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Sero-prevalence of Avian Influenza in Broiler Flocks in District Gujranwala (Pakistan)

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ABSTRACT

In the current project the Sero-prevalence of Avian Influenza (AI) was monitored in broiler flocks in the area of Gujranwala, Pakistan. For this purpose serum samples and cloacal swabs were collected from the 100 suspected and healthy farms in and around Gujranwala. Serum samples were subjected to Hemagglutination Inhibition (HI) Test and Agar Gel Precipitation Test (AGPT) for sero-prevelance of AI. Tissue samples and cloacal swabs were sent to the NARC (National Agricultural Research Centre) for isolation of AI. Only three farms located at Wazirabad road, Pasroor road and Lahore road were positive for AI with sero-prevalence of 20, 50 and 30%, respectively. In the population study the upper limit of the broiler population was 15,000 and lower limit was 1500 birds. So the mean was 3870 birds. There was no affect of feed, vaccination schedule and breed on the prevalence of AI. The most affected age of broiler population was between 26 and 38 days. AI vaccine was not carried out at the broiler farms. But almost all broiler breeder farmers vaccinated their birds against both H_7 and H_9 subtypes of AI, which showed satisfactory results. © 2011 Friends Science Publishers

Key Words: Sero-prevalence; Avian influenza; Broiler; Gujranwala

INTRODUCTION

AI is a contagious viral disease cosmopolitan in occurrence that affects the chickens of all ages with variable mortality ranging from 30% to 100%, depending on the pathogenicity of the AI virus. AI has emerged as a disease with significant potential to disrupt commercial poultry production resulting in extensive losses. All strain of the chicken flocks including broiler, layers and layer breeders are affected with the AI virus (Shane, 2002). The significance of AI is enhanced due to its zoonotic importance and many studies indicate that influenza viruses have emerged as much more economically serious pathogens of the poultry industry, than they had been in the previous years (Chen *et al.*, 2004).

Avian as well as mammalian influenza viruses belong to the family orthomyxoviridae that consists of genera that are influenza type 'A', 'B' and 'C'. All avian, equine, porcine and most of the human influenza infections are caused by type 'A' influenza viruses (Banbura *et al.*, 2000; Alkhalaf, 2010). Influenza type 'A' viruses are roughly spherical or filamentous particles, 80-120 nm in diameter. The nucleo-capsid has a helical symmetry and is enclosed within a protein matrix, the surface of which is covered by two types of glycoprotein projections or spikes with which Haemagglutinin and Neuraminidase activities are associated.

AI is common in poultry in many countries of the world. In Pakistan outbreak of AI was first time recorded in October 1994. The disease affected broiler breeders in Mansehra, Abbotabad, Rawalpindi and adjoining areas killing approximately one million birds. The causative agent was confirmed as AI virus H_7N_3 type (Naeem & Hussain, 1995). In 1996, outbreak of AI in boilers breeders and in commercial layers was suspected in various areas of the Punjab. This outbreak did not cause considerable loss but was responsible for low production and immune-suppression. In research laboratories, the causative agent was isolated and characterized as AI virus H_9N_2 type. Keeping in view the virulence of this virus for poultry, this was also included in locally prepared vaccines. The AI vaccines containing locally isolated H_7N_9 types have been extensively used since 1996 (Muhammad *et al.*, 1997). However, due to poor biosecurity and intensive farming, the problem of AI started reappearing in Karachi in 1999 mainly in broilers. This time, the disease was controlled by vaccination and strict biosecurity measures. However, later on due to poor biosecurity and non-usage of vaccine the AI was endemic in Karachi by the end of 1999 (Naeem *et al.*, 1999).

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In 2001, outbreak of a respiratory syndrome in broilers and layers, in Karachi and Abbotabad was recorded, the morbidity was up to 100% and mortality was up to 50%. In effected birds, the trachea was hyperemic and lungs were congested, while in the complicated cases other lesions like peritonitis, enteritis and hepatitis, were also found. The HA agent was consistently recovered in allanto-amniotic fluid when samples prepared from morbid trachea and lungs, were inoculated in 9-days-old embryos. The HA agent was confirmed as AI (H_9 type) by using HI test with AIV-H_9 specific antisera (Muhammad *et al.*, 2001).

In October 2003, the AI hit the commercial layers in Karachi and caused heavy mortalities. The disease was mainly recorded in non-vaccinated birds. The Pakistan Poultry Association in January 2004 organized a meeting of the National Disease Control Committee in Karachi and discussed various approaches to investigate and control the problem. The samples obtained from the dead and the morbid birds were collected and processed in various diagnostic laboratories. The causative agent was isolated and characterized as AI subtype H_7. AI viruses isolated so far from poultry in Pakistan has been subtyped as H_7N_3 and H_9N_2. In February 2005, a respiratory problem of unknown etiology was observed in broiler and broiler breeder flocks in some areas of Gujranwala, Pakistan and its surrounding districts. In some of these outbreaks clinical signs and postmortem lesions resembled of that of AI. Considering no epidemiological studies in these areas the present project was initiated to investigate sero-prevalence of H_7 and H_9 in these birds. It is hoped that the information generated would be useful for the development of control strategies of this newly emerging problem throughout the country.

MATERIALS AND METHODS

Avian Influenza (AI) is causing substantial losses in domestic poultry throughout the world, for last few decades. Outbreaks of AI have been also reported from Pakistan and cause heavy economical losses to local poultry industry.

Collection of epidemiological data: A total of 100 poultry farms (both suspected & healthy) located in various poultry raising areas of the Gujranwala including, Hafizabad road, Alipur road, Pasroor road, Sheikhupura road, Lahore road, Sambarial road, Gujrat road and Batala Sharam Singh road were visited. Epidemiological data from each flock was collected on a predesigned Performa. The signs, symptoms, vaccination program, history of the disease and medication against microbes at each farm were recorded. External appearances of the carcass and postmortem findings were also recorded.

Collection of serum samples: Blood samples from 10 birds of each poultry farms were collected in sterile syringes, then allowed to clot at 25°C for four hours and the sera were separated in clean sterilized bottles. The serum samples were placed in ice in a thermos flask before shipment to the laboratory of the Department of Veterinary Microbiology, University of Agriculture, Faisalabad (UAF). The serum samples were tested immediately in the laboratory for serology and the remaining serum samples were stored at –20°C till further processing.

Collection of cloacal swabs: Cloacal swabs were collected from the typical diseased birds showing signs and symptoms of the disease. At least 10 cloacal swabs were collected from 10 different birds of each farm. The swabs were immediately dipped in the transport medium separately and swabs were placed on ice in the thermos flask before shipment to NARC for isolation and identification of the causative agent.

Collection of tissue samples: Morbid samples such as trachea were collected in sterilized bottles. Samples were placed on ice in the field later held in a refrigerator before shipment to the NARC for isolation and identification of the causative agent.

Raising of Hyper Immune Serum

New castle disease virus: The commercially available oil based vaccine (Imposet; Rhone Merieux) was inoculated in 20 birds at the rate of 0.3 mL/bird subcutaneously at days 1, 7 and 21. These birds were kept in the Dept. of Vet. Mic., UAF. 5mL of blood was collected from each bird 28-day post injection, in the sterile disposable syringes. Syringes were kept undisturbed in the slanting position for one hour then transferred to the refrigerator for overnight. Next day each serum sample was collected in plastic bottle separately and stored at –20°C in deep freezer for further use.

Avian influenza virus serotypes: Inactivated AI virus subtypes of H_7, and H_9 were used to raise the hyper immune serum. The above-mentioned serotypes in vaccine were given to 20 birds at the rate of 0.3 mL/bird subcutaneously at days 1, 7, and 21. These birds were kept in the Dept of Vet. Mic., UAF. 5 mL of blood was collected from each bird 28 days post-boosting. The blood was collected in sterile disposable syringes. The syringes were placed undisturbed in the slanting position for one hour, then were transferred to the refrigerator for overnight. Next day each serum sample was collected in plastic bottle separately and stored at –20°C in deep freezer for further use.

Agar gel precipitation test: AGPT was used for the diagnosis of the causative agent as described by Beard (1978).

Haemagglutination (HA) test: AI viruses H_7 and H_9 were checked for their haemagglutinating activity and 4HAU was calculated by using the following procedure (Allan Gough, 1974). The HA titer was the reciprocal of the highest dilution showing haemagglutination. The end point titer and 4HA units of AI viruses H_7 and H_9 type were calculated.

Haemagglutination inhibition (HI) test: HI test was conducted for each serum sample according to the technique described by Allan Gough, (1974), by using 4HA units of AI Virus, H_7, and H_9 serotypes of AI virus. The raised hyperimmune sera against ND was subjected to HI test by using 4HAU of H_7 and H_9 subtypes of AIV obtained from NARC and the result showed that there was no cross

reaction, so it was confirmed that the virus given by NARC did not show false positive results (ND positive as AI positive).

RESULTS

In this study, 100-broiler chicken farms were visited. From each farm, blood sample and cloacal swabs were collected from the birds, which were randomly selected. Tissue samples were collected from recently dead birds and also from sick birds. Tissue samples, lung and trachea were transported to the Department of Microbiology, University of Agriculture, Faisalabad, Pakistan and processed in the laboratory. Tissue samples and cloacal swabs were sent to the NARC for isolation and confirmation of AI virus. The serum was separated from blood and stored at -20°C. These sera samples were tested by AGPT. The positive samples showing precipitation band in the agar gel, were tested by HI test. From 100 farms, 1000 serum samples were tested; out of 100 farms, 2 farms were sero-positive (Table I).

Description of Studied Areas

Wazirabad road: Thirty farms located at Wazirabad road were visited and serum samples, cloacal swabs and tissue samples were collected. Serum was separated from blood samples, and then tested. Totally 300 serum samples were tested. All these farms were negative, except one farm in Dhub Cheema. From that farm 10-samples were tested, out of ten, 3 were sero-positive. (Table II).

Hafizabad road: Ten farms were also visited at Hafizabad road, showing signs and symptoms confusing with AI. These farms were located in Ladhaywala Warraich, Chalal Kalah, Pupnakha, Maan, Herdhu Pur, Qazi Kot, Ugo Chak, Noor Pur, Nokhar and Kiladidar Singh. Serum samples, cloacal swabs and tissue samples from those farms were collected. Totally 100 serum samples were tested. All samples were negative (Table II).

Ali pur road: Many broiler farms are located at Ali Pur road, which showed respiratory problems. From these farms 10 farms were visited. These farms were located in Gondala Wala, Kot Baray Khan, Saaday Chak, Baig Chak, Kalaskay, Bohraywale, Kot Jahangir and Ali Pur. Serum samples from those farms were tested, all samples were negative (Table II).

Pasroor road: Around the Pasroor road some farms were showing mixed type of respiratory problem. 10 farms in Jandayala Baghwala, Tulvandi Musa Khan, Mukhal Sindwan, Pero Chak, Sulakhanabad, Dubirge, Dherowale and Nazaam Chak were checked. One farm located in Tulvandi Musa Khan was sero-positive for AI, all other farms were negative. At the positive farm out of ten, 5 samples were sero-positive (Table II).

Sheikhupura road: Some farms at Sheikhupura road were reported to have a respiratory outbreak, with signs and symptoms similar of AI. These farms located in the Tatlaywale, Herchoke, Ghumanwala, Magouh Chak, Jahan Shah, Chachoke and Mustafaabad were tested. From these ten farms 100 serum samples were tested and all were negative (Table III).

Table I: Farm based prevalence of AI in Gujranwala

Area	No. of Farms Examined	No. of Sample Tested	No. of Farms Positive	Percentage of farms positive (%)
Wazirabad Raod	30	300	1	3.33
Hafizabad Road	10	100	--	--
Ali Poor Road	10	100	--	--
Pasroor Road	10	100	1	10
Sheikhupura Road	10	100	--	--
Lahore Road	10	90	1	10
Sambarial Road	5	50	--	--
Gujrat Road	5	50	--	--
Batala Sharam Singh Road	10	100	--	--
Total	100	1000	3	3

Lahore road: Some farmers reported respiratory outbreak at their farms, not recovering with antibiotics. These farms were located in Aaman Abad, Aadhoray, Chadyala, Khila Naveed Singh, Bhatti Kay and Attava. 10 farms were visited. One farm located in Chadyala was seropositive for AI. Three samples were positive, out of 10, at that farm (Table III).

Sambarial road: Around the Sambarial road many poultry farms are located some of them were showing respiratory disease. These farms were visited, located in Sohdra, Chak Saatyah, Kot Shah Muhammad, Lavarewala and Aziz Chak. Five farms were visited all the farms were negative for AI (Table III).

Gujrat road: Around Gujrat road many dense poultry raring areas are present. Some of these were showing clinical signs like AI. Five were located in Bhatti Kay, Khasray and Phalokee were checked. From these five farms no sample was positive for AI (Table III).

Batala sharam singh road: Some farms at Batala Sharam Singh road were reported to have a respiratory outbreak, with signs and symptoms similar of AI. Farms located in Batala, Kila Khazana, Machikay, Akbaryah and Sansarah Ghorayah were studied. From these 10 farms no sample was positive for AI (Table III).

Percentage Sero-prevalence of Avian Influenza

Wazirabad road: At Wazirabad road, out of 30 farms, one farm was sero-positive. From that farm 10 serum samples were tested, out of ten, two serum samples were sero-positive, which showed that sero-prevalence at affected farm along Wazirabad road was 20% (Table IV).

Pasroor road: At Pasroor road, out of 10, one farm was sero-positive. From that farm 10 serum samples were tested, out of ten, five serum samples were sero-positive, which showed sero-prevalence 50% (Table IV).

Lahore road: At Lahore road, out of 10, one farm was sero-positive. From that farm 10 serum samples were tested, out of ten, three serum samples were sero-positive, which showed, 30% sero-prevalence (Table IV). From these three affected farms, 30 serum samples were tested, first by

Table II: Prevalence of AI in farms in different localities

Locality	Area	No. of Farms Tested	No. of Samples Tested	No of Farms Affected	Percentage of farms positive (%)
Wazirabad Road	Dhub Cheema	7	70	1	14
	Roop Chand	3	30	--	--
	Adil Ghar	4	40	--	--
	Dhilum	2	20	--	--
	Kolar	4	40	--	--
	Kot Waris	2	20	--	--
	Banka Cheema	5	50	--	--
	Kot Noora	3	30	--	--
Hafizabad Road	Ladhaywala Warraich	1	10	--	--
	Chahal Kalah	1	10	--	--
	Pupnakha	1	10	--	--
	Maan	1	10	--	--
	Herdhu Pur	1	10	--	--
	Qazi Kot	1	10	--	--
	Ugo Ckak	1	10	--	--
	Noor Pur	1	10	--	--
	Nokhar	1	10	--	--
	Kaladidar Singh	1	10	--	--
Ali pur road	Gondala wala	1	10	--	--
	Kot Bahray Khan	1	10	--	--
	Saaday Chak	1	10	--	--
	Baig Chak	1	10	--	--
	Kalaskay	1	10	--	--
	Bohraywale	2	20	--	--
	Kot Jahangir	2	20	--	--
	Ali Pur	1	10	--	--
Pasroor Road	Jandyala Baghwala	1	10	--	--
	Tulvendi Musa Khan	1	10	1	100
	Mukhal Sindwan	3	30	--	--
	Pero Chak	1	10	--	--
	Sulakhanabad	1	10	--	--
	Duburge	1	10	--	--
	Dherowale	1	10	--	--
	Nazaam Chak	1	10	--	--

Table III: Prevalence of AI in farms in different localities

Locality	Area	No. of Farms Tested	No. of Samples Tested	No of Farms Affected	Percentage of farms positive (%)
Sheikhupura Road	Tatlaywale	1	10	--	--
	Herchoke	1	10	--	--
	Ghumman Wala	4	40	--	--
	Majoh Chack	1	10	--	--
	Jahan Chak	1	10	--	--
	Chachoke	1	10	--	--
	Mustafaabad	1	10	--	--
Lahore Road	Aaman Abad	1	10	--	--
	Aadhoray	1	10	--	--
	Chadyala	5	50	1	20
	Khila Naveed Singh	1	10	--	--
	Bhatti Kay	1	10	--	--
	Attava	1	10	--	--
Sambarial Road	Sohdra	1	10	--	--
	Chak Saatyah	1	10	--	--
	Kot Shah Muhammad	1	10	--	--
	Lavarewala	1	10	--	--
	Aziz Chak	1	10	--	--
Gujrat Road	Bhatti Kay	1	10	--	--
	Khasray	2	20	--	--
	Phalokee	2	20	--	--
Batala Sharam	Batala	2	20	--	--
Singh Road	Kila Khazana	2	20	--	--
	Machikay	2	20	--	--
	Akbaryah	2	20	--	--
	Sansara Ghorayha	2	20	--	--

AGPT then by HI test. Out of 30, 10 samples were sero-positive, which showed the cumulative sero-prevalence at affected farms was 33.3% (Table IV).

Parameters of the Farms Affected

Wazirabad road: Farm that was sero-positive for AI along Wazirabad road was located in Dhub Cheema. The population of the farm was 2000 birds and the age of the birds was 30 days. The mortality at the farm at that day was 2.5%. Vaccination they carried out at the farm was; ND vaccine at 3rd day, IBD vaccine at 5th day, boaster of ND at 14th day, vaccination against HPS was performed at 19th day. The birds were showing respiratory signs, diarrhea was also present (Table V).

Pasroor road: Affected farm at Pasroor road was located in Tulvandi Musa Khan. The population of the farm was 8000 birds and the age of the birds was 33-days. The mortality at the farm was 3%. Vaccination schedule they followed was; ND vaccine at 4th day, IBD vaccine at 5th day, boaster of ND at 13th day, vaccination against HPS at 18th day. These birds were also showing respiratory signs like coughing, sneezing and cyanosis of comb, etc. (Table V).

Lahore road: Seropositive farm for AI was located in Chadyala. The population of the farm was 3700 birds, and the age of the birds was 31-days. The mortality was 4%. Vaccinations they carried out at the farm were; ND vaccine at 4th day, IBD vaccine at 5th day, ND booster at 13th day and vaccination against HPS at 18th day (Table V).

The relationship of following parameters with the prevalence of AI was recorded, which are described below:

1. Highest limit of broiler population studied: 15,000 birds.
2. Lowest limit of broiler population studied: 1500 birds.
3. Mean population at individual farm: 3870 birds.
4. Effect of feed on sero-prevalence: No.
5. Effect of age: Mostly 26-38 days.
6. Effect of vaccine: No.

AI vaccine was not carried out at the broiler farms. But almost all the broiler breeder farmers vaccinate their birds against both H_7 and H_9 strains of AI, which showed satisfactory results.

DISCUSSION

AI is causing substantial loses in domestic poultry throughout the world, for last few decades. Sometimes it causes an asymptomatic infection and sometime an acute, fatal disease of chickens, turkey and many other avian species. Fowl plague, which is categorized in HPAI, is one of the most nefarious members of the AI viruses with H_7 antigen and any of several N antigens. Recently H_5 is also included in HPAI (Lu *et al.*, 2004).

Table IV: Percentage sero-prevalence at affected farms

Area	No. of Farms Positive	No. of Sample Tested	No of Samples Positive	Sero-Prevalence (%)
Wazirabad Road	1	10	2	20
Pasroor Road	1	10	5	50
Lahore Road	1	10	3	30
Total	3	30	10	33.3

Table V: Different parameters of affected farms

Parameters	Wazirabad Road	Pasroor Road	Lahore Road
Age	30-days	33-days	31-days
No. of birds	2000	8000	3700
Mortality (%)	2.5	3	4
Vaccination schedule followed			
ND	3rd day	4th day	4th day
IBD	5th day	5th day	5th day
ND	14th day	13th day	13th day
HPS	19th day	18th day	18th day

Serological diagnosis is important when clinical specimens are not available or when the laboratory does not have the resources of virus isolation (WHO, 2002; Hadipour *et al.*, 2011). There are different sero-diagnostic tests, which can be used to monitor the sero-prevalence of AI, which include PCR, ELISA, AGPT, HI, etc. In the current study, the AGPT and HI were used to determine the sero-prevalence of AI, as recommended by FAO. In our project antigens used were of sero-types H_9 and H_7. These sero-types were isolated from Pakistan, H_9 was isolated in 1996 from Punjab and H_7 was isolated in 2003 from Karachi. Both of these sero-types were used in AGPT and HI tests.

In our study 100 farms were visited located in the dense poultry rearing areas of district Gujranwala. These farms showed respiratory problem confusing with AI. Only three farms present in different localities were sero-positive for AI. The signs and symptoms were similar to that of AI which is described above. These farms were located along Wazirabad road, Pasroor road and Lahore road. Percentage sero-prevalence at these farms was 20, 50 and 30%, respectively. Naeem *et al.* (1999) reported influenza outbreak in northern areas of Pakistan in 1999 with 10-20% mortality and with decrease egg production from 10 to 75%. The AI virus was of H_9N_2 subtype and since then the disease has been repeatedly reported from various poultry rearing areas in the country. The disease caused by subtype H_7N_3 caused high mortality among the affected flocks. Wegdan *et al.* (2007) found high rate of 86.4% of antibodies in chicken serum against AI virus type 'A' in Sudan. Similarly serological prevalence of 31.6% of AI was recorded in Nigeria with no history of vaccination against this virus (Wakawa, 2009). Serological examination on the basis of HI test showed that out of 14 sampled layer farms, 3 farms had antibodies against H_7 subtype, 2 against H_9, 3 against both H_7 and H_9, while remaining seven farms were negative for antibodies to AI in heavily populated areas in Toba Tek Singh in Pakistan (Numan & Siddique, 2005).

In the current project we found that incidence of AI was low in the chicks whose parent flocks had suffered from AI in their later ages. Other studies found there was ample evidence of horizontal transmission of AI viruses but little evidence indicated that the viruses can be transmitted vertically. Our findings are in alignment with Beard *et al.* (1984) who failed to isolate the AI viruses from hen eggs that were experimentally infected with H_5N_2 virus. In our studies we found that birds were dying without any predisposing illness but the mortality percentage was far less than those observed in the outbreak. Edema of the face and comb region, vesicles and necrosis of the comb were seen. The clinical signs of AI are highly variable. In HPAI, some time birds dye without showing any clinical sign (Ficken, 1989). Nervous signs were observed in experimentally and naturally infected birds (Kobaayashi *et al.,* 1996). Edema of the face and comb region is also observed (Acland *et al.,* 1984). These differences may be attributed to the difference in virulence of the infecting strains. Per acute signs observed by Ficken *et al.* (1989) were similar to those, which were observed in the current project under field conditions. Moreover, in the affected flocks, pattern of the disease, signs and symptoms and postmortem observations were indicative of a disease complex of various pathogens such as ND, IB, Coryza and AI. AI (H_9N_2) virus in collaboration with IBD and some unidentified bacterial species caused high mortality in infected flocks (Muneer *et. al.,* 2001).

In the present study the convalescent sera from the birds of the infected area contained antibodies to H_7. This further confirmed that the causative agent of this outbreak was AIV H_7 type. There are 16 HA antigens and 9 types of NA antigens in AI virus. Each serotype may contain any of the combination of H and N antigens. These findings were in full agreement with Naeem and Hussan (1995) who reported that the antibodies against the same H_7 isolate confirmed the virus involved in the outbreak was of H_7 type. Muneer *et al.* (2001) also found high HI titres against AI virus type H_9N_2 from the convalescent sera of bird suffering from acute respiratory distress in Karachi.

In our study openly constructed poultry houses were visited for conducting the sero-prevalence study of AI. In open houses there are increased chances of spread of infectious diseases like AI due lack of bio-security measures. Poorly controlled movement and lack of standard bio-security measures caused AI to become endemic in Europe and some areas of Asia (Stubbs, 1948). Due to poor bio-security and congested poultry colonies, AI reappeared in Karachi in 1999 mainly in broiler flocks. It became endemic in Karachi by the end of 1999. The disease was controlled by vaccination and strict bio-security measures (Naeem *et al.,* 1999).

Our study indicated that poultry farmers practiced vaccination against ND, IBD and HPS, while no vaccination was conducted against AI. Serological study for quantification of antibodies to AIV in vaccinated flocks in central Punjab (Pakistan) has shown satisfactory level of protection against AIV-subtype H_7N_3 in commercial layer and breeder flocks (Numan *et al.,* 2005). Other studies have documented that the vaccinated flocks cannot be considered influenza virus-free, but vaccine use typically reduces the amount of virus shed in experimentally vaccinated and challenged birds thereby, reducing shedding and potential transmission of the virus to other birds (Halvorson *et al.,* 1987). The presence of H_9N_2 and H_7N_3 in poultry is a continuous threat for the emergence of more pathogenic strains of influenza viruses. For this purpose there is a constant need to carry out a coordinated surveillance for influenza viruses both in birds and humans in the country. The documented sero-prevalence of AI in broilers with a history of respiratory tract infection was 54.54% and in broilers without a history of respiratory tract infection was 70.14% (Naeem *et al.,* 2003).

Our study found that 26-38 days of age of broiler was the high hit age for AI infection, while growing birds were moderately affected and brooding age reveled high resistance, this was indicated by high mortality at 26-38 days of age of broiler. Similarly, Brugh (1996) also observed the effect of age in the pathogenicity of AIV. His observations indicated that hosts of older age were more susceptible to AIV than the younger ones and the recovered birds show poor growth in their future life.

The primary source of the AI outbreak could not be identified, although involvement of wild water fowls were suspected as they act as asymptomatic carriers of AI and they transmit the disease from infected countries to Karachi during winter migration and then the disease might be brought to Gujranwala by mutual trade of poultry products including eggs and chicks. Further-more poultry workers and veterinarians would also be involved in the spread of the disease and the transportation of egg trays, equipments and other fomites may be the suspected source. Lack of prompt quarantine measures during the initial phase of outbreak and the movement of the infected birds to the disease free areas has resulted in the wide spread transmission of the AI virus within the country. Similar observations have also been reported (Becker, 1966; Lovov *et al.,* 1984) confirming that AI outbreak results from a single source and humans are primarily responsible for its spread. Since infected birds can excrete high levels of virus in their feces, transmission may be accomplished by anything contaminated with the feces e.g., birds, mammals, feed, water, equipment, supplies, cages, clothes, delivery vehicles, etc., through these agencies viruses are readily transported to other areas. Poultry workers, transportation and marketing services also have their share in transmission of virus. Infected birds, infected poultry products and the humans are the main sources for the spread of AI (Homme *et al.,* 1970).

In conclusion, it is the need of the time that the source of the spread of AI and the effect of Mycotoxins on the spread of AI should be traced out. Vaccination and sero-monitoring of the flocks is highly recommended.

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