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Study of Disease Outbreak in Layer Flocks in and Around Sammundri Area

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Abstract

A respiratory disease outbreak was reported in layer flocks in and around Sammundri area of Punjab. Postmortem of the affected birds were performed and morbid organs (trachea, lungs, air sacs, spleen, proventriculus and brain) were collected and processed for isolation and identification of etiological agent. The samples were inoculated in 9-days old embryonated hen eggs. The harvested amnio-allantoic fluid (AAF) agglutinated chicken RBCs and was subjected to inhibition of haemagglutination (HI) and Agar Gel Precipitation test (AGPT) with specific antisera of Newcastle Disease Virus (NDV), H_9 and H_7 serotype of Avian Influenza (AI) virus to identify the etiological agents. The haemagglitination (HA) activity of AAF collected from the samples of Sammundri and Gojra areas were inhibited with antisera of NDV while antiserum of AIV H_9 inhibited the HA activity of AAF of Kamalia area. AGPT with known antisera confirmed the presence of NDV in the outbreaks of Sammundri, Gojra where as $AIVH_9$ in the outbreaks of Kamalia area. Isolated NDV was pathotyped as velogenic on the basis of Mean Death Time (MDT), Intracerebral Pathogenicity Index (ICPI) and Intravenous Pathogenicity Index (IVPI).

The serum samples collected were analyzed for antibodies to NDV, H_7, H_9 serotype of AI and *Mycoplasma gallisepticum (MG)*. The sera of sammundri and Gojra areas were found positive to MG, NDV while that of Kamalia to MG and H_9 serotype of AI. Bacteriological culture indicated *E.coli* and *Salmonella* infection in the flocks. The clinical signs, postmortem lesions, serological analysis and bacteriological examination indicated that a number of infectious agents like NDV, H_9 serotype of AI along with, *E.coli* and *Salmonella*, were responsible for the recent respiratory outbreaks in layer flocks.

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Key words: Respiratory distress, AGPT, HI, $AIVH_9$, NDV, layer

Introduction

Poultry industry in Pakistan is being confronted with numerous disease problems including infectious diseases. Among these infectious diseases, Newcastle disease (ND), Infectious bronchitis (IB), Infectious bursal disease (IBD), Avian influenza (AI), and Marek's disease (MD) separately or in combination with each other are causing major set back to the poultry industry. In the recent years, respiratory diseases probably are the main hazards to the industry causing considerable economic losses (Simon, 1986).

Intensification and concentration of flocks in limited area, multiage groups rearing at the same farm and improper managemental practices are supporting factors in the spread of infectious diseases.

During November-December 2006, an outbreak of respiratory distress causing great economic losses in layer flocks in and around Sammundri area was recorded. The signs, symptoms and postmortem lesions were suggestive of a combination of different diseases. So, the area was surveyed with the objective to record signs and symptoms, lesions and to isolate and identify the causative agent on the basis of its cultural and serological characters. The data obtained and information gathered will help to adopt preventive measures against the anticipated respiratory outbreaks in future.

Materials and Methods

A total of 60 layer farms in and around Sammandri area were visited and detailed history of the flocks including age, breed, feed and egg production, medication, vaccination schedule and management conditions of the farms were noted. Samples were collected from dead and morbid birds showing signs of respiratory distress for laboratory investigations.

Collection of Samples

A total of 10 morbid layer birds showing typical signs of respiratory distress from each farm were necropsied and morbid organs like trachea, lungs, air sacs, head, spleen, kidneys and liver were collected in polythene bags. Blood samples were also collected and sera were separated. The samples were transported in cold chain system to disease diagnostic lab, Poultry Research Institute (PRI), Rawalpindi for further investigations.

Processing of Samples

The organs of different areas were weighed separately and triturated with equal volume of normal saline (0.9 % NaCl) The triturate was centrifuged at 1000 x g for 15 mins. The supernatant was separated and filtered through 0.22 μm syringe filter (Millipore ,Germany) and preserved at -20 °C till further use.

Inoculation of Embryonated hen Eggs

A total of six hundred 9 days old embryonated hen eggs were obtained from Breeding and Incubation section PRI, Rawalpindi. These eggs were divided into three groups, A, B, and C containing 200 eggs each. Each group of eggs were inoculated with 0.1 ml of filtrate of Summandari, Gojra, and Kamalia areas respectively along with negative inoculated control (sterile normal saline) through amnio-allantoic route and un-inoculated control. (Alexander 2000). All the eggs were incubated at 37 °C and candled daily for 5 days. On each day the dead embryos were removed and amnioallantoic fluid (AAF) was harvested.

Haemagglutination (HA) and Haemagglutination Inhibition (HI) assays

The harvested AAF was processed for HA activity against 1% washed chicken red blood cells (RBCs). The HI test was performed with known sera (wheybridge UK) against Newcastle disease virus, H_7 and H_9 serotypes of Avian Influenza virus. The serum samples collected from layer flocks were also subjected to HI test to estimate the antibody titers against NDV, H_7, and H_9 serotype of AI. The spot plate agglutination (SPA) test for *Mycoplasma gallisepticum* screening was also performed with SPA antigen (Intervet international).

Agar Gel Precipitation Test

Agar Gel Precipitation Test was performed against NDV, AIV H_7, and AIV H_9 antisera to detect the presence of multiple isolates in amnio-allantoic fluid. Appearance of precipitation lines between antisera and AAF were indications for positive samples.

Bacterial Culture

Direct organ culture was performed for the isolation of bacterial pathogens on Nutrient broth, Nutrient agar, MacConky agar, Thioglyconate agar and PPLO agar.

Results and Discussion

A respiratory disease complex spread in layer flocks in and around Sammundri area was characterized by respiratory distress, open mouth breathing, coughing, nasal discharge, torticollis, diarrhoea, swollen heads, and drop in egg production.

History of the farms visited revealed average mortality ranging from 10 to 45 percent in the infected flocks. Postmortem examination of mortile and morbid birds revealed hemorrhages on proventriculus, tracheal congestion, exudates in trachea, air sacculitis, caseated pus in the lungs, spleenomegaly and nephritis.

The signs, symptoms and postmortem lesions were simulating the findings of the outbreak reported previously (Hasan *et al*., 2002). These were indicative of various pathogens such as Newcastle disease, Infectious bronchitis, Avian influenza, and chronic respiratory disease (Alexander and Parson, 1980, Jordon 1990 and Shane 1995). All the effected flocks were treated with heavy doses of broad spectrum antibiotics such as Quinolones, Gentamycin, lincospectin etc but all the measures to control this malady were failed. The samples were, therefore, collected and processed in disease diagnostic laboratory PRI, Rawalpindi for isolation and identification of etiological agents.

The inoculations of the collected samples caused death of chicken embryo within 45 hours through amnioallantoic route. The harvested amnioallantoic fluid (AAF) exhibited haemagglutination activity with chicken RBCs. The HA activity was not influenced when AAF was passed through syringe filter (0.22μm). These results depicted the presence of haemagglutinating viral agent. The HA activity was not influenced within 2 hours at room temperature but it was vanished on overnight incubation, indicating that the causative agent was a virus having haemagglutinin and neuraminidase molecules on its surface. Similar type of molecules has been described by Webster and Cambell (1972) in Avian Influenza and ND viruses. These findings further indicated that the virus was a member of paramyxovirus or orthomyxovirus group which were characterized by using monospecific antisera (Alexander,1986, Beard, 1970). The standard positive serum against H_9 serotype inhibited the HA activity of Kamalia isolate which was indicative of H_9 serotype of Avian influenza virus in the respiratory disease problem while the standard positive sera against NDV inhibited the HA activity of Sammundri and Gojra isolates. However the HA activity of Sammundri, Gojra and Kamalia isolates was not inhibited by known antiserum of AIVH_7

(Table-1).These results indicated the presence of NDV and H_9 in the Sammundri, Gojra and Kamalia respectively.

Agar Gel Precipitation Test with standard known sera confirmed the presence of NDV in the respiratory outbreaks of Sammandri and Gojra and AIVH_9 in Kamalia area (Table-1).

Newcastle disease virus isolates from Sammundri and Gojra areas were pathotyped as velogenic, on the basis of Mean Death Time (MDT), Intracerebral Pathogenicity Index (ICPI) and Intravenous Pathogenicity Index (IVPI) (Beard and Hanson , 1984) (Table-3).

The sera collected from the morbid flocks indicated low, nonprotective anti-ND-HI antibody titres in the NDV vaccinated flocks of Sammundri and Gojra (Table-2) which may be due to immunosuppression (Giamborne and Clay;1986) .In immunosuppression the antigen is not properly processed by the antigen processing cells (APC), hence there is lack of transformation of T-lymphocytes into lymphoblasts leading to the failure of the stages essential of antibody production (Abbas *et al*.,1991).Protective anti-ND-antibody tires were observed in the sera of kamalia flocks (Table-2). Presence of non protective antibodies against H_9 in Kamalia areas was suggestive of field exposure to H_9 serotype of AIV. However anti -H_7- antidody titres were absent in the sera of all the areas indicating absence of AIV H_7 serotype (Table-2).

Sera of the flocks were also found negative against Mycoplasma *gallisepticum antigen* (Table-2).

Bacteriological cultural and biochemical examination of respiratory organs of clinically morbid birds yielded pathogenic *E.Coli* and *Salmonella* while no *mycoplasma* growth was observed even after 14 days post culturing.These pathogns could be the contributing factors of mortality in the flocks (Rajashekar *et al* ., 1998, Tablante *et al* .,1999)

Velogenic Newcastle disease virus and AIV H_9 isolates with secondary infection of *E.coli* and *salmonella* were responsible for respiratory outbreak in the layer flocks of Sammundri, Gojra and Kamalia areas of Pakistan. In view of the fact that Newcastle disease outbreaks are not uncommon even in the vaccinated flocks, therefore, it is recommended that inactivated vaccine may be prepared from velogenic strain of ND and used, as it is more immunognic in nature. It is also suggested that oil based H_9 serotype Avian influenza vaccine should be included in the vaccination schedule to prevent setback of poultry industry in future. For this purpose field veterinarians and the farmers should be educated frequently.

Table1:- Inhibition of HA activity and Agar Gel Precipitation Test of isolates with known antisera.

Isolate of areas	Inhibition of HA activity with known antisera of			AGPT with known antisera of		
	NDV	AIVH_7	AIVH_9	NDV	AIVH_7	AIVH_9
Sammundri	+	-	-	+	-	-
Gojra	+	-	-	+	-	-
Kamalia	-	-	+	-	-	+

Table2:- Antibody status of flocks to various known antigens.

Samples of areas	No. of samples	Anti-Mg-SPA	GMT of HI titres against(log2)		
			NDV	AIVH_7	AIVH_9
Sammundri	200	-	2.3	0.0	5.8
Gojra	200	-	2.5	0.0	6.2
Kamalia	200	-	8.12	0.0	3.2

Table3:- Pathotypes of Newcastle disease virus isolated from Sammundri and Gojra areas.

Isolate of areas	MDT	ICPI	IVPI	Results
sammundri	40	1.6-1.7	1.5	Velogenic
Gojra	45	1.2	2.0	Velogenic

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