CURRENT RESPIRATORY DISEASE PROBLEM AND THE PROBES IN CHICKEN

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ABSTRACT

Recently, high mortality was recorded in broiler flocks in various areas of Pakistan. The samples from six broiler flocks were studied. The blood samples collected were analyzed for antibodies to Newcastle Disease Virus (NDV), Avian Influenza Virus (AIV), Infectious Bronchitis Virus (IBV), Infectious Bursal Disease Virus (IBDV), *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS) and Salmonella organisms (SPG). It was found that the samples had no antibodies against NDV, AIV, MG, MS and SPG but variable levels of antibodies were recorded against IBV and IBDV. Bacteriological examination of the respiratory organs of clinically sick birds yielded Haemophilus and pathogenic *E. coli*. The absence of any pathogen activity in filtrates of 0.2 and 0.1 *u*m inoculated through CAM and CAS routes in embryonated eggs ruled out the possibility of the involvement of AIV and NDV. Unfiltered homogenate and 0.45 *u*m filtrate activity indicated the presence of Mycoplasma in the homogenate. It is concluded that: 1. The problem primarily resulted from the interplay of Mycoplasma, IBDV, IBHV and IBV, 2. Quality of the chicks ir carrying vertical Mycoplasma infection played basic role in the development of the problem, 3. The associated bacterial pathogens i.e., Infectious Coryza and Colibacillosis played precipitating role in the problem, 4. Extreme environmental temperature played a conducive role in the episode and 5. Predisposing role of mycotoxins in the malady cannot be overlooked.

Key Words: Broilers, respiratory disease, outbreak.

INTRODUCTION

Amongst different prevalent poultry diseases, respiratory diseases with variable severities are the most common under intensive rearing system. This indicates a variety of stresses constantly posed to the poultry. Moreover poor managemental procedures and unethical vaccination practices aggravate the stresses to add up the losses many folds in the shape of morbidity, poor growth, mortality, decreased production and increased cost on vaccination and treatment.

Recently an obnoxious respiratory disease complex flared up in almost all the poultry rearing areas of the country that resulted into different school of thoughts as far as etiology and control measures are concerned. So this study was conducted to investigate the problem for suggesting proper preventive and therapeutic measures.

MATERIALS AND METHODS

Preliminary study and sample collection

A total of six broiler flocks having the birds of different breeding strains with different age groups, located separately around Rawalpindi/Islamabad were visited for recording the clinical history and collection of samples for further study. The study included both clinically affected and unaffected flocks. For the convenience of description, the flocks were designated as I, II, III, IV, V and VI. The affected flocks, I to III having the age of 3 - 5 weeks were in the acute phase of the disease, while the unaffected flocks, IV to VI were at the age of 6 weeks. A total of 120 blood samples (20 from each flock) were collected. The serum from each blood sample was separated in sterile sample tubes, heat inactivated at 56°C for 30 minutes and stored at -20°C for further investigations.

Bacterial culture

Trachea, lungs, liver, spleen and bursa of Fabricius were collected from the freshly dead chicks with typical symptoms and lesions of the respiratory disease problem from different flocks. Direct organ culture was performed for the isolation of bacterial pathogens on Nutrient broth, Selenite broth, Nutrient agar, MacConkey agar, Brilliant Green agar, Triple sugar iron agar media and Chocolate agar. Biochemical and sugar test were also preformed on the bacterial isolates to identify the organisms.

Clinical pathology

The slides were prepared from the intestinal scrapings and examined microscopically for clinical finding of coccidial oocysts and accumulation of urate/oxalate in intestines.

Serology

Sera were analysed through different tests for antibodies to Newcastle Disease Virus (NDV), Avian Influenza Virus (AIV), Infectious Bronchitis Virus (IBV), Infectious Bursal Disease Virus (IBDV), *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS) and Salmonella organisms (SPG). Haemagglutination Inhibition (HI) test was performed for the detection of antibody titres against NDV and AIV, Agar Gel Precipitation (AGP) test for IBDV and IBV, and Serum Plate Agglutination (SPA) for MG, MS and SPG.

Chicken inoculation

The morbid tissues were ground to prepare a 10% homogenate and centrifuged at 3000 rpm for 10 minutes. The supernatant was used for disease transmission in susceptible birds. Intratracheal and subcutaneous routes were tried to reproduce the disease with the tissue filtrate alone and in combination with IBD and avian adeno viruses field and vaccinal strains in four weeks old broiler chicks.

Chicken embryo inoculation

The infected tissue suspension was prepared and filtered through 0.45um, 0.20um and 0.10um syringe filters. The filtrates were used further for chick embryo inoculation through yolk sac (YS), chorio-allantoic sac (CAS) and chorio-allantoic-membrane (CAM) routes. Six days old chick embryos were inoculated through Yolk-Sac (YS) route and nine days old chick embryos were inoculated through CAM and CAS routes.

Histopathology

Histopathological procedures were adopted to examine different tissues collected from affected birds including trachea, lungs, liver and spleen. The liver tissue of the chicken embryos inoculated through YS route was also examined microscopically.

Therapy

The affected flocks were treated keeping in view the clinical findings of the disease. In most of the cases, chicks were showing clinically mixed infection involving viral, bacterial and protozoal pathogens alongwith the affects of mycotoxin in the feed. In selected cases a formalinized auto-vaccine was prepared by using 10 per cent filtrate of affected tissues homogenate including liver, lungs, spleen and bursa of Fabricius (Ahmad *et al.*, 1990).

RESULTS AND DISCUSSION

Early respiratory disease complex is an acute disease characterized by depression, respiratory distress and increased mortality in 2 to 3 weeks old broiler chickens. Postmortem lesions include airsacculitis, fibrinous pericarditis and perihepatitis. Colisepticaemia is primary cause of the death (Tablante *et al.*, 1999). In current respiratory disease problem out-break could be seen anytime after first week of age. Swollen heads, bloody nasal discharge, sneezing, coughing, uneven growth, ruffled feathers, varying rate of morbidity and mortality were amongst the prominent findings of the problem. Bloody nasal exudation was the unique finding in the study. Air saculitis, caseated pus in the lungs and air sacs, pericarditis, perihepatitis and nephritis were noted. Some degree of bursal swelling along with hemorrhages on thigh and chest were also noted.

Bacterial culture

Bacteriological examination of the respiratory organs of clinically sick birds yielded Haemophilus and pathogenic *E. coli* which could be primary cause of the death (Rajashekar *et al.*, 1998; Tablante *et al.*, 1999).

Clinical pathology

While examining the slides of intestinal scrapings under the microscope, urate and oxalate crystals were frequently found, indicating urinary disfunction. Coccidial oocysts of different stages were another frequent finding.

Serology

The flock-wise findings indicated that birds of each flock had uniform level of antibodies against all the pathogens (Table 1). Antibody titres against NDV and AIV were near to zero. It is worth mentioning that amongst all the vaccines, ND vaccine was the most frequently used vaccine in all types of birds. The use of ND vaccine was to such an extent that did not carry any technical justification. Giamborne and Clay (1986) reported that broilers vaccinated against NDV with low mean HI titre were not protected against the virulent NDV challenge. Newcastle disease continues to be a serious economic threat to the poultry industry resulting in increased flock mortality and losses of production in breeding, laying and broiler chickens. Plate agglutination reaction was negative against MG, MS and SPG. The absence of antibodies to endemic pathogens is indicative of very high degree of immunosuppression in the birds due to many reasons including increased prevalence of IBD, MG and mycotoxin etc. The role of mycotoxins in the malady cannot be overlooked and needs further investigation.

Table 1: Antibodies to various infectious agents in the sera of broiler chicks affected/unaffected with respiratory disease problem.

Flock	No. of samples	Plate agglutination			GMT of HIT		AGPT	
		MG	MS	SPG	NDV	AIV	IBDV	IBV
I*	20	-	-	-	7	0	+	+
II*	20	-	-	-	0	0	+	+
III*	20	-	-	-	0	0	+	+
IV**	20	-	-	-	0	0	+	+
V**	20	-	-	-	0	0	+	+
VI**	20	-	-	-	0	0	+	+

* Affected flock; ** Unaffected flock

Table 2: Prevalence of antibodies against IBV Variants.

Sample	Nos. of Sample	IBV Variants positive samples				
		H120	D274	IB4-91	D1466	M41
A*	8	2	5	7	6	8
B*	8	1	5	4	3	8
C**	8	5	4	5	6	8

* Affected flock; ** Unaffected flock.

Table 3: Isolation of respiratory disease pathogen through filtration/embryo inoculation.

Filtrate	Route of Inoculation					
	YS		CAM		CAS	
	Died*	HA**	Died*	HA**	Died*	HA**
0.45 um	+	+	-	-	+	+
0.20 um	+	-	-	-	-	-
0.10 um	-	-	-	-	-	-
Unfiltered	+	+	-	-	+	+
-ive control	-	-	-	-	-	-

* Embryo died within 24 - 48 hours postinoculation; ** Heamagglutination (HA) reaction.

Table 4: Vaccination done with auto-vaccine for respiratory disease problem at different farms showed positive response

Sr .No.	Date	Batch	Farm	Location	No. of birds
1	22 MAY 99	1398	SARWAR SHAH	TAXILA	2300
2	22	1399	IQBAL	B.KHUO	1500
3	29	1402	ABRAR KHAN	AJK	700
4	29	1402	MANSUBDAD	ATTOCK	2000
5	31	1403	SHARAZ	WHA CANT	4500
6	2 JUNE 99	1404	AKSAR	G.KHAN	3000
7	2	1405	AKSAR	G.KHAN	1600
8	3	1407	YOUSAF	RAKH MOR	1500
9	3	1408	NIAZ	COHASHARIF	1000
10	4	1410	NOSHAD	MARDAN	2400
11	11	1413	ASIF	K.SYIADA	2000
12	14	1415	SALEEM	SAGRI	2500
13	14	1416	S.RABIULLAH	HARIPUR	5000
14	14	1417	SAJJID	MURDAN	2500
15	28	1423	ABDULHALEEM	WHA CANT	2500
16	30	1424	HANIF	P. GHAP	1200
			TOTAL		36200

All the tested sera showed the presence of antibodies against the IBDV (Table 1). Age of chickens at exposure influences clinical response to virus. Chickens exposed between 3 and 5 weeks of age develop an acute disease. The birds younger than 3 weeks of age, do not show acute disease but reduced immunocompetence (Sharma, 1985). IBD virus has many serotypes (Jackwood *et al.*, 1985). It is also possible that the serotype used as vaccine in broilers did not provide adequate protection against the heterologous challenge.

Weak precipitating lines after 24 hours on Noble agar against all vaccinal antigens of IBV were observed in different samples (Table 2). The presence of antibodies to various stains of IBV in the tested sera are attributed to exposure of birds to IB virus under field conditions (Muneer *et al.*, 1992).

Chicken embryo inoculation

Tissue filtrate passing through 0.45um filter caused embryonic death within 48-72 hours postinoculation through yolk-sac (YS) and chorio-allantoic sac (CAS) routes and gave HA positive reaction with chicken and rat RBCs. The filtrate passed through 0.2um caused death of one embryo inoculated through YS route. CAS fluid was negative for HA activity but embryo hepatocytes were having intranuclear inclusion bodies. The filtrate passed through 0.1um filter did not cause the death of chicken embryo through any route (Table 3). The absence of any pathogenic activity in filtrate of 0.2 and 0.1 *um* inoculated through CAM and CAS routes rule out the possibility of the involvement of AIV and NDV. Unfiltered homogenate and 0.45 *um* filtrate activity indicate the presence of Mycoplasma in the homogenate. Vertically transmitted mycoplasma infection may have primary role in early respiratory disease complex (Bradbury, 1998).

Chicken inoculation

The disease was reproduced only in one group after two weeks of inoculation through subcutaneous route with affected tissue filtrate additionally combined with IBD hot strain vaccinal virus. The affected birds showed clinical picture of swollen heads, sneezing, nasal discharge, ruffled feathers and depression.

Histopathology

Intranuclear and intracytoplasmic inclusion bodies were observed in hepatocytes of the affected birds and chick embryos inoculated with tissue filtrate. Lymphocytic infiltration in trachea, spleen and liver was frequent. Epithelial layer was found intact in trachea and no inclusion body was found in tracheal rings.

Therapy response

All the cases responded to the antibiotic/ antibacterial therapy along with symptomatic applications. Formalinized auto-vaccine prepared in selected cases showed encouraging results (Table 4).

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