# Effects of Passive Immunity in Pheasants at Wildlife Breeding Center, Jallo Park, Lahore, Pakistan

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**Abstract:** A disease of an unknown etiology (Syndrome) broke out as epidemic infecting chicks of peafowl / pheasants at Wildlife Research Center, Jallo park, Lahore and caused 100% mortality. To investigate the causative agent, mutant / resistant bacterial strains were cultured from the pus collected from the ocular region of the infected peafowl and pheasant chicks. This bacterial strain was found resistant to the latest broad spectrum antibiotics viz: Tylosin, Doxycycline, Colistin, Amoxixillin, Tribrissen, Gentamycin, Furazolidone, Quinolone. Passive immunity induction plan was developed to control the bacterial disease. For this purpose, pus and blood taken from infected chicks were injected to the healthy birds to obtain antiserum which was later injected to the infected chicks. Antibiotics, Glucose and Texiron were mixed with antiserum to strengthen the immune system. A total of 250 chicks were treated and 73% survival was obtained. This supported the hypothesis that passive immunity is an effective tool against mutant / resistant bacterial strains. Identification of the mutant / resistant bacterial strains, preparation of vaccine / antibiotics and passive immunization with antiserum are the most important factors as management strategies in wildlife and poultry sectors.

**Keywords:** Captive breeding, epidemics, passive immunity, pheasant chicks, traditional antibiotics

## INTRODUCTION

Punjab wildlife and parks department is striving for the captive breeding, management and propagation of wildlife in the province. During 1978-81, the department prioritized captive breeding of peafowl and pheasants on commercial scale to make pheasants as street birds and rehabilitate the abandoned habitats. To meet the objectives, Wildlife Breeding Centre at Jallo Park was established for captive breeding and where necessary incubation and brooding facilities were installed. Eggs were property collected, stored, disinfected and set in incubators. Hatchery services were also being extended to Lahore Zoo, Lahore. Fifteen species of pheasants have been housed at the centre (Table 1).

| Sr. No. | Common Name | Zoological Name |
|---|---|---|
| 1 | Blue Peafowl | *Pavo cristatus* |
| 2 | Black Shoulder Peafowl | *Pavo cristatus negripennis* |
| 3 | Java Green Peafowl | *Pavo muticus* |
| 4 | Emerald Peafowl | *Pavo cristatus* |
| 5 | White Peafowl | *Pavo cristatus* |
| 6 | Pied Peafowl | *Pavo cristatus* |
| 7 | Ring Necked Pheasant | *Phasianus colchicus* |
| 8 | Green Pheasant | *Phasianus versicolor* |
| 9 | Silver Pheasant | *Lophura nycthemera* |
| 10 | Reeves Pheasant | *Syrmaticus reevesii* |
| 11 | Lady Amherests Pheasant | *Chrysolophus amherstiae* |
| 12 | Grey Peacock Pheasant | *Polyplectron bicalcaratum* |
| 13 | Palawan Pheasant | *Polyplectron emphanum* |
| 14 | Argus Pheasant | *Argusianus argus* |
| 15 | Siamese Fireback | *Lophura diardi* |

Table 1: Pheasant species housed at Wildlife Breeding Centre, Jallo Park, Lahore.

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Family Pheasinidae of Galliformes is selectively prone to many fatal bacterial and viral diseases. Bacterial strains such as mycoplasma and salmonella are the death causing agents among birds of this family. Broad-spectrum antibiotics act as selective force to induce mutations in bacterial strains resulting in resistance to traditional antibiotics. Such cumulative resistance is alarming and un-controllable due to non availability of effective antibiotics to cure out-break of epidemics due to mutant / resistant bacterial strains.

The symptoms of syndrome appeared in five to ten days old chicks which included severe irritation in the nasal and ocular region, pus filling up in the cornea leading to blindness and ultimate death. Broad spectrum antibiotics viz: Tylosin, Doxycycline, Colistin, Amoxixillin, Tribrissen, Gentamycin, Furazolidone, Fluemequin were given which were found in-effective to control the problem (unpublished data). In general, one cannot determine the primary etiology of the syndrome (unknown disease) from clinical symptoms and symptomatic treatment of the infection (Sonne *et al*., 2011).

The immune system plays a major role in protecting birds against invading pathogens (Sharma, 1999, 2003). The primary lymphoid organs of the bird include the bursa of Fabricius and the thymus (Sharma, 1999, 2003). Development of B lymphocytes in the bursa of Fabricius distinguishes the avian immune system from mammalian immune systems (Glick, 1967). There are higher percentage of immigrant B cells in the avian thymus compared to that of other animals (Sharma 1997). Once cells are functional, they leave the primary lymphoid organs and migrate to the secondary lymphoid organs (spleen, bursa of Fabricius, bone marrow, Harderian gland, conjunctiva associated lymphoid tissue (CALT), gut associated lymphoid tissue (GALT), and the bronchial associated lymphoid tissue (BALT), which are the principal sites of antigen induced immune responses (Sharma, 1999, 2003). The Harderian gland, which is a distinct structure located over the eyes, is full of plasma cells secreting IgM and IgA (Sharma, 1997).

The development of immune system in birds begins early during embryogenesis. The thymus develops from an epithelial outgrowth of the pharyngeal pouches and the bursa of Fabricius from an outgrowth of the cloacal epithelium starting at day five of incubation (Sharma, 2003). Bursal precursor cells can be detected in the embryo around day seven of embryonation (Sharma, 1999). IgM, IgG, and IgA can be detected on days 10, 14, and 16 of embryonation respectively (Sharma, 1999).

Lymphocytes with surface IgG develop on day 21 nearly at the time of hatching, whereas IgA cells first appear in the intestine by three to seven days after hatching mainly responsible for immunity (Tizard, 1996). B cells represent the humoral component of acquired immunity by producing antibodies against an antigen approximately five days post exposure (Tizard, 1996; Sharma, 2003). Although avian and mammalian IgG have similar biological functions, avian IgG is larger than its mammalian counterpart and lacks a genetically encoded hinge (Sharma, 1999, 2003). Because of the differences between avian and mammalian IgG, avian IgG is often referred to IgY (Leslie and Clem 1969; Magor *et al*., 1998, Sharma, 1999, 2003). Molecular data suggest that IgY may be the ancestral precursor of mammalian IgG and IgE (Parvari *et al*., 1988).

The present experiment was designed to immunize chicks through passive immunity. Prior of this, no work has been undertaken on passive immunity in pheasants in Pakistan. The results of this study will be helpful in controlling out-break of fatal epidemics. It will provide additional information in the existing captive management practices for Pheasinidae in particular and Galliformes in general.

## MATERIALS AND METHODS

Two chicks (one ring neck pheasant and one common peafowl) were examined at Grand Parent Poultry Pvt. Ltd. Laboratory in Faisal Town, Lahore. The pathogens were cultured but their strains could not be identified as Mycoplasma or Salmonella. It was a new un-identified bacterium or mutant strain. It was resistant to eight broad-spectrum antibiotics viz: Enrofloxacin, Amoxicillin, Doxcyclin, Furuzolidone, Tribrissen, Colistin, Gentamycine Fluemequin and was 100% fatal. To control this fatal out-break; passively immunizing plan of chicks was set to save precious wildlife species. The study was undertaken from 15$^{th}$ August to 30$^{th}$ September, 2007 and data collected was analyzed.

For antiserum preparation ring-necked pheasant (2 females) and one black shoulder peafowl were injected blood and pus taken form infected chicks. Pus was also applied locally in the ocular region for direct transmission of pathogens. These birds were watched for 10 days to develop antibodies against the syndrome. Venous blood was collected in the syringe and placed vertically for separation and settling down of blood protein. The yellow colored serum was collected as antiserum for further transfusion to chicks.

Chicks of peafowl and pheasant infected with syndrome were segregated and divided into two groups as control and treated. The treated group was further divided into two subgroups; one with antiserum injection through veins and the other

through subcutaneously. After three consecutive doses, the antiserum was re-in forced. A mixture of glucose, antiserum and Texiron at the ratio of 10:03:01 ml, respectively and 0.1 ml of mixture was injected into infected chicks in treated groups.

Antiserum injection was given in phases. During phase-1, peafowl chicks (n= 20) were selected, which were severely infected and have refused feeding. Antiserum was given for three consecutive days with an interval of three to five days. In the second phase, 17 ring necked pheasants with similar conditions as peafowl, were selected and treated with antiserum.

A total of 250 chicks were treated as injections were given intravenously and subcutaneously. The blood shortage was overcome by addition of new blood donors from healthy stocks. The injections were given in batches of 50 birds (Table 2).

| Number of Days | Number of Chicks |
|---|---|
| 1 | 50 |
| 2 | 50 + old 50 new addition |
| 3 | 100 + old 50 new addition |
| 4 | 50 new |
| 5 | 50 + old 50 new addition |

only a few mortalities were noted in immunized chicks. In the later stage, the antiserum was modified and reinforced by mixing with glucose and Texiron (Table 3)The overall survival of 73% among chicks was a significant achievement against syndrome and proved that when there is no other option of treatment; passive immunization is successful to control mortality in infected chicks of pheasants. Adding glucose in antiserum was helpful in providing instant energy (ATP) to restore activities among severely sick and lethargic chicks and antibiotics (Texiron) to control secondary bacterial infections. Protection through passive immunity is land mark in poultry industry. It may be exercised in precise, collaborated and well-organized manner.

Table 3: Survival rate among passively immunized chicks of ring necked pheasants and peafowl.

| Treatment | Antiserum | No. of chicks | Survival Rate | Control (without antiserum) |
|---|---|---|---|---|
| 1 | Antiserum | 37 | 100% | 10 (all died) |
| 2 | Antiserum | 50 | 60% | 10 (all died) |
| 3 | Re-enforced Antiserum | 250 | 60% | 30 (all died) |
| **Overall Survival: 73%** | | | | |

## CONCLUSIONS

The results showed that passive immunization is very effective tool to cope with emergency and mutant/resistant strains of bacteria that are resistant to routine antibiotic treatments. It can be extended to various fields of human and livestock sectors to control deadly diseases. Immediate steps are required to control out-break of new mutant/resistant strain of bacteria failing which will be a menace for wildlife, poultry and human.

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