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# ISOLATION & CHARACTERIZATION OF BACTERIA, FUNGI AND PARASITES FROM INFECTED MIGRATORY BIRDS (KOKKARE BELLURU PELICANS)

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## Abstract:

Pelican, Pelecanus belonging to the Pelecanidae family are defenseless to different infection by an assortment of pathogens. They succumb to illness due its low immunity and unhealthy environment. Avian infections contribute to an important set of problems within the life cycle balancing system and live stock production. Various pathogens are known and have been studied. More studies have been done on infection that commonly caused in bovine such as Botulism, Psittacosis and few others. Fungal diseases have been studied such as Aspergillosis, Candidiasis, Cryptococcosis and few others. Parasitic diseases have also been studied such as Helminth and arthropod. Pelicans are infected by number of pathogens and they succumb to diseases. For this study, we considered the most common infections Botulinum, Aspergillosis and Helminth, Nematode (Ringworm) & Arthropod parasitic infections.

By plating technique infected sample of Pelican were screened for bacteria and fungi, using Agar-Agar, Potato Paste and Sheep blood and incubated at 25'C. Once the visible colonies were formed on the plate, they were characterized by colony morphology.

To further identify the fungi, mycelial studies were done. The fungi culture slide were prepared by tease mount technique using lacto phenol cotton blue stain [LPCB]. The slides were then analyzed to characterize the fungi. The identification was done as per the guidelines recommended by Larone (1976) and Al-Doory (1980). And to identify bacteria, the bacterial culture slide were prepared by Gram's staining using Crystal violet, Gram's iodine, 90% alcohol and Safrannin suggested by Danish bacteriologist Hans Christian Gram, who developed the technique (1882).

To further identify the microbes present in water, samples were collected and plating technique was done.
Molluscs as being rare food for Pelicans were also collected and examined by bacterial staining technique.

Identification of the parasitic infection in Bird, were analyzed by the food chain of birds, (Pelicans feed on molluscs, arthropods which may cause parasitic infections). They sometimes even feed on maggots. Investigation on the food cycle parasitic infection detected.

The organism found to be ***Fusarium*** **mycotoxin**, i.e., Deoxynivenol mycotoxin.

Further studies have to be carried out in order to study the mechanism and role involved in pathogenesis.

# Introduction:

Pelicans are a genus of large water birds that make up the family Pelecanidae. They are characterized by a long beak and a large throat pouch used for catching prey and draining water from the scooped up contents before swallowing. Pelican, any of seven or eight species of water birds in the genus *Pelecanus* constituting the family Pelecanidae ( order Pelecaniformes), distinguished by their large elastic throat pouches. Pelicans inhabit lakes, rivers, and seacoasts in many parts of the world. With some species reaching a length of 180 cm (70 inches), having a wingspan of 3 meters (10 feet), and weighing up to 13 kg (30 pounds), they are among the largest of living birds.

Pelicans eat fish, which they catch by using the extensible throat pouch as a dip-net. The pouch is not used to store the fish, which are swallowed immediately. One species, the brown pelican (*Pelecanus occidentalis*), captures fish by a spectacular plunge from the air, but other species swim in formation, driving small schools of fish into shoal water where they are scooped up by the birds.

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The spot billed pelican (*Pelecanus philippensis*) is a globally threatened species that has suffered a rapid decline in population during that past seventy years. The annual mid winter water-fowl census conducted by the Asian Wetland bureau has reported not more than 5000 birds in the whole of South Asia, in its 1993 report. Of the ten known nesting sites in India the Pelicinary at Kolamuru village in AP and one at Kaziranga in Assam have now been virtually abandoned.

No current information is available from Northern Sri Lanka where there used to be nesting sites in mangroves.
The spot-billed pelican or Grey pelican (*Pelecanus philippensis*) is a member of the pelican family. It breeds in southern Asia from southern Pakistan across India east to Indonesia. It is a bird of large inland and coastal waters, especially large lakes. At a distance they are difficult to differentiate from other pelicans in the region although it is smaller but at close range the spots on the upper mandible, the lack of bright colors and the grayer plumage are distinctive. In some areas these birds nest in large colonies close to human habitations.[1]

In current migration of Birds from Florida to Kokkare Belluru, more than 300+ have dead, in this study we found out the cause for the death of Pelicans. In North America (2004-2005) American White Pelicans (93) dead due to Avian cholera, Aspergillosis, Botulism (type C, E and suspect), Chlamydiosis, emaciation of unknown etiology, possible Newcastle disease, trauma,, toxicoses and West Nile virus encephalitis.

The spot-billed pelican is a relatively small pelican but still a large bird. It is 125–152 cm (49–60 in) long and a weight of 4.1–6 kg (9.0–13.2 lb). It is mainly white, with a grey crest, hind neck and a brownish tail. The feathers on the hind neck are curly and form a grayish nape crest. The pouch is pink to purplish and has large pale spots, and is also spotted on the sides of the upper mandible. The tip of the bill (or nail) is yellow to orange. In breeding plumage, the skin at the base of the beak is dark and the orbital patch is pink. In flight they look not unlike the Dalmatian pelican but the tertials and inner secondaries are darker and a pale band runs along the greater coverts. The tail is rounder

The newly hatched young are covered in white down. They then moult into a grayish speckled plumage. The spots on the bill appear only after a year. The full adult breeding plumage appears in their third year.

They are very silent although at their nests they can make hisses, grunts or snap their bills.Some early descriptions of nesting colonies have claimed them to be distinctive in their silence but most have noted colonies as noisy.

Like most other pelicans, it catches fish in its huge bill pouch while swimming at the surface. Unlike the great white pelican it does not form large feeding flocks and is usually found to fish singly or in small flocks. Groups may however sometimes line up and drive fish towards the shallows. When flying to their roosts or feeding areas, small groups fly in formation with steady flapping. During the hot part of the day, they often soar on thermal. They may forage at night to some extent.

The birds nest in colonies and the nest is a thick platform of twigs placed on a low tree. The breeding season varies from October to May. In Tamil Nadu, the breeding season follows the onset of the northeast monsoon. The courtship display of the males involves a distention of the pouch with swinging motions of the head up and down followed by sideways swings followed by the head being held back over the back. Bill claps may also be produced during the head swaying movement. The nests are usually built alongside other colonial water birds, particularly painted storks. Three to four chalky white eggs is the usual clutch. The eggs become dirty with age. Eggs hatch in about 30–33 days. The young stay in or near the nest from three to five months. In captivity the young are able to breed after two years. Like other pelicans, they cool themselves using gular fluttering and panting.

**Avian Bacterial Diseases:**

Birds are susceptible to various kinds of bacterial diseases - usually caused by a lack of hygiene or stress -- but some birds have genetic immunity and instead become carriers of these diseases, able to infect other birds.

However, there are times carrier birds can become sick if they are faced with infection triggers like age (very young or old birds), ill health due to other infections or diseases, environmental or emotional stress, or anything else that temporarily lowers a bird's immunity to bacteria.

**Symptoms and Types**

Symptoms for a bird will depend on the type of bacteria, its location in the body and the organs it is affecting. Common symptoms in most bacterial diseases include listlessness, weight loss and loss of appetite.

More specifically, stomach infections show digestive symptoms, such as a lack of appetite, and diarrhea. Liver infections display digestive and urinary problems. Lung infections can affect and lead to breathing difficulties, nasal discharges, and eye infections. Finally, nervous system infections will cause tremors and seizures in birds.

**Causes**

There are many bacteria which cause infections in birds. Among them: *E. coli*, *Pseudomonas aeruginosa, Serratia marcescens, Salmonella, Mycobacteria, Clostridia, Klebsiella, Enterobacter, Proteus, Citrobacter, Pasteurella are all species of bacteria which affect birds.*

*Pasteurellabacteria* is found in animals -- like cats or rats -- and they pass on the infection to the bird through biting. Some common bacterial infections in birds are avian tuberculosis (mycobacteriosis), psittacosis (chlamydiosis or parrot fever), and Clostridial diseases (Ducks and Pelicans)

**Treatment**

The veterinarian will test the infected bird, and diagnose the bacteria causing the infection. The treatment will then consist of antibiotics either by food, water or injection, and relieving the stress of the infected bird. The bird's environment needs to be thoroughly cleansed, as well.

**Prevention**

The following are a list of precautions which should help prevent bacterial disease in your bird

- Quarantine any new bird
- Do not overcrowd birds
- Avoid creating stressful environments
- Keep the bird's living area well ventilated
- Provide a nutritionally balanced diet
- Store feed hygienically
- Regularly disinfect cage, utensils and nest boxes
- Maintain regular veterinary visits for your bird.

**Clostridium Botulism:**

***Clostridium botulinum*** is a spore-forming obligate anaerobic bacillus that produces seven potent neurotoxin, A to G. It exists as a spore in the environment, and requires anaerobic conditions to grow and produce toxin.

The seven neurotoxin are designated by letters A through G. Type C is responsible for most animal cases although Type D is occasionally seen in dogs and cattle. Type B can occur in horses, and type A and type E are found in mink and birds. Types A, B, E, F, and rarely G affect humans. All types produce the same symptoms but the toxin type is important if antiserum is used for treatment.

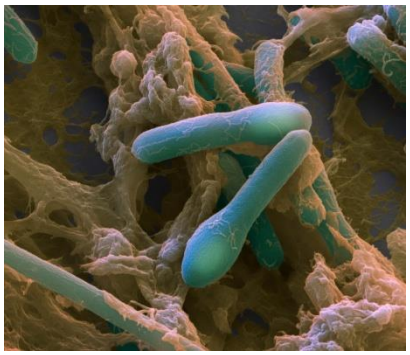


Fig.1

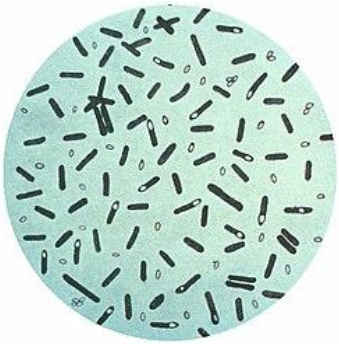


Fig.2

Fig.1: Electron microscopic observation of *Clostridium botulinum*

Fig.2: Compound microscopic observation of *Clostridium botulinum*

***Transmission:***

Disease can result from ingestion of preformed neurotoxin or from bacterial growth in anaerobic tissues as when wounds are contaminated with spores.

*Clostridium botulinum is* ubiquitous and spores are found in soil, fresh and coastal waters and in the intestinal tracts of animals, including fish. Animals are usually infected through ingestion of feed contaminated with pre-formed toxin. Cases of botulism in horses and cattle are primarily associated with infected forage, especially silage. Outbreaks in waterfowl and wild birds occur sporadically in Ohio, usually in late summer. The disease is associated with decomposing feed and carcasses, especially in a water source. Tissues from dead animals can be toxic if ingested by other animals.

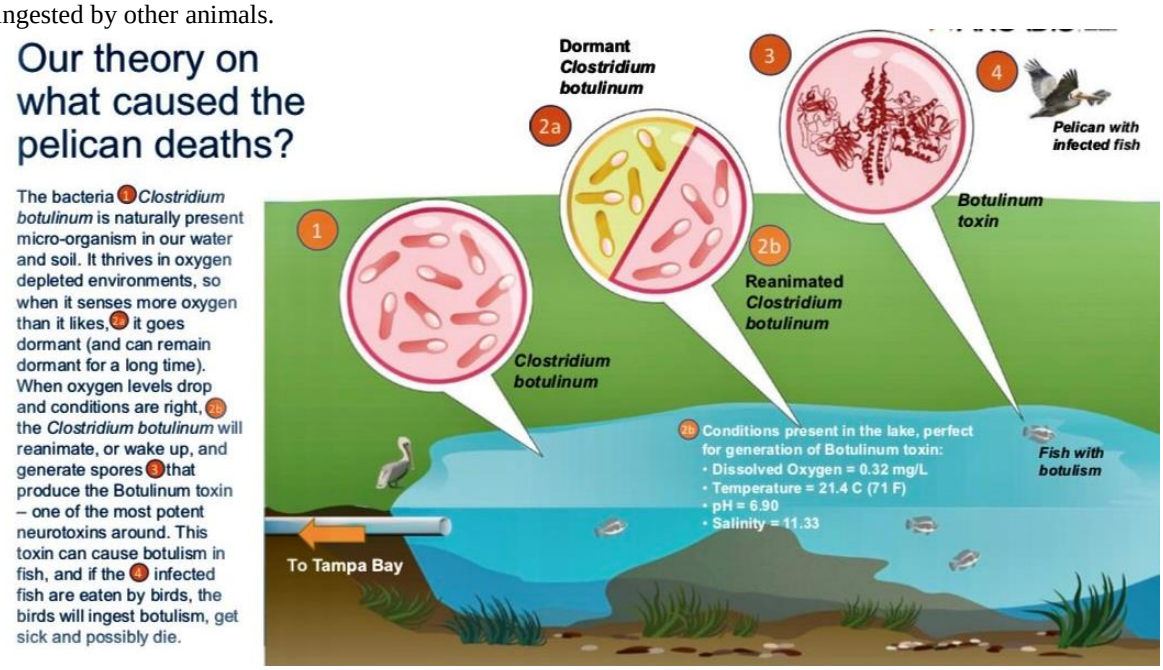



Fig.3: Causes and Transmission

**Clinical signs***:* The incubation period ranges from a few hours to two weeks with most developing signs in the first two days. Botulism causes progressive motor paralysis including in coordination, drooling, muscle tremors, weakness, paralysis, difficulty chewing and swallowing, and visual disturbances. Death typically results from paralysis of the cardiac or respiratory muscles. Additional signs are species specific.

| Species | Most common toxin type | Clinical signs |
|---|---|---|
| Birds | Type C, rarely Type E | Flaccid paralysis is usually seen in the neck ("limber neck"), wings, legs, and eyelids. Drowning may occur due to neck paralysis. May see large die offs |

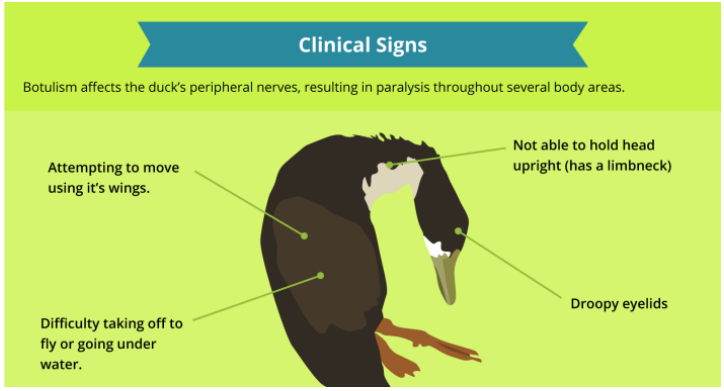



Fig.4: Clinical Sign

***Diagnostics*:**

- ELISA, mouse inoculation: identifies toxin in feed, stomach contents, feces, vomits, blood or fetus
- Culture: from stool or tissues

**Case classification:**

- Suspected: a clinical case with signs consistent with botulism
- Probable: a clinically suspect case with laboratory evidence from a screening or invalidated test
- Confirmed: a case that meets confirmatory testing criteria determined by a state or federal diagnostic laboratory

**Avian Skin Infections:**

Much like humans, birds suffer from skin infections. In birds, they can be due to injury or infection and usually results in redness and swelling. And if the bird continuously pecks at the infection, it may be ulcerate.

**Symptoms and Types**

In general, symptoms affecting the skin include:

- Itching
- Redness
- Swelling

If your bird is pecking an skin area more than usual, check for infection and take the bird to the veterinarian for proper diagnosis and medication.

Bacterial skin infections- caused by bacteria, such as bacilli, *staphylococci* and *streptococci*. Bumble-foot (pododermatitis) is caused by staphylococci.

**Avian Fungal Infections**

There are several types of fungi that affect humans and animals (including birds), but the most common is the ***Aspergillus*** fungus. This fungus is usually associated with seed, corn cobs, old food, humid areas, air conditioners, blow heaters and wet cages.
It also occurs in individuals that have a poor level of natural resistance or a damaged immune system. Your bird may show dropping changes, because the ingested fungus irritates the bowel. This fungus may also produce a toxin which causes liver disease. Can notice a dark green dropping when this occurs. When the fungus is inhaled it produces a squeaky voice, sneezing, coughing or breathing difficulties. Both the inhaled and ingested form are potentially life threatening. The culture test identifies the exact type of fungus, so we can identify where it has come from, how best to treat it and how to prevent it from recurring.
Loss of or changes in voice are possible.

**Treatment**

An anti-fungal treatment must commence immediately, because this is a potentially life threatening disease. Fungilin is the medicine used to treat fungal infections. This should be given directly by mouth, or may sometimes be added to the drinking water. As well, remove all seed, grit, seed bells and fruit, from the cage. Disinfect the cage with a Water Cleanser and start your bird on sterile seed. Dry heat may be advised in the form of a bar heater. The above recommendations protect your bird from re-injection by killing or removing any fungal spores from the environment.

**Aspergillosis**

Aspergillosis is a fungal infection caused by the *Aspergillus* fungus, which can be found in damp or wet seed mixtures, in birds' nesting materials, or in landfills. Spores inhaled into the lungs and air sacs of birds eventually cause pneumonia and bronchitis. Sick birds experience labored breathing, weakness, and diarrhea, but will continue to take food at feeding stations until they die.

**Avian Aspergillosis**

Airway and respiratory tract diseases are very common in pet birds. One such disease commonly is Aspergillosis, which is a fungal infection of the bird's respiratory tract.

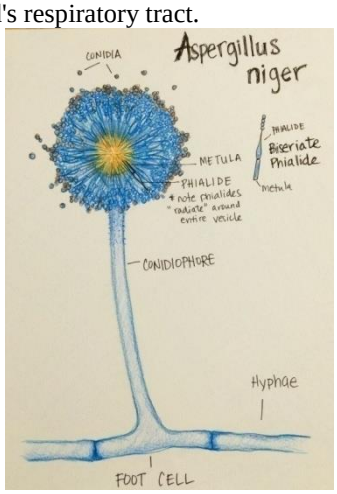



Fig.5: *Aspergillus niger*

Fig.6: Electron Microscopic Observation

**Symptoms and Types**

The symptoms of the disease are dependent on the form of the infection. For birds, the fungal spores lodge in the *air sacs* of the lungs. But, it can also involve the bronchi, *trachea*, and *syrinx* (voice box) of the bird. If not treated quickly, *Aspergillus* can even spread to other organs. There are two forms of Aspergillosis disease found in birds.

1. Acute Aspergillosis occurs in young and newly imported birds. It is severe and of short duration. Birds will have a lack of appetite, breathing difficulties, and if not treated in time, the infected bird may die. When the air sacs get inflamed, the problem is called airsacculitis. A veterinary examination will find a bird's lungs and air sacs congested with white *mucus*; the lungs may also have nodules.
2. Chronic Aspergillosis occurs in older, captive birds. Infection occurs over a long time and birds will shows signs of listlessness, depression, weakness, and will have trouble breathing. The symptoms will only become apparent after the infection has been present in the lungs for some time. The changes and problems for these birds are severe, and may be permanent. There may be bone changes and a misshaping of the *upper respiratory tract* -- nose, trachea, and syrinx. The lungs will be severely damaged, due to the long-term infection, and it can easily be spread to other organs and systems. If the central nervous system becomes infected, the bird may show tremors, loss of coordination and paralysis.

**Causes**

Aspergillosis disease is caused by the fungus *Aspergillus*, and its spores are what cause respiratory problems in birds. The fungal spores can be present in contaminated food, water, nest boxes, incubators, other nesting material, and unventilated areas. However, birds can also catch the infection from the environment.

Fungal infection is common in birds with vitamin A deficiency, malnutrition, stress and in various other weakened states. The fungal spores enter the bird's lungs and are especially infectious when the bird's immunity is low.

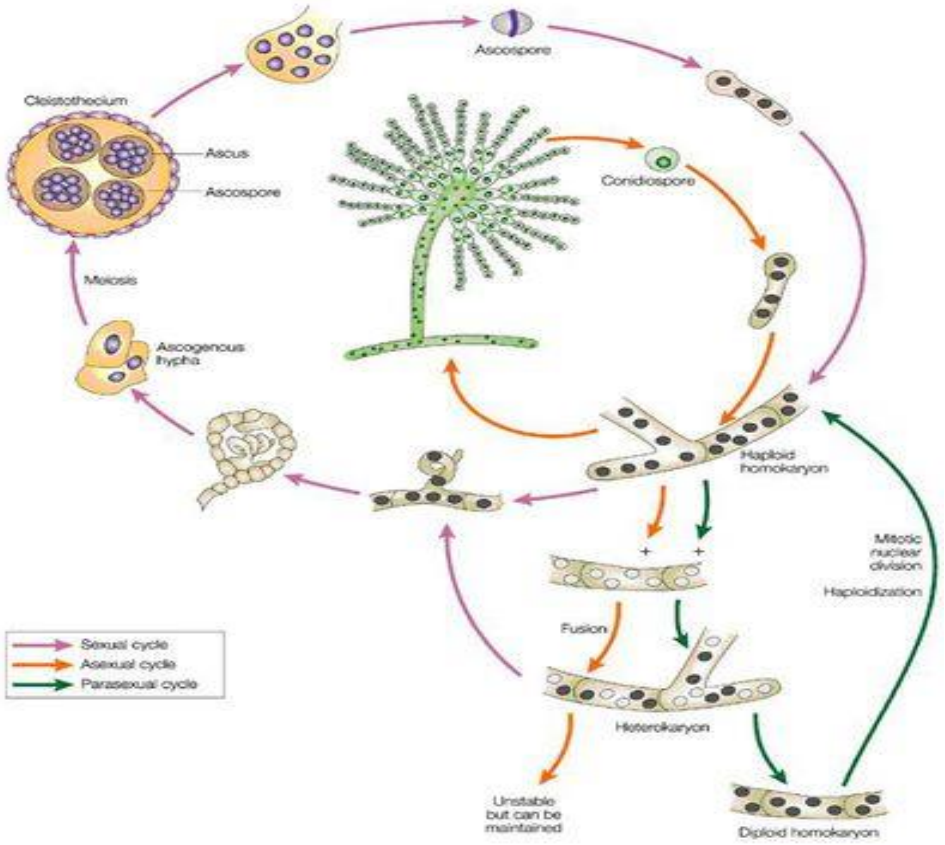



Fig.8: Life Cycle of *Aspergillus niger*

**Treatment**

After proper diagnosis (and if treated early), the veterinarian can cure Aspergillosis disease with anti-fungal drugs. And because the symptoms of this disease are similar to other respiratory infections, you must be vigilant and take your bird to the veterinarian if any of these symptoms become apparent.

**Prevention**

Aspergillosis disease in birds can be prevented with a few simple precautions: you should maintain good hygiene, nutrition and ventilation for your bird.

**Candidiasis:**

Candidiasis is a fungal infection caused by *Candida sp*., most commonly *Candida albicans* that affects many species. Candidiasis is common in neonatal psittacine birds. It occurs primarily as a result of immunosuppressant and trauma to the ingluvies. Hypothermia, feeding too cold or too hot formula which results in crop-burn, poor nutrition, long-term antibiotic therapy, hypovitaminosis A and systemic illness from other causes are all predisposing causes. The bird may present with inflammation and delayed emptying of the ingluvies. On examination of the crop surrounding structures, there are normally grey-white lesions and a whitish necrotic material present. There may also be thickening of the crop wall.

Fig.12 Candidiasis under a Compound Microscope

**Parasitic Infections**

**Helminth (Worms)**

**Tapeworms**

It is asymptomatic. It may absorb nutrients from host causing bird to be unthrifty and have diarrhea. It is most common in Finches African grays (15-20% of imported birds), Cockatoos (10-20% of imported birds) and Eclectus parrots. There is no direct correlation between eosinophilia (increase in eosinophils in blood which are a type of white blood cell which characteristically increases in parasitic infections) and parasitism. Generally infections are non- pathogenic, although large numbers of worms can cause impaction. With severe infection, birds may die following a period of weight loss and diarrhea. Some feel that it may be a cause of feather picking in Old World birds. Tapeworms require 3 an intermediate host (such as insects or mites which carry the parasite) so infections are uncommon in birds that do not have access to ground.

Diagnosis is by identification of proglottids (tapeworm segments) or whole worms in feces. Individual eggs may not be noted in routine fecal samples unless proglottids in feces have ruptured.

Treatment - Praziquantel (Droncit).

**Flukes**

Flukes are rarely reported in imported birds. These are usually Old World species and should be self-limiting because the intermediate hosts of origin (usually an arthropod) are not present in the US. Hepatic (liver) trematodes have been described in cockatoos. They are periodically seen in raptors.

Diagnosis is through identification of characteristic egg in feces.

Treatment - Fenbendazole, Praziquantel, Ivermectin may be used in combination with one of the aforementioned drugs.

**Roundworms**

Most common parasites found in birds maintained in enclosures with access to ground. Infections are common in budgies and cockatiels. Have a direct life cycle (passed directly from one bird to the next). Eggs require 2-3 week period for embryonated larvae to form in egg and become infective. It is viable for long periods in moist warm environments. Resistant to disinfectants, but can be controlled with steam and flaming. Embryonated eggs which are ingested are directly infective. Periodically test birds that have tested positive for roundworms and periodically worm any outdoor breeding flock. Clinical signs with severe infestation may include distended abdomen, weight loss, diarrhea, malabsorption, intussusceptions (telescoping of a portion of the intestinal tract), blockage and death.

Diagnosis: Through fecal examination/flotation.

Treatment - Pyrantel pamoate, Fenbendazole.

**Arthropods**

Biting Lice

Can cause prurit Kokkare belluruis (itching) and poor feather condition. Parasites can be observed directly or eggs (nits) attached to feathers. Most species are host specific and die quickly when they leave the host. Dusting with pyrethrins can control infestation.

**Mites:**

Feather Mites

Numerous feather mites have been described in birds. It is seen in newly acquired or imported birds. They have specific microhabitats including specific portions of the feather. Generally non-pathogenic in host adapted species but can cause clinical problems in non-host adapted species or with heavy infestations when mites move from feathers to skin. Quill mites reside in the pulp of developing feathers and cause damage its growth.

Diagnosis is through visualization of mites, examination of feather pulp with quill mites.

Treatment - Topical pyrethrins, Ivermectin, removal of affected feathers with quill mites.

## Objectives:

1. To isolate and characterize bacterial and fungal pathogen in an infected Pelican:

- To isolate and characterize bacteria, the samples should be grown on NA and incubated for 24 hours at 37'C. After the growth the bacteria need to be isolated and characterized by colony morphology, then the bacteria staining by Gram's Staining Technique. Then identification microorganisms by microscopic observation under 10x and 40x.

- Morphology of bacteria:

1. Depending on their shape, bacteria are classified into several varieties 1. Cocci (from kokkos meaning berry) are spherical or oval cells

2. .Bacilli (from baculus meaning rod) are rod shaped cells

3. Vibrios are comma shaped curved rods and derive their name from their characteristics vibratory motility.

4. Spirilla are rigid spiral forms.

5. Spirochetes (from speira meaning coil and chaite meaning hair) are flexuous spiral forms

6. Actinomycetes are branching filamentous bacteria, so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from actis meaning ray and mykes meaning fungus)

7. Mycoplasmas are bacteria that are cell wall deficient and hence do not possess a stable morphology. They occur as round or oval bodies and as interlacing filaments.

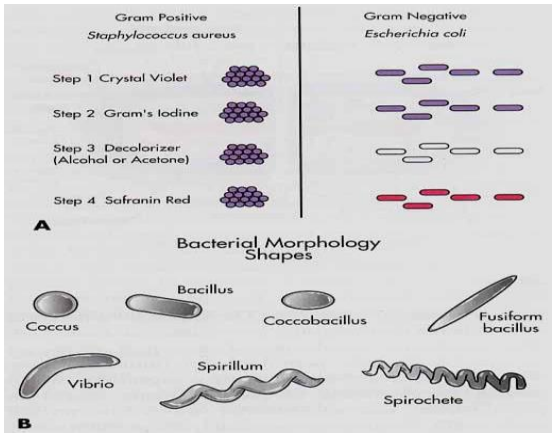



Fig.9 Bacterial Morphology

- To isolate and characterize fungi, the samples should be grown on SDA and incubated for 4 days at 25'C. After the growth the fungi need to be characterizes by colony morphology technique and stain using Tease Mount Technique.

- Morphology of fungi:

1. Yeasts: Unicellular, Nucleated rounded fungi

2. Yeast like fungi: Grow partly as yeasts and partly as elongated cells resembling hyphae which are called pseudo hyphae.
3. Molds**:** Multicellular**,** Filamentous with hyphae and produce conidia [spores]
4. Dimorphic fungi: Occur in 2 forms: Molds (Filaments) Yeasts

-- Fungi Clinical Classifications:

1. Superficial mycosis

2. Subcutaneous mycosis

3. Systemic mycosis- Primary pathogens & Opportunistic pathogens

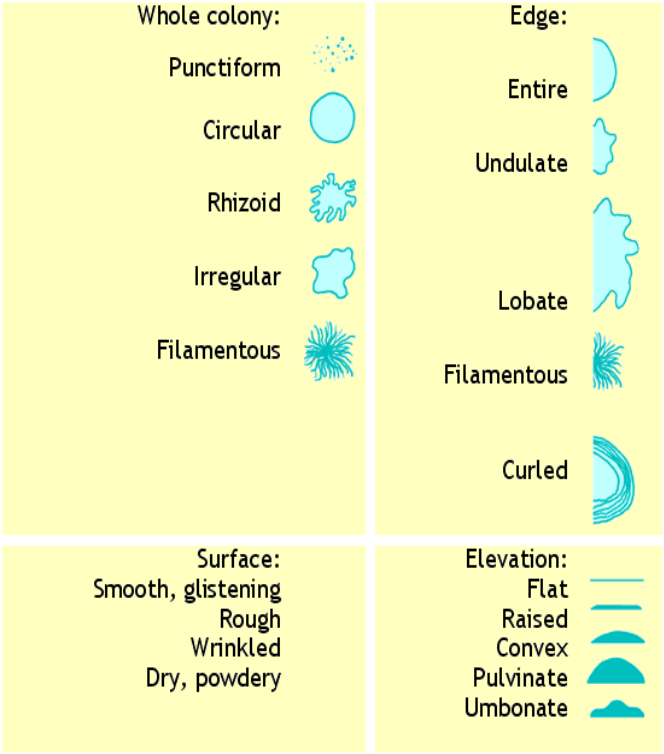



Fig.10: Fungal Morphology

2. To identify the parasite in an infected Pelican:

- To identify the parasitic infection in Bird, need to analyze the food chain of the birds, as they rarely feed on molluscs, arthropods. They sometimes even feed on maggots. By this parasitic infection can happen, on investigation on the food cycle and food chain the parasitic infection can be detected.

## Research Methodology and Culturing Techniques

**Apparatus:**
Glass Petredish – 10nos, Pipette 10ml – 5nos, Pipette 1 ml – 5nos, bent glass rod -1nos, Inoculation loop, tissue paper, gloves, newspaper, thread, cotton plug, conical flask 250 ml- 3 no's, measuring cylinder, test tubes 20 no's, spirit lamp

**Chemicals:**
Peptone, Beef extract, Yeast extract, Sodium chloride, Agar, Distilled water, Alcohol

**Stains:**
Lacto phenol cotton blue stain, Methylene Blue for Fungal Staining.
Crystal Violet, Gram's Iodine, 90% Alcohol and Safrannin for Bacterial Staining.

**Instrument:**
Autoclave, Laminar Air flow

**Sampling:**
The sample of the infected Pelican was aseptically collected in a sterilized container wearing gloves. The sample is then immediately refrigerated for optimum growth of the culture and to avoid cross contamination. The samples were then labeled them accordingly noting down, date, time, location, symptoms involved for the infection.

**Preparation of media:**

**AGAR-AGAR, POTATO PASTE and SHEEP BLOOD**:

**Agar-Agar Media**: Agar-Agar was washed thoroughly in water. Boil the water till it melts and settles down. Drain the water and pour the gel to the petriplate and culture to it and swirl clockwise and anti clockwise. Liquefies at 98'C and solidifies at 42'C.

**Potato Paste**: Cook two potatoes and squash it and to that add boiling water and make fine paste. Pour the paste to the petriplate and add the culture sample to it. It solidifies.

**Sheep Blood**: Take debfibrinated blood and pour 3/4$^{th}$ of the petriplate and add culture sample to it and swirl clockwise and anti clockwise. Allow it for solidification.

# Culturing techniques:

The samples were cultured on different media, Agar-Agar media. The media is taken out then pour to petredish by pour plate method in an aseptic condition, before it solidify the sample need to be added and then keep aside for solidification. The petredish kept for incubation (Fungal culture plate for 7 days).

## Plating technique:

### 1. Plating into agar.

The sterilized medium which has been autoclave is taken and cooled to 40$^0$C. For **pour plating** method 1 ml of the inoculums from appropriate dilution was poured into the sterile petriplate. To that around 20 ml of the medium is poured and swirled well for uniform distribution. The plates were labeled appropriately and kept on a flat surface for solidification.

Image1. Agar-Agar Media in Petriplate

Image2.Agar-Agar Media in Test tube

For **spread plate method**, the medium is solidified by pouring them into sterile petriplate. To the solidified medium 0.1ml of the inoculums was added from appropriate dilution. The inoculums were spread evenly with the sterilized bent glass rod. The plates were incubated at 25$^0$C for more than a week to isolate fungal culture.

## Analysis of parasites:

The swabs were collected and mixed with saline because plain water could cause the protozoan parasites to rupture or swell or swell making identification difficult. Flotation can be done using with routinely used solutions. Then the solution can be examined microscopically.

**Microscopic observation of fungi:**

The fungal cultures that were isolated were observed microscopically to identify its characteristics. Tease mount technique of fungal culture was used. Two drops of lacto phenol cotton blue stain was placed on a clean glass slide. With a help of a sterile forceps, a small amount of hyphal mass was placed from the culture plate to the slide. Then hyphal mass were teased apart using sterile needle. A cover slip was gently placed onto the specimen with the help of a needle. Then the slides were observed under a microscope.

**Microscopic observation of bacteria:**

The bacterial cultures that were isolated were observed microscopically to identify. Gram's staining technique of bacterial culture was used. A drop of saline is added on the clean glass slide and the isolated culture were smeared on the saline and then heat fixed. Initially the Crystal violet is added and kept for 1 minute, then Gram's iodine was added and kept for 1 minute and added 90% alcohol and kept for 30 seconds and added Safrannin and kept for 2 minutes. In the last rinsed the culture slide with distilled water. Blot the slide using blotting paper and allow to air dry. After drying then culture was oil immersed. Then the slides were observed under a microscope.

**Microscopic observation of parasite:**

The parasitic cultures were observed microscopically to identify. After floating with saline the culture can be examined under a microscope.

## Results and Observation:

**Observation of culture growth:**

**Day1**:

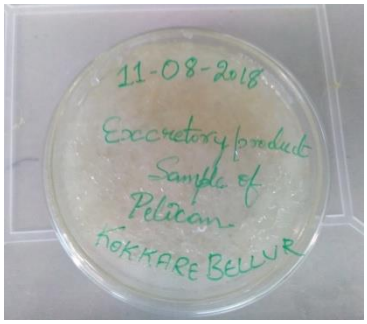



Image 3 Labeled Petriplate Image 4 Screw-capped Test Tube

**Day 2**:

Image 5 Image 6

**Day 5:**

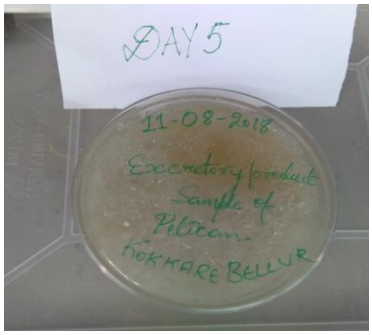



Image 7


Image 8

**Day 7:**

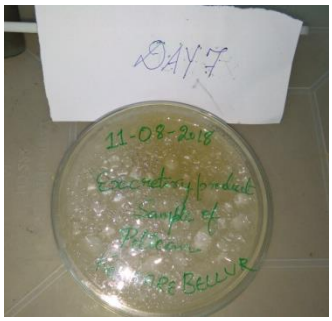



Image 9

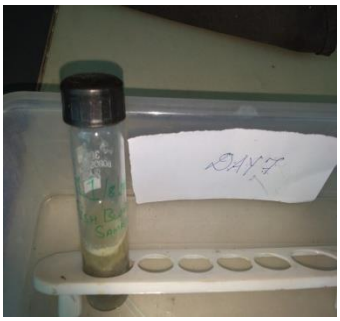
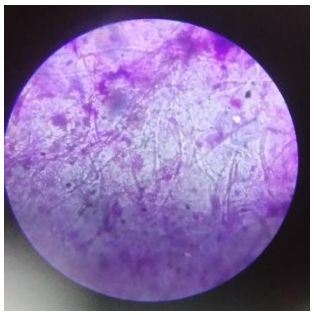



Image 10

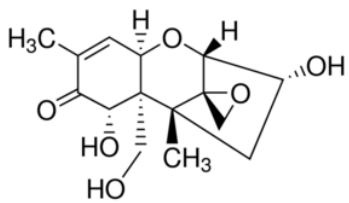
The culture grown on the Agar-Agar media plate was yellowish in colour. The culture was isolated and smeared on a clean glass slide aseptically observed under a microscope at 10X. The results were observed were mycelium and hyphae of fungi shown in Microscopic observation 1.

Organism found to be ***Fusarium*** sp., that is Fusarium mycotoxin. The toxin identified was Deoxynivenol mycotoxin



Microscopic observation1
(Mycelium & Hyphae)

Microscopic observation2
(Fusarium mycotoxin)




**Deoxynivenol mycotoxin**

No growth seen in screw-capped test tube.

## Discussion and Conclusion:

By plating technique infected sample of Pelican were screened for bacteria and fungi, using Agar-Agar media and incubated at 25'C. Once the visible colonies were formed on the plate, they were characterized by colony morphology.

Identification of fungus mycelial studies were done. The fungi culture slide were prepared by tease mount technique using lacto phenol cotton blue stain [LPCB]. The slides were then analyzed to characterize the fungi. The identification was done as per the guidelines recommended by Larone (1976) and Al-Doory (1980). And to identify bacteria, the bacterial culture slide were prepared by Gram's staining using Crystal violet, Gram's iodine, 90% alcohol and Safrannin suggested by Danish bacteriologist Hans Christian Gram, who developed the technique (1882).

Identification of the parasitic infection in Bird, were analyzed by the food chain of birds, (Pelicans feed on molluscs, arthropods which may cause parasitic infections). They sometimes even feed on maggots. Investigation on the food cycle parasitic infection detected.

The organism found to be ***Fusarium* mycotoxin**, i.e., Deoxynivenol mycotoxin. *Fusarium* mycotoxins frequently contaminate cereal grains. It is considered as one of the most important trichothecenes and found in all kinds of grains, such as wheat, rye, barley and oats. In general, the acute form of DON mycotoxicosis rarely occurs in birds under normal conditions. However, if diets contain low levels of DON (less than 5 mg DON/kg diet), lower productivity, impaired immunity and higher susceptibility to infectious diseases can occur.

Deoxynivalenol (DON) is also called vomitoxin and produced by *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum* . DON is the most common contaminant of feedstuffs worldwide. It was found in cereal grains (wheat, maize, barley, oat and rye and less often in rice, sorghum and triticale). DON contaminates mainly corn and wheat, while small grains, such as oats, rye and barley, have less DON contamination. *Fusarium graminearum* and *F. culmorum* can survive in the leaves of the cold season and be a source of infection for the new crop.

## Conclusion:

By the conclusion of the investigation, the birds were dying and dead due the consumption of cereals like groundnuts which has infected by fungal toxin i.e., mycotoxin. Mycotoxin has many varieties, where in the investigation the mycotoxin found to be ***Fusarium* mycotoxin.**

Further studies have to be carried out in order to study the mechanism and role involved in pathogenesis.

**Appendix:**

**Pictorial representation**

Dead Birds:

Fig 1

Fig 2

Bird Having Food:

Fig 3

Sample Collection:

Fig 4

## Culturing:

1.Pour plate and Spread plate method of the serially diluted fecal matter sample that has kept for incubation.

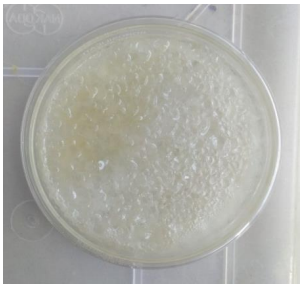
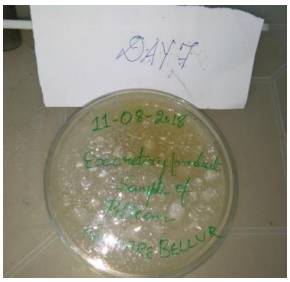


Fig 5 Fig 6

2.Agar-Agar plates after 7 days of incubation at $25^0$ C.



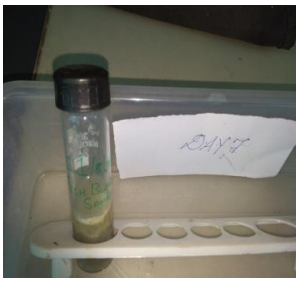




Fig 7 Fig 8

3.Tease Mount technique of staining fungal culture for identification

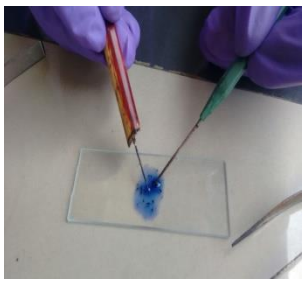
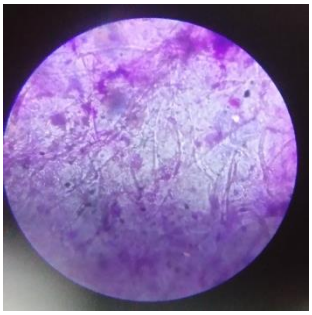
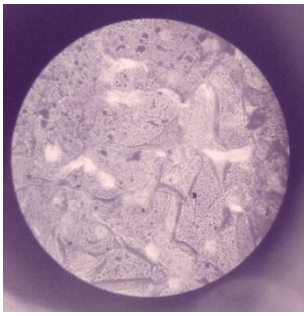


Fig 9

4.Microscopic Observation



Microscopic observation1
(Mycelium & Hyphae)



Microscopic observation2
(Fusarium mycotoxin)

Fig 10

**Media:**

Agar-Agar Media

Dried Strips of Agar-Agar is immersed in boiling water till it solidifies, it will become gelatinous.

1. Lactophenol Cotton Blue

Lactophenol Cotton Blue is used as a staining solution for fungi.

| | |
|---|---|
| Phenol crystals | 20.0gm |
| Cotton blue | 0.050gm |
| Lactic acid | 20.0ml |
| Glycerol | 20.0ml |
| Distilled water | 20.0ml |

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