gives them Protection from their enemies.

### **Seasonal migration**

Some birds migrate at different seasons of the year for food or breeding, called seasonal migration. e.g., cuckoos, swifts, swallow etc. They migrate from the south to the north during summer. These birds are called summer visitors. Again there are some birds like snow bunting, red wing, shore lark, grey plover etc. which migrate from north to south during winter. They are called winter visitors.

### **Geospatial Technologies:-**

#### **Global Positioning Systems (GPS):-**

A satellite-based geolocation system that functions worldwide and is accessible to the public via GPS units. Bird migration is the regular seasonal, large-scale, often long-distance movement between breeding and wintering grounds. Migration behaviour is a critical indicator for evaluating environmental health. By use of GPS important stopover and wintering locations can be identified and one can take action to save these key locations to protect endangered species.

#### **Remote Sensing: -**

Science of acquiring information about a physical phenomenon of an object or surface of the earth measured at a distance without being in physical contact with the object of interest. Detection and discrimination of objects or surface features means, detecting and recording of radiant energy reflected or emitted by objects or surface material. Different objects return different amount of energy in different bands of the electromagnetic spectrum, incident upon it. This depends on the Property of material (structural, chemical, and physical) surface roughness, angle of incidence, intensity and wavelength of radiant energy. Remote sensors are

of two types Active Remote Sensors (Laser altimeter, Lidar and Radar), and Passive Remote Sensors (Accelerometer, Radiometer and Spectrometer).

### **Geographic Information Systems (GIS):-**

Information systems enable the creation, organization, and presentation of data in a spatially referenced form, as well as the production of maps and charts.

## **Components of GIS**

### **Hardware:-**

Hardware is Computer on which GIS software runs. Some of the hardware components are Motherboard, Hard driver, processor, graphics card, printer and so on. These all component function together to run GIS software smoothly.

### **Software:-**

Software provides tools to run and edit spatial information. It helps to query, edit, run and display GIS data. Few GIS software are ArcGis, ArcView 3.2, QGIS, SAGA GIS etc.

### **Data:-**

Data is generally known as fuel for GIS. GIS data is combination of graphic and tabular data. Graphic can be vector or raster. Raster image store information in a cell based manner. It can be aerial photo, satellite image, Digital Elevation Model (DEM). Raster images normally store continuous data. Vector data are discrete. It stores information in x, y coordinate format. There are three types of Vector data: Lines, Points and Area.

### **People:-**

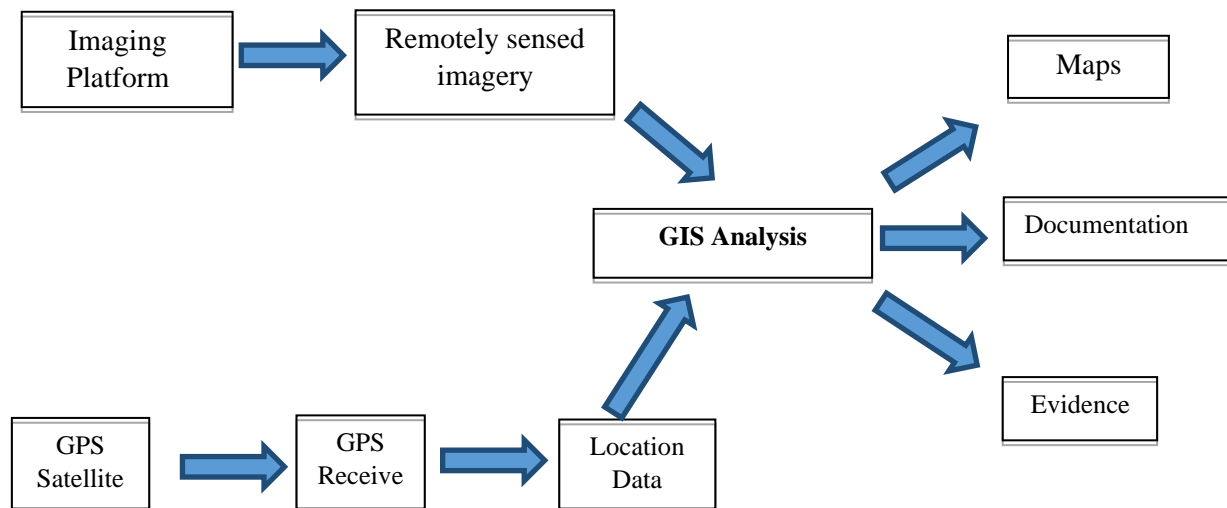
People are user of Geographic Information System. They run the GIS software.

### **Methods:-**

For successful GIS operation a well-designed plan and business operation rules are important. Methods can vary with different organizations.

## **Use of GIS in Migratory Birds**

In avian research and conservation, the GIS is a very powerful tool for the studying bird distributions, population densities, home range characteristics, and habitat quality. With the GIS we can map where birds are most active, home range sizes and overlap, and where birds build nests with behaviour. Satellite telemetry allows researchers to track birds across state, regional, national, and even international boundaries. It synthesizes different types of geospatial data, reveals spatial patterns and simplifies confirmation of observations by others.



### Research and Monitoring

Targeted research is necessary to understand the ecology of flagship migrants throughout their lifecycles, identifying the key barriers and threats (full lifecycle planning). Through targeted research, we can understand the migratory connections linking sites, regions, local communities, local governments and Partners. Wider land-use issues can be understood, facing migratory birds through targeted research and the best management practices can be implemented. The threats can be assessed, posed by climate change to the flagship migrants, and promotion of the role that migratory birds can play in monitoring climate change. Regular monitoring and evaluation of the conservation status of flagship migratory species and the networks of critical sites on migratory flyways can be done by using geospatial techniques. Capacity building should be done for flyway-scale conservation including the strengthening of local and national capacity at critical points on the flyways and the strengthening of collaboration and support between Bird Life Partners and their allies. The technological advances within the areas of remotely sensed and other spatial data, increased computing power for large datasets, and geographical information systems (GIS) may provide us with a means to develop solutions to balance our management goals.

## EFFECT OF CLIMATE CHANGE ON MIGRATORY BIRD

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### **Bird Migration**

Bird migration is one of the great wonders of the natural world. Migratory birds fly hundreds and thousands of kilometres to find the best ecological conditions and habitats for feeding, breeding and raising their young. When conditions at breeding sites become unfavourable, it is time to fly to regions where conditions are better.

The most beautiful migratory birds coming to India during the winter and summer season are-

1. Siberian Cranes
2. Greater Flamingo
3. Bluethroat
4. Great White Pelican
5. Asiatic Sparrow-Hawk
6. Harriers
7. Pacific Golden Plover
8. Wagtails
9. Starling

Migratory birds have the perfect morphology and physiology to fly fast and across long distances. Often, their journey is an exhausting one, during which they go to their limits. The Red Knot has one of the longest total migration routes of any bird, travelling up to 16,000 kilometres twice a year. It breeds in Siberia and overwinters on the west coast of Africa, some even going down to the tip of South Africa.

It is truly amazing how migratory birds can navigate with pin-point accuracy. Exactly how migrating birds find their flyways is not fully understood. It has been shown that they are able to orientate by the sun during the day, by the stars at night, and by the geomagnetic field at any time. Some species can even detect polarized light, which many migrating birds may use for navigation at night.

Migrating birds follow set routes that include suitable habitats where they can stop to rest and refuel along the way. Migration is a perilous journey and involves a wide range of threats, often caused by human activities. Hungry, exhausted birds may arrive at a stopover site, only to find that it has been destroyed by farming or urbanisation. Every year, millions of birds are illegally killed by hunters, or collide with man-made structures such as power lines. Climate change is causing habitats to shift or disappear. World Migratory Bird Day (Second Saturday in May and October) has a global outreach and is an effective tool to help raise global

awareness of the threats faced by migratory birds, their ecological importance, and the need for international cooperation to conserve them.

### **Impact of Climate Change on Migratory Bird:**

Climate change has the potential to disrupt conditions which are required for migration throughout their annual cycle: on their breeding grounds, in their non-breeding range, and along migratory routes between the two.

Migration and reproduction of many avian species are controlled by endogenous mechanisms that have been under intense selection over time to ensure that arrival to and departure from breeding grounds is synchronized with moderate temperatures, peak food availability and availability of nesting sites. Climate change is causing mismatches in food supplies, snow cover and other factors that could severely impact successful migration and reproduction of avian populations unless they are able to adjust to new conditions.

### **Global Climate Change**

Global mean temperatures are expected to increase by 1.4–5.88 °C (Houghton et al. 2001). These temperature alterations and associated changes in diurnal and seasonal temperature fluctuations and precipitation patterns are likely to cause major shifts in world climates. Some existing climates are likely to disappear and novel climates are likely to appear (Williams et al. 2007).

Human-induced climate change has begun to affect our planet and the organisms that live on it. Many migrating birds are very sensitive to environmental changes and are already being affected by climate change. Increasing temperatures, changing vegetations and extreme weather conditions lead to significant changes of the birds' essential habitats. In many cases these are likely reasons for the decline of bird populations and changes in migration patterns.

Climate change is likely to impact migratory birds in a number of different ways. Increased storm frequency, lowered water tables, higher drought frequency, sea level rise and habitat shifts resulting from climate change could all have a dramatic impact on migratory birds. Currently, several different scenarios exist about the impact of climate change will have on the migratory birds and although it is difficult to make certain predictions, some of these include:

#### **Loss of habitats:**

One of the major effects of climate change is the loss of habitats. The habitats of migratory birds are in danger to change and to disappear due to increasing temperatures, flooding or desertification. Coastal wetland areas that migrating birds use for nesting and foraging are an example. During their migration, birds rely on these areas to provide food and resting places. There they can refuel and repose before continuing their long journeys. Rising sea levels due to climate change cause the flooding of these habitats and they are lost. Without these stop-over places, the birds have insufficient reserves to continue and have difficulties to completing their journeys.

The loss of habitats continues through increasing desertification. Increasing populations lead to a demand for more land to grow crops and graze animals and to the intensified use of land. This contributes to further land degradation and leads to expansion of desert areas. Climate change worsens the effect by a decrease in rainfall. As a consequence of the dryness, the desert expands further. The crossing of the Sahara is one of the most challenging parts of the birds' journey because they have no possibility of resting. The Sahara expansion, combined with destruction of the habitats, gradually makes it nearly impossible for African-Eurasian migrants to cross this ecological barrier successfully.

Among other habitats, many breeding habitats are also strongly affected. For example the Siberian tundra where many goose species breed. They use the tundra's rocky bedrock for breeding and raising their offspring. However, climate change is rapidly changing its vegetation structure: The increasing temperatures make the permafrost soil thaw, enabling forest to expand into the tundra. This new green carpet changes the habitat completely and makes it impossible for the geese to breed.

### **Patterns of migration**

The migration rhythm of birds is usually more fixed and they struggle with readjustment to change in temperatures. Because of this rigidity they suffer more from the impacts of climate change than other birds.

Global warming influences the routes of many migratory birds and their annual migration rhythm. A lot of migratory birds change their routes, shorten or completely cancel their journey as a result of changing temperatures. For example, some small bird species do not winter in Spain, France or in the north of Africa anymore. Instead, they prefer to stay in England, where they breed. Cranes, which normally migrate to Spain and Portugal, stay in Germany, accompanied by Starlings. Unfortunately their inactivity has severe consequences. They are not used to low temperatures and in case of a hard onset of winter, most of them won't survive.

### **Competition for breeding places**

Warm weather increases the competition for breeding places. Mild winters help resident birds to survive. They start to use food resources and breeding places of long distance migrants. As a consequence, long distance migrants might find their breeding grounds occupied by a large number of resident birds. This increases the competition between the species for food and breeding grounds.

### **Food shortages**

The warm spring temperatures in some regions have led to an earlier arrival of many birds. Thus, they start to breed earlier. At the same time, increasing temperatures also make the vegetation bloom and insects hatch earlier. Unfortunately, these shifts are not in line with each other. The vegetation bloom and insect peak occurs even before the young birds hatch. As a result of this mismatch, the birds cannot provide enough food for their offspring.

### Threat to migratory birds

Climate change and habitat loss are the two greatest threats to biodiversity in the present century. Climate change amplifies the danger to migratory birds. The earth's climate has been changing throughout time. Some of the earth's bird species were able to adapt to these changes, while others could not and have become extinct as a result. This is a natural process. However, the climate change we are experiencing today is different: human-induced global warming is happening at an accelerated speed and it is becoming increasingly difficult for many bird species to keep up with the resulting shifts. In addition, adapting to climate change becomes even more difficult for some species, because other man-induced threats add to the challenge. The world's remaining habitats are also lost or degraded for many other reasons, such as pollution, fragmentation, or conversion and overbuilding. Furthermore, migratory bird species also suffer from barriers like wind farms or competition from newly introduced alien species. Species that are already on the decline due to these factors are especially vulnerable to climate change. In other words, for some species, climate change may give these already very vulnerable species the final push towards extinction. Regarding the different threats migratory birds have to face, one thing becomes apparent: climate change is already happening and it endangers migratory birds. Especially long distance migrating birds feel the effect; as they are less flexible than other birds, they suffer most.

The process climate change cannot be stopped completely. We have the possibility to interfere. Everyone can join the fight against climate change every day: reduce the energy you use, turn off the lights, reduce heating and take up cycling.

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## ECOTOXICOLOGY AND ITS IMPACT ON WETLAND AVIFAUNA

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### Introduction

The term *ecotoxicology* was first coined by Truhaut in 1969 as a natural extension from *toxicology*, the science of the effects of poisons on individual organisms, to the ecological effects of pollutants. Pesticides and other contaminants that get into the natural environment can affect wild plants and animals. The science of studying these effects is called ecotoxicology (Newman & Unger, 2003). *Ecotoxicology* is a mix of ecology, toxicology, physiology, analytical chemistry, molecular biology, and mathematics. *Ecotoxicology* looks at the impacts of contaminants including pesticides on individuals, populations, natural communities, and ecosystems. Ecotoxicology employs ecological parameters to assess toxicity. In a more restrictive but useful sense, it can be defined as the science of assessing the effects of toxic substances on ecosystems with the goal of protecting entire ecosystems, and not merely isolated components.

The impact of anthropogenic activity and its toxic impact on ecosystem can understood by the following table of events–

Date	Contaminant(s)	Effects
1850s	Industrial revolution; soot from coalburning	Industrial melanism of moths
1863	Industrial wastewater	Toxicity to aquatic organisms; first acute toxicity tests
1874	Spent lead shot	Ingestion resulted in death of waterfowl and pheasants
1887	Industrial wastewater	Zones of pollution in rivers established by species tolerance
1907	Crude oil spill	Death of thousands of puffins
1924	Lead and zinc mine runoff	Toxicity of metal ions to fish
1927	Hydrogen sulphide fumes in oil field	Large die-off of both wild birds and mammals
1950s	DDT and organochlorines	Decline in American robins linked to DDT use for Dutch Elm disease; eggshell thinning in bald eagles ( <i>Haliaeetusleucocephalus</i> ), osprey ( <i>Pandion haliaetus</i> ), and brown pelicans ( <i>Pelecanus occidentalis</i> ) linked to DDT; and fish-eating mammals at risk



1960s	Anticholinesterase pesticides	Die-offs of wild birds, mammals, and other vertebrate species
1986	Radioactive substances from Chernobyl nuclear power station	Worst nuclear incident in peacetime, affecting a wide variety of organisms and ecosystems
1990s	Complex mixtures of potential endocrine disrupting chemicals, including PCBs and organochlorine pesticides	Abnormally developed reproductive organs, altered serum hormone concentrations, and decreased egg viability in alligators from contaminated lakes in Florida
Source: Hoffman et al., 2002		

Compounds like pesticide may directly affect something far from the site of application. Pesticides that are bound to soil particles may be carried into streams with runoff. Pesticide drift may travel many miles in the wind. Sunlight, water, microbes, and even air can break down pesticides. Insecticides such as DDT, chlordane, and dieldrin don't break down easily, and they are still found in soil, plants, and animals. Persistent pesticides may travel long distances in the air or water, or even in living organisms such as migrating birds or fish. Researchers have found pesticide residues in alpine lakes and snow, many miles from where the pesticides were applied (Bradford et al., 2010; Hageman et al., 2010). Pesticides have even been found in the Arctic and Antarctic environments, probably carried there by currents in the atmosphere or oceans (Bidleman et al., 1993; Halsall, 2004).

What has been learned since, as demonstrated by the risks that face many trans-border avian migrants, has clarified the need for greater international cooperation and harmonization of pesticide use. Where a large portion of a species population occupies a small geographical area, either in the course of its migration or on wintering grounds, any localized contaminant or non-contaminant impact can have potentially serious consequences for that population. In Argentina, monocrotophos, one of the most acutely toxic pesticides to birds, remained the second highest use OP throughout the world through the mid-1990s, resulted in the death of an estimated 20,000 Swainson's hawks (*Buteo swainsoni*). Reproductive impairment due to *bioaccumulation* of selenium in fish and aquatic birds has been an ongoing focus of fish and wildlife research, not only in the western United States but also in other parts of the world. Selenium is a naturally occurring semi-metallic trace element that is essential for animal nutrition in small quantities, but becomes toxic at dietary concentrations that are not much higher than those required for good health. The partial meltdown of the 1000 Mw reactor at Chernobyl in the Ukraine released large amounts of radiocesium and other radionuclides into the environment, causing widespread contamination of the northern hemisphere, particularly Europe and the former Soviet Union (Hoffman et al., 2002a).

Reports of anthropogenic environmental contaminants affecting free-ranging wildlife began to accumulate during the industrial revolution of the 1850s. Early reports included cases of arsenic and lead-shot ingestion and industrial smokestack-emission toxicity. Mortality in waterfowl and ring-necked pheasants (*Phasianus colchicus*) from ingestion of spent lead shot

was recognized as early as 1874 when lead-poisoned birds were reported in Texas and North Carolina (Forbes and Sanderson, 1978). Eggshell thinning, related to DDT and ultimately dichloro-diphenyl-dichloroethylene (DDE), was determined to be an important factor in reproductive failure in European and North American birds of prey as well as in brown pelicans (*Pelecanus occidentalis*) (Blus et al., 1972). This and similar research played a major role in the cancellation of many highly persistent pesticides. But, some factors other than agricultural practices may pose toxic hazards to wildlife. *Secondary poisoning* can occur if an animal eats another animal that has been fatally poisoned by a pesticide, and predator dies as a result of the poisoned prey. This is also called *relay toxicosis*. Pesticide residues build up in organisms and in food webs. *Bioaccumulation* can occur if residues build up faster than the organism can break them down and excrete them. Bioaccumulation in aquatic animals where the pesticide is taken in from the water is called *bioconcentration* (Newman and Unger, 2003).

Many of the endocrine disruptor reports in wildlife are based upon observed adverse reproductive and developmental effects rather than direct evidence of endocrine-modified function or defined endocrine pathways. A wide variety of chemicals have been reported as potential endocrine disruptor chemicals (EDCs) and these include polycyclic aromatic hydrocarbons; polychlorinated and polybrominated biphenyls, dibenzop-dioxins and dibenzop-furans; organochlorine pesticides and fungicides; some non-organochlorine pesticides; complex environmental mixtures; and a few metals. Collectively, there is strong evidence of altered reproductive and developmental processes in wildlife and also wildlife avifauna exposed to endocrine disruptors. Potential mechanisms of action for EDCs are diverse. EDCs may interrupt multiple pathways along the hypothalamic-pituitary–target-tissue axis, potentially disturbing the normal synthesis, transport, release, binding, action, biotransformation, or elimination of natural hormones in the body. EDCs may alter the hypothalamic-pituitary axis, which can have widespread effects through the disruption of endocrine functions downstream of the hypothalamus. There is increasing evidence that EDCs may disrupt endocrine function by influencing the regulation/release of the pituitary tropic hormones. EDCs can alter the rate at which hormones are metabolized.

Effects can also occur on larger ecological scales than that of the individual. For example, predator-prey relationships may be changed by pesticides and other contaminants. When pesticides remove one of the species at the bottom of a food web, many other species may be affected.

With increasing loss of habitat the quality and fate of the remaining habitat becomes increasingly critical to the survival of species and ecosystems. Species that are endangered or at risk and that occupy a very limited geographical area could be easily decimated by a single event such as an oil or chemical spill or misapplication of pesticides. Furthermore, on a temporal basis where a large portion of a species population occupies a small geographical area, either in the course of its migration or on wintering grounds, any localized impact, whether pesticide-related (e.g., as reported by Hooper and co-authors, Chapter 25) or not, has the potential for serious consequences to populations. For these reasons the balance between shrinking habitat and anthropogenic stressors becomes increasingly crucial to sustain both ecosystems and species diversity (Hoffman et al., 2002a).

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## ADVANCED LABORATORY AND MICROBIOLOGICAL DIAGNOSTIC MODALITIES FOR AVIAN DISEASE DIAGNOSIS

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The diagnostic capabilities of modern clinical microbiology laboratories have improved rapidly and have expanded greatly due to a technological revolution in molecular aspects of microbiology and immunology. Advanced Techniques in Diagnostic Microbiology provides a comprehensive and up-to-date description of advanced methods that have evolved for the diagnosis of infectious diseases in the routine clinical microbiology laboratory.

Reliable and rapid techniques are needed by national and regional diagnostic laboratories to screen for important pathogens.

### **Polymerase chain reaction(PCR)**

(PCR) is a method widely used to rapidly make millions to billions of copies of a specific DNA sample, copies of very small amounts of DNA sequences are exponentially amplified in a series of cycles of temperature changes. PCR is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand. Because DNA polymerase can add a nucleotide only onto a preexisting 3'-OH group, it needs a primer to which it can add the first nucleotide. At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies (amplicons). Applications of the technique include DNA cloning for sequencing, gene cloning and manipulation, gene mutagenesis; construction of DNA-based phylogenies, or functional analysis of genes; diagnosis and monitoring of hereditary diseases.

### **Real-time PCR**

Real-time PCR is the latest improvement in the standard PCR technique to be implemented in veterinary laboratories. This technique is a single-capillary, closed assay that greatly decreases the problem of cross-contamination between samples. The fluorescence readings are plotted by computer software and results can be transmitted electronically, eliminating the need for post-PCR reaction analysis by electrophoresis. Two common methods for the detection of PCR products in real-time PCR are (1) non-specific fluorescent dyes that intercalate with any double-stranded DNA and (2) sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter, which permits detection only after hybridization of the probe with its complementary sequence.

The development of extraction methods such as the magnetic bead technique has made it possible to use real-time PCR to test large numbers of samples in a matter of hours during

disease outbreaks. Realtime PCR has been adapted for use in the field through the use of portable thermocyclers and lyophilised reagents. The PCR is also used extensively for the genotyping and phylogenetic analysis (relatedness) of veterinary pathogens.

### **ELISA (enzyme-linked immunosorbent assay)**

The assay uses a solid-phase type of enzyme immunoassay (EIA) to detect the presence of a ligand (commonly a protein) in a liquid sample using antibodies directed against the protein to be measured. In the most simple form of an ELISA, antigens from the sample to be tested are attached to a surface. Then, a matching antibody is applied over the surface so it can bind the antigen.

This antibody is linked to an enzyme and then any unbound antibodies are removed. In the final step, a substance containing the enzyme's substrate is added. If there was binding the subsequent reaction produces a detectable signal, most commonly a color change.

### **Immunohistochemistry (IHC)**

IHC is the most common application of immunostaining. It involves the process of selectively identifying antigen (proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues.

### **Microarray technology**

Originally developed for the mapping of genes, it is being used to detect a wide variety of veterinary pathogens. Specific oligonucleotides are bound to small solid supports such as glass slides, silicon chips or nylon membranes. Extracted DNA or complementary DNA is labelled with a fluorescent dye and then hybridized with the microarray. Specific patterns of fluorescence are detected by a microarray reader which allows the identification of specific gene sequences found only in the veterinary pathogen of interest.

This technology has the potential to identify the presence of agents of interest at the serotype or subspecies level, or to differentiate agents that cause similar clinical signs, for example, vesicular lesions.

### **Nanotechnology**

The term nanotechnology' is broadly defined as systems or devices related to the features of nanometre scale (one billionth of a metre). This scale of technology as it applies to diagnostics would include the detection of molecular interactions.

The small dimensions of this technology have led to the use of nanoarrays and nanochips as test platforms. One advantage of this technology is the potential to analyze a sample for an array of infectious agents on a single chip. Applications include the identification of specific strains or serotypes of disease agents, such as the identification of specific influenza strains, or the differentiation of diseases caused by different viruses but with similar clinical signs, such as vesicular viral diseases.

Many research groups are considering the use of chip assays that detect a number of agroterrorism agents in each sample. Small, portable platforms are being designed to allow pen-side testing of animals for diseases of concern. Another facet of nanotechnology is the use of nanoparticles to label antibodies.

These labeled antibodies can, then be used in the various assay to identify specific pathogens, molecules or structures. Examples of nanoparticle technology include the use of gold nanoparticles, nanobarcodes, quantum dots (cadmium selenide) and nanoparticle probes.

### **Loop-mediated isothermal amplification (LAMP)**

LAMP is a single-tube technique for the amplification of DNA and a low-cost alternative to detect certain diseases.

LAMP is an isothermal nucleic acid amplification technique. In contrast to the polymerase chain reaction (PCR) technology, in which the reaction is carried out with a series of alternating temperature steps or cycles, isothermal amplification is carried out at a constant temperature, and does not require a thermal cycler.

LAMP is widely being studied for detecting infectious diseases such as tuberculosis, malaria, sleeping sickness, and SARS-CoV-2.

### **Biosensors:**

Biosensor is analytical tools for the analysis of bio-material samples to gain an understanding of their bio-composition, structure and function by converting a biological response into an electrical signal. The analytical devices composed of a biological recognition element directly interfaced to a signal transducer which together relates the concentration of an analyte (or group of related analytes) to a measurable response.

### **Viral disease**

#### **Avian Influenza**

Avian influenza (AI) is a viral disease characterized by respiratory signs, depression and reduced feed and water intake. In egg laying birds there is a decline in egg production. AI viruses can be classified into two categories: low pathogenic (LPAI) that typically causes little or no clinical signs in birds and highly pathogenic (HPAI) that can cause severe clinical signs and/or high mortality in birds.

Confirmation of AI cases requires laboratory tests such as serology (AGID and/or ELISA) and virus detection (real-time RT-PCR and/or virus isolation)

#### **Marek's Disease**

Marek's disease (MD) is a herpesvirus-induced neoplastic disease of chickens characterized by infiltration of various nerve trunks and/or organs with pleomorphic lymphoid cells.

Characteristics of MD lesions of importance in differential diagnosis include nerve involvement (when present), the absence of bursal lesions or, rarely, diffusely thickened bursas, and pleomorphic lymphocytes comprising lesions, some of which exhibit MATSA and only few of which are positive for immunoglobulin M (IgM).

### **Avian Leukosis/Sarcoma Viruses**

The avian leukosis (ALV)/sarcoma group are retrovirus-caused, neoplastic diseases of semimature or mature chickens.

Diagnosis of ALV-J is achieved by gross and histopathologic examination of tumors and by virus isolation from cloacal or vaginal swabs or tumors. Although PCR tests have been developed, the virus mutates frequently requiring the production of new primers.

### **Chicken Infectious Anemia**

Chicken infectious anemia (CIA) is a disease of young chickens characterized by aplastic anemia, generalized lymphoid atrophy, subcutaneous and intramuscular hemorrhage, and immunodepression.

1. Serologic assays to detect antibodies such as the ELISA, virus neutralization test, and indirect immunofluorescence.
2. PCR is the test of choice for identification of CIA virus in cell cultures and chicken tissues

### **Fowl Pox**

Fowl pox is a slow-spreading viral disease of chickens, turkeys, and many other birds characterized by cutaneous lesions on unfeathered skin of the head, neck, legs, and feet and/or by diphtheritic lesions in the mouth, upper digestive and respiratory tracts.

The diagnosis can be confirmed by demonstrating intracytoplasmic inclusion bodies in stained sections or in scrapings of the lesions. Electron microscopy may be helpful in these cases.

### **Hepatitis E Virus**

Hepatitis E Virus causes a disease known as Hepatitis-splenomegaly (HS) syndrome in both layer and broiler-type chickens. It is characterized by increased mortality and decreased egg production. Dead birds have hemorrhagic livers, with clotted blood around the liver or abdominal cavity and splenomegaly.

1. Immunohistochemistry (IHC) on tissues can also be used for diagnosis.
2. Serology by ELISA and AGID are other methods that can be used for diagnosis of HS.
3. Currently, the diagnosis of avian HEV is made primarily based on the detection of virus RNA by RT-PCR either in the feces or liver samples.

## **Infectious Bronchitis**

Infectious bronchitis (IB) is an acute, highly contagious viral disease of chickens characterized by respiratory signs (gasping, sneezing, coughing, and nasal discharge), severe renal disease associated with nephrotropic strains, and a marked decrease in egg production.

1. Several procedures including serum-virus neutralization (VN), enzyme-linked immunosorbent assay (ELISA) and modified hemagglutination inhibition (HI) are available, but only VN and to some extent HI tests (due to cross-reactions) are serotype specific.
2. The fluorescent antibody technique or electron microscopy can be used on tracheal samples for rapid diagnosis of IB but do not differentiate the serotype.

## **Infectious Bursal Disease**

Infectious bursal disease (IBD) is an acute, contagious, viral disease of young chickens characterized by inflammation followed by atrophy of the Bursa of Fabricius and variable degrees of immunosuppression.

PCR, paired serologic testing with rising titers using the ELISA, agar-gel precipitin, or virus neutralization can be used to confirm the diagnosis.

## **Infectious Laryngotracheitis**

Infectious laryngotracheitis (ILT) is an acute viral disease of chickens, and, rarely, pheasants and peafowl characterized by marked dyspnea, coughing, gasping, and expectoration of bloody exudate.

Demonstration of viral antigen or DNA in clinical samples, usually tracheal epithelium, by the use of the fluorescent antibody, immunoperoxidase, electron microscopy, DNA hybridization techniques, antigen capture ELISA and PCR.

## **Newcastle Disease**

ND is currently defined as: “an infection of birds caused by a virus of avian paramyxovirus 1 (APMV-1)

1. Virus isolation and subsequent pathogenicity testing,
2. RT-PCR – demonstration of viral RNA and subsequent typing,
3. Serology - demonstration of increasing titer of Newcastle antibody in the flock from onset to convalescence by ELISA or HI.

## **Bacterial disease**

### **Avian Tuberculosis**

Avian tuberculosis is a slow-spreading, usually chronic, granulomatous infection of semimature or mature birds, characterized by progressive weight loss and, ultimately, by emaciation and death.



An enzyme-linked immunosorbent assay (ELISA) has been developed for the detection of mycobacterial antibodies in serum and this test has greater promise in the detection of avian tuberculosis in individual exotic birds and aviaries.

### **Botulism (Limberneck; Western Duck Sickness)**

Botulism is an intoxication caused by ingestion of the toxins of *Clostridium botulinum*. Botulism is caused by ingestion of the preformed toxins of *C. botulinum* in feeds, foods, dead poultry, or toxin-containing maggots. Clinical signs include drowsiness, weakness, and progressive loss of control and flaccid paralysis of the legs, wings, neck, hence the term 'limberneck'.

ELISA is by far the most commonly applied immunoassay format in the detection of botulinum neurotoxins.

Mouse lethality assay- The standard procedure for the detection of botulinum toxin is the mouse lethality assay.

PCR and probe hybridization protocols applied for the detection and identification of *Clostridium botulinum*.

Pulsed-field gel electrophoresis- Macrorestriction pattern analysis by PFGE has become one of the most powerful genetic typing tools in the diagnostics of food-borne pathogenic bacteria.

### **Fowl Cholera**

Fowl cholera is an infectious disease of poultry as an acute septicemic disease with high morbidity and mortality. The etiologic agent is *Pasteurella multocida*. An enzyme-linked immunosorbent assay (ELISA) is used for identification.

### **Spirochetosis**

Spirochetosis or nonrelapsing borreliosis is a septicemic disease of most poultry and many other birds. Acute cases are characterized by depression, cyanosis, diarrhea, and leg weakness progressing to paralysis and death.

The spirochetes can be identified in Giemsa-stained blood smears (Fig. 1) or by dark-field or phase-contrast microscopy of blood and other fluids. Spirochetes can be concentrated in the buffy coat of centrifuged blood.

### **Fungal disease**

#### **Aspergillosis**

Aspergillosis is an acute or chronic disease, primarily affecting the respiratory system. The most common etiology is *Aspergillus fumigatus* but *A. flavus* can be involved.

Microscopic examination reveals septate, branching hyphae within lesions. Hyphae can be seen in fresh preparations cleared with 10% KOH or stained with lactophenol cotton blue.

In histologic sections, special stains (methenamine-silver, PAS, Gridley) are useful for demonstrating fungi in tissues. Nodules in the lungs usually appear as granulomas containing fungal hyphae.

### **Candidiasis**

(Thrush; moniliasis, crop mycosis, sour crop, muguet, soor, levurosis)

Candidiasis is a disease of the digestive tract caused by the yeast-like fungus *Candida albicans*. The disease generally involves the upper digestive tract and usually occurs as a secondary infection. *Candida albicans* grows readily on Sabouraud's dextrose agar.

## **DISINFECTION AND MEASURES FOR CARCASS DISPOSAL DURING MASS MORTALITY IN BIRDS**

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Effective cleaning and disinfection is an essential component of good hygiene and thus one of the key biosecurity measures for disease control. This should be carried out from time to time to reduce the build-up of pathogenic organisms and a disinfectant known to be effective against a large range of pathogens. Approved disinfectants like chlorine dioxide and peracetic acid for disinfection or sterilization may be used.

Hygiene and sanitation play a major role in any effective disease control programme for poultry production premises. Poultry premises and buildings should comply with requirements for isolation from the environment and strict observance of principles of hygiene and disease prevention (e.g. restrictions on movement of staff, equipment and vehicles). A poultry site must be prepared methodically for the entry of each new batch (removal of birds, litter and manure; vector and rodent control; dry and wet cleaning; disinfection; fumigation). Attention should be paid to the terminal sanitation of houses and equipment after depopulation (physical and chemical cleaning, pressure washing, disinfection, fumigation). Particular care should be exercised in the performance of sanitary procedures after a disease outbreak.

### **Commonly used disinfectants**

Disinfectants are the chemical compounds which are commercially sold and each has its own properties for specific applications. The various disinfectants are as follows:

1. Cresols: They are derived from the distillation of petroleum. They are cheap and effective biocides when applied to soil or buildings. They cannot be used where there are live chickens, processed meat or eggs as they are going to be tainted by the chemical's odour.
2. Organic phenols which are recommended for use in hatcheries for equipment decontamination.
3. Quaternary ammonium compounds also known as "QAT". They are highly suitable to decontaminate equipment, housing, and in hatcheries on the condition that an anionic detergent is used before applying QAT.
4. Chlorine compounds: They are mostly used in processing plants and to purify water on livestock farms. Hypochlorite is effective only when pH value range between 6.5 and 7.5 in organic matter free water and you need to expose it to inactive bacteria for 10-20 minutes.
5. Formalin: It is a corrosive and carcinogenic compound that is recommended for fumigating eggs in cabinets designed for this purpose. However, there are special precautions to be followed by the applicator to avoid being exposed and injured.

Immediate disposal of dead and diseased birds is an important and effective tool in preventing the dissemination of any disease. Disposal methods include the use of burial pits, tanks, burial in trenches, burning, rendering and composting. Regular visual inspection, together with routine testing by microbiological monitoring methods, is very effective in checking the efficacy of cleaning and disinfection.

When animals die or are slaughtered on farms, carcasses and parts that are unfit for use as food should be disposed of properly. Safe and environmentally responsible disposal of animal carcasses, whether an individual death or during significant mortality events, is an essential consideration.

Information on the safe and lawful disposal of carcasses can be obtained from local environmental protection agencies. Because state and federal regulations regarding the science of disposal of animal carcasses and biohazard waste is constantly changing, this discussion only addresses carcass disposal and not associated biohazard wastes such as bandaging materials. When the circumstances under which death has occurred suggest a transmissible disease or toxic hazard, the nearest animal health official should be notified immediately.

As general precautions, persons handling carcasses and disinfectants should wear protective clothing and be properly equipped to complete the tasks of disposition and disinfection. Premises should be promptly cleaned in a manner that prevents any infectious or toxic health hazard to domestic or wild animals or people.

The method of disposal should preclude contamination of soil, air, and water. Hides and other parts of animals that have succumbed to infectious diseases or toxins should be safely disposed of and not retained for use.

### **Rendering of carcasses**

Ordinarily, rendering is a safe, rapid, and economic method of disposal of carcasses. Renderers are required to use equipment and methods that prevent health hazards. Local regulations specify requirements for transportation of carcasses to rendering plants. During transportation, biosecurity must be considered to avoid spreading infectious agents into the environment.

### **Burial of carcasses**

When a site acceptable to the local environmental protection agency is available, burial is usually the preferred method of disposal. In selecting a burial site, it is necessary to consider the adequacy of soil depth and to avoid underground electrical cables, water pipes, gas pipes, septic tanks, and water wells. The prevention of secondary toxicosis or exposure to infectious agents must be considered (eg, the burial of a carcass infected with blastomycosis could potentially contaminate the soil and groundwater, putting scavengers at risk).

The burial pit or trench should be at least 2.3 m wide and 3 m deep (7 × 9 ft). The pit is a cave-in hazard and must not be entered without proper shoring, and any other appropriate precautions should be taken. At this depth, 1.3 m<sup>2</sup> (15 ft<sup>2</sup>) of floor space will accommodate a mature bovine or equine carcass, 5 mature pigs or sheep, 100 mature chickens, or 40 mature turkeys. For each additional meter (3 ft) in depth, the number of animals per 1.3 m<sup>2</sup> of floor space may be doubled. Contaminated litter, soil, manure, feed, milk, or other material should be placed in the pit with the carcasses and covered with at least 2 m (6 ft) of soil. The covering soil should not be compacted. Decomposition and gas formation cause cracking, bubbling, and leaking of fluids from a compacted burial site. The soil should be mounded and neatly graded.

Certain landfills are licensed to accept animal carcasses. Check with the landfill in your area. If this is allowed, all biosecurity practices should be followed during transportation.

### **Burning of Carcasses**

Burning carcasses in an open site should be done only when legally permitted. Burning poultry carcasses should be considered only when burial is not feasible. The burn site should be away from public view and on flat, open ground that is clear of buildings, hay or straw stacks, overhead cables, and shallow underground pipes or cables. Locations upwind from houses, farm buildings, roads, or populated areas, and those from which precipitation runoff may contaminate the environment, should be avoided.

Carcasses must be placed on a quantity of combustible supporting materials sufficient to reduce them completely to ashes. The material must also be arranged in a manner to permit an adequate flow of air to the fire. Gasoline or other highly volatile combustible liquids should not be used.

To prepare the fire bed, an area of ground should be staked out to accommodate the number of carcasses to be burned: 8 × 3 ft for 100 mature chickens, or 40 mature turkeys. The fire bed burns best if at a right angle to the prevailing wind.

Under favorable conditions, burning should be complete in 48 hours. Additional combustible material should be added as needed. When the fire has died out, the ashes should be buried and the area cleaned, graded or plowed, and prepared for seeding.

### **Composting carcasses**

Developed for use on poultry farms, composting has been successfully used for swine, cattle, horse, sheep, and goat carcasses. The proper balance of material (oxygen, moisture, nitrogen, and carbon) is required for this natural degradation process to reach a temperature of 130°-150°F, which is sufficient to kill most disease-causing organisms, allowing the end product to be suitable for use as a soil amendment. However, toxins and any medications or drugs in the animal at the time of death will not necessarily be degraded

through this process. Care should be taken to protect the compost from excessive rain and to secure it from predators.

### **Other Disposal Method for Carcasses**

Tissue digestion, fermentation, and dry extrusion methods have been developed to process certain dead animals and animal waste, destroy pathogenic organisms, reduce volume, and produce feedstuffs. Tissue digestion, both alkaline and thermal degradation, may be available in animal diagnostic laboratories. Local environmental protection agencies and state agriculture departments should be consulted concerning the acceptability of these and other possible alternative disposal methods.

### **Disinfection of Premises after Carcass Disposal**

Removal and safe disposal of manure, feed, and debris by burial or burning, followed by thorough scraping and cleaning of all buildings and equipment, must precede the application of chemical disinfectant. Except for steam cleaning, cleaning with aqueous solutions is practical only at temperatures above freezing. A cleaning agent such as trisodium phosphate or sodium carbonate dissolved in hot water will facilitate cleaning. All traces of the cleaning agent must be rinsed away with clear water before disinfectant is applied because some may inactivate the disinfectant. Provision must be made to contain and safely dispose of cleaning solutions, rinse water, and disinfectant.

Disinfectants recommended for general use on surfaces free of organic matter are sodium or calcium hypochlorite (1,200 ppm available chlorine), iodine, phenol, and quaternary ammonium compounds. Newer disinfectants use a combination of products (eg, quaternary ammonia and glutaraldehyde) to enhance efficacy. The selection of the best disinfectant for a particular situation, as well as the contact time and concentration required for the concerning pathogen, is complex and beyond the scope of this discussion. The CDC has an excellent overview in their [disinfection and sterilization guidelines](#). In addition, information on disinfectants for specific animal disease agents can be obtained from state or federal animal health agencies. Disinfectants should bear the approval statement of the EPA in the USA or of a similar agency in other countries. Label instructions for application must be followed.

For the disinfection of buildings, it is advisable to use formalin 4% end solution (commercial formalin 37.5% solution diluted 1:8 in water) with propylene glycol. The propylene glycol is essential to enable the formaldehyde to penetrate pores, cracks and spaces between metal plates where joints are riveted or welded together.

The best procedure is to disinfect equipment in special premises and then return it to the rearing area when disinfection is complete. Small equipment, and equipment which can be dismantled, may be placed in a special plastic or stainless steel bath or container (containing a solution of iodine, phenolic or quaternary ammonium compounds) for no more than 2 min. In tropical countries, poultry house equipment may be placed in the sun after cleaning for further disinfection.

Electrical equipment (waterproofed), egg-handling equipment and other large equipment should be disinfected in accordance with the recommendations provided by the manufacturers of the equipment and the disinfectant. Fuse boxes should be disinfected by hand, using a cloth soaked in disinfectant. All fuses should be removed before disinfection.

All accessory decontamination equipment (e.g. rakes, shovels, scrapers, brushes, trucks, tractors, manure spreaders and bucket loaders) should be cleaned and disinfected after use and stored in a secure location.

The water system should be decontaminated using commercial disinfectants, carefully following the recommendations. Dismantled tubes should be filled individually with a water disinfectant and soaked for 24 h.

Water pipes which cannot be dismantled should be filled with commercial disinfectant through the pressure tank, left for 24 h, and then flushed through with fresh water, using the highest pressure available. Disinfection of silos should be generous, using 6% formaldehyde with propylene glucol.

Disinfection of dirt floors is virtually impossible. In situations where dirt floors cannot be concreted, fumigation can be performed (under a nylon or polythene cover sheet) using methyl bromide at a rate of 100 g per meter cube for 24 h. Alternatively, disinfectant could be applied to the floor at a rate of 4 l per 10 meter square.

Formaldehyde gas used on dirt floors is effective only on the surface, as fumigation is unable to affect pathogens at a depth of more than 2 cm.

The efficacy of a disinfectant depends on the duration of contact with the soiled environment. Most disinfectants are dissolved in water and contact lasts until the applied disinfectant solution is dry.

The contact time of disinfectants has been increased several fold with the advent of foaming techniques. Foam takes a lot longer to dry and, consequently, the antimicrobial activity of the disinfectant is greatly increased.

Ultra-violet (UV) radiation is not an effective method of destroying microorganisms in poultry production environments. UV light can disinfect by damaging the nucleic acid of pathogens, but this is only effective when the source of light is positioned close to the surface to be treated; the surface must be free of dust and exposed to direct rays.

Before entering a disinfected area or touching disinfected equipment, all personnel must change into clean clothes and clean, disinfected rubber boots, and must wash and disinfect their hands. Clean overalls must hang on the 'disinfected' side of the barrier, and personnel must wear these whenever crossing to this side of the barrier. Used overalls must be removed and placed on the 'dirty' side for washing. Boots or shoes used on the 'dirty' side must be removed, and new rubber boots put on, when passing the barrier into the 'disinfected' area. Before entering the 'disinfected' zone, personnel must stand in a boot disinfectant bath for 20 sec, during which time they may wash and disinfect their hands. Only when these procedures have been completed can personnel enter the premises and take part in the work.

## ONE HEALTH-AVIAN DISEASES OF ZONOTIC IMPORTANCE

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### Zoonosis

Rudolf Virchow in 1880 first introduced the word zoonosis to include collectively the diseases shared in nature by man and animals. According to the WHO, zoonoses can be defined as “any disease or infection caused by all types of agents (bacteria, parasites, fungi, viruses and unconventional agents) transmissible from vertebrate animals to humans and vice versa”. The transmission of these agents can occur through direct and indirect methods and through food, water or the environment. Humans are closely associated with animals in agriculture, as companions and in the natural environment which appears as a major public health problem around the world. During recent decades, the onset of newly emerging infections, reemerging and resurging infections and wildlife originated zoonoses has been increased, such as Ebola virus, West Nile virus and Newcastle Disease (ND). Recently, the emergence of severe acute respiratory syndrome (SARS) and highly pathogenic avian influenza (HPAI) viruses have shown that zoonosis can pose a significant health risk among population (Rabozzi *et al.*, 2012). The recent example is the novel coronavirus that causes COVID-19, has been spread as a global pandemic.

### Avian diseases of zoonotic importance

When handling birds, whether poultry or wild birds, it is important to remember that there are several avian diseases which can be transmitted to humans. Chlamydiosis, salmonellosis, avian influenza and avian tuberculosis infections may cause serious or life-threatening conditions.

### Types of avian zoonotic diseases

#### A. Viral Zoonoses

1. Avian Influenza (AI)
2. Newcastle Disease (ND)
3. West Nile Virus

#### B. Bacterial

1. Chlamydiosis / Psittacosis
2. Salmonellosis
3. Avian Tuberculosis
4. Colibacillosis
5. Erysipelas
6. Pasteurellosis

#### C. Fungal

1. Histoplasmosis

### Avian Influenza (Bird Flu)

Avian Influenza (AI) has occurred in Asia, Africa, the Pacific and Europe. The strain H<sub>5</sub>N<sub>1</sub> has major concern in human infection which comes under Highly Pathogenic Avian Influenza (HPAI). Transmission of the organism occurs by direct contact with infected birds,



contact with surfaces contaminated with discharges from infected birds and eating raw or undercooked poultry and their products. There is serious threat that the H<sub>5</sub>N<sub>1</sub> virus will adapt over time and be able to spread person to person. The incubation period for H<sub>5</sub>N<sub>1</sub> in humans is believed to be from 3 to 7 days followed by a rapid onset of pneumonia, cough and fever. Other typical symptoms include sore throat, muscle aches, chest pain, lethargy, vomiting and diarrhoea. The mortality rate in humans with this virus is about 60%. In human risk can be minimized by wearing gloves, mask and protective clothing. To minimize risk in pet birds keep them indoors and don't allow contact with wild birds.

### Newcastle disease

It is caused by *Avian paramyxovirus-1* which is primarily infection of bird. Transmission in bird and human may occur by direct contact with infected birds, inhalation or ingestion of contaminated material. Virus can survive in the environment, especially in feces. Birds show symptoms of uncoordinated movement, head twisted over back, weakness, muscle tremors, paralysis of one or both wings and legs. Conjunctivitis and possibly mild flu like symptoms are present in human. Human risk can be minimized by strict sanitary condition viz. wear gloves, mask and protective appliances along with disinfect equipment and supplies. Immunize susceptible birds and follow quarantine measures on arrival of new flock.

### West Nile virus

The pathogenic organism belongs to arbovirus genus *Flavivirus* and belongs to the Japanese encephalitis virus antigenic complex; which is single-stranded RNA virus which has wide host range including domestic species, wild mammals and humans. Birds are reservoir host. The virus is transmitted by mosquito vector (*Culex* species). Virus has been detected in dead birds of crows and jays having inflammation of heart and brain. In human incubation period is about 3-14 days. There are no any symptoms in 80% human infections and remaining 20% infections reported flu-like symptoms including fever, fatigue, headache, muscle or joint pain. In severe neuro-invasive disease (<1%) meningitis, encephalitis and poliomyelitis is reported which may be fatal.

### Chlamydiosis

*Chlamydia psittaci* is an obligate intracellular bacterial organism that excreted in the feces and nasal discharges of infected birds. The disease is also referred as parrot fever and ornithosis. Chlamydiosis is primarily transmitted in human by the inhalation of contaminated fecal dust, bird bites, mouth-to-beak contact and during handling of infected bird plumage and tissues. Bird to bird, from feces to bird, and from bird to human and human-to human transmission can occur. Infection is spread by carrier birds that remain carrier for years. *C. psittaci* can survive drying and be transmitted on contaminated clothing and equipment. Incubation period in human is about 5 to 14 days, severity varies from inapparent illness to systemic illness with severe pneumonia, flu-like symptoms, nonproductive cough and pneumonia. In human risk is managed by controlling airborne infection while cleaning poultry houses, handling infected poultry and poultry products, wear protective clothing and masks.

## Salmonellosis

Bacteria of genus *Salmonella-S.typhimurium* is the most common in the environment and are associated with gastrointestinal tract of animals namely reptiles, mammals and amphibians including birds. Transmission occurs by direct contact, contaminated food or water. All species of birds are susceptible showing signs of ruffled feathers, droopiness, diarrhoea, severe lethargy, incoordination, convulsions and results in heavy mortality. Fewer than 15 serotypes are responsible for the majority of human infections. The incubation period for salmonellosis varies between 6 and 72 hours, although most cases have an incubation period of 12 to 36 hours. Common clinical symptoms in all species include diarrhoea, vomiting, and a low-grade fever. Other symptoms include dehydration, weakness, septicemia, and headaches. Most cases of salmonellosis are mild and do not require the administration of antibiotics or other drugs. Personal hygiene measures should be followed strictly to minimize occupational hazard. Wash hands thoroughly with soap and water right after touching animals and their food. Don't let children and older immune-compromised individuals handle or touch high-risk animals. Animal habitats and their contents should be carefully cleaned and disinfected.

## Avian tuberculosis

*Mycobacterium avium* is the causative agent for avian tuberculosis and all avian species are susceptible to infection along with human and livestock species. The organism spreads via inhalation of aerosols, fecal-oral route, handling and contact with contaminated objects and surfaces so exposure can occur through birds coughing and sneezing as well as from dirty bedding and litter material. In man, lesions may occur in any organ system but commonly it occurs in the lungs. Clinical signs in humans are asymptomatic to night sweats, fever, weight loss, abdominal pain, fatigue, diarrhoea and cough in immune-compromised people. Wear protective masks, gloves and follow sanitary measures when exposed to dust of poultry cages. Eradication is difficult in birds due to the chronic carrier state, intermittent shedding and the organism is resistant to many antibiotics. The infected bird must be treated for a long period using a combination of drugs. People who are infected with human tuberculosis should not own birds, since these people may serve as a source of infection for their pet birds.

## Colibacillosis

Colibacillosis is caused by an *Escherichia coli* infection. Similarly to salmonella, *E. coli* are found in the intestinal tract of animals as a part of the normal bacterial flora. The major mode of transmission is fecal/oral route. In poultry, *E. coli* causes septicemia, diarrhoea, mastitis, pyometra, chronic respiratory disease, synovitis, pericarditis, colisepticemia, infectious cellulitis and salpingitis. In human the principal symptoms are profuse and watery diarrhoea, abdominal colic and vomiting. Certain strains of *E. coli* viz. enterotoxigenic, enteropathogenic and verotoxigenic strain (O157:H7, O104:H4, O121, O26, O103, O111, O145 and O104:H21) produce potentially lethal toxins. *E. coli* isolates from commercial poultry are often resistant to a variety of antibiotics so culture and sensitivity test may assist treatment. Vigorous sanitation program viz., Egg fumigation, dust control in house, routinely remove dead birds from house

should be followed at breeder house and hatchery along with avoid stress and immunosuppression among birds. Human infection can be prevented by wearing protective clothing viz face cover and gloves when handling birds, eggs or body tissues and follow strict hygienic practices.

### **Erysipelas**

Erysipelas is caused by *Erysipelothrix rhusiopathia*, which is a non-motile, Gram-positive, facultative anaerobe, catalase-negative, rod-shaped bacterium. It grows aerobically and anaerobically and does not produce endotoxin. In human, organisms enter through minor skin abrasions from contaminated dead flesh and surrounding area. It causes generalized illness, with skin lesions (erysipeloid) and joint disease. Less commonly, it can result in sepsis which is often associated with endocarditis.

### **Fowl cholera (Pasteurellosis)**

Fowl Cholera is a serious, highly contagious disease caused by the bacterium *Pasteurella multocida* in a diverse range of avian species including chickens, turkeys, and water fowl. The bacterium is a Gram-negative rod or coccobacilli, non-motile, penicillin-sensitive, oxidase-positive, facultative anaerobe organism. Transmission occurs in man by an animal bite, scratch, or lick but infection without epidemiologic evidence of animal contact may occur which leads to acute pneumonia or septicemic disease.

### **Histoplasmosis**

Histoplasmosis is caused by fungus *Histoplasma capsulatum* which grows in soil having dropping of bird and bat because bird droppings are primarily a nutrient source for the growth of the organism already in the soil. Birds do not appear to be infected by *H. capsulatum*; organism can be carried on the wings, feet, and beaks of birds. Transmission occurs by inhalation of spores when contaminated soil/ bedding material is disturbed and causes lung infection in human. Symptoms appear 3-17 days after exposure. Most infected people are asymptomatic or have only mild flu-like symptoms. In acute form there is respiratory symptoms, malaise, fever, chest pains, dry or non-productive cough; chronic form resembles TB; in immune compromised persons most severe and rare form occurs which affects multiple organ systems and is fatal unless treated.

In this light, we can affirm that the risk of zoonoses, has been probably underestimated in past years. This has been highlighted by epidemics that originated from the animal management centers. The example of HPAI clearly shows that any emerging disease may rapidly, become pandemic, causing a public health concern. Therefore, emerging and re-emerging diseases represent priorities for prevention and the creation of an early warning system that is specifically targeted at predicting the risk of an epidemic along with detection of early signs of its onset (Rabozziet al., 2012).

### **One health approach to control zoonosis**

In a globalized world, the interaction between human, animal and environment was very high which offers the opportunity for the emergence and spread of disease that could adversely impact animal health, human health, or both. Dealing with the risks that arise at the

interface between animals, humans and the environment to control zoonotic diseases demands integrated action from both human and animal health sectors along with additional key inputs from the environment sector. One Health movement addresses risks, including zoonoses, at the human–animal–environment interface, and requires the development of innovative partnerships at the political, institutional and technical levels. The US Agency for International Development (USAID) launched the Emerging Pandemic Threats Programme “PREDICT”. PREDICT utilizes a One Health approach focused on quick diagnosis and detection of zoonotic threats, collaboration between different health care agencies and early response to potentially zoonotic threats at their source ideally before they emerge in people.

### **One health status in India**

In the Indian context, the one health approach is strategically gaining importance from all stakeholders such as public health professionals, veterinarians, health-care providers, policymakers, and researchers. The successful implementation of the one health model involves integration and collaboration between multiple sectors of agriculture, animal health, and human health. In India, a National Standing Committee on Zoonoses was formed in 2007. The Food Safety and Standard Act, India limits the contaminants, naturally occurring toxic materials, antibiotic residues, pesticides, heavy metals, veterinary drug residues, etc.,. With increasing population, a large number of people are in contact with pet and farming animals, making the country a hotspot for emerging zoonotic diseases. While there is an increased focus on the prevention and prediction of diseases in human health other than diagnosis, treatment, and rehabilitation, the animal health sector lacks proper surveillance and reporting of animal diseases and laboratory diagnosis (Aggarwal and Ramachandran, 2020).

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