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Epidemiology of Newcastle Disease in Rural Poultry in Faisalabad, Pakistan

MUHAMMAD YOUSUF KHAN[1], MUHAMMAD ARSHAD, MUHAMMAD SHAHID MAHMOOD AND IFTIKHAR HUSSAIN
Department of Microbiology, University of Agriculture Faisalabad, Pakistan
[1]Corresponding author's e-mail: dr.khan.fsd@gmail.com

ABSTRACT

The present study was conducted in randomly picked eight villages around two Tehsiles, Gojra and Samundri in Faisalabad from January 2009 to June 2009 to investigate epidemiology of Newcastle disease ND in rural poultry in the rural poultry. Data obtained through of questionnaires revealed that lack of vaccination, unavailable cold chain facilities, very poor knowledge about ND, multi-age of birds, improper dwellings at congested and dirty houses in harsh climatic conditions, insufficient feeding, unethical disposal of dead birds in premises and ignorance to other basic farming utilities result in highly fatal outbreaks and enormous mortality. Out of forty affected samples, collected from two outbreaks virus was isolated and identified through Haemagglutination (HA) and Haemagglutination Inhibition (HAI) test and was characterized on the basis of Mean Death Time (MDT) and Intracerebral Pathogenicity Index (ICPI) and found velogenic. About 33% prevalence of NDV antibodies was recorded through HAI test after the collection of the randomly selected eggs from the non-vaccinated but ND survivor hens during the outbreaks. These protective levels of antibodies were found to safeguard the hens for long time.

Key Words: Newcastle disease; Haemagglutination; Haemagglutination Inhibition; Mean death time; Intracerebral pathogenicity index; Epidemiology; Faisalabad

INTRODUCTION

Poultry eggs and meat are valuable source of protein in the era of protein insufficiency in Pakistan. The products from rural poultry are always ranked higher by the consumer due to delicious taste. Poultry industry in Pakistan is the back bone of our commercial as well as rural economy. It contributes significantly to nation's GDP. There are about 1105.91 million poultry birds in Pakistan, among, which rural poultry is about 152.44 millions. It plays a vital role in the village economy with the contribution of up to 3611 million eggs and 100.41 metric tons of the total poultry meat (Anonymous, 2008-09).

Newcastle disease is the top ranking disease of rural poultry in Pakistan. Seroprevalence of ND in unvaccinated rural poultry in district Faisalabad was recorded as 40.5% (Arshad, 2000). Azhar (2006) reported that 41.33% of un-vaccinated rural chicken were found positive for the presence of antibodies against ND virus. The morbidity and mortality may reach up to 100% in the severe forms of the disease and un-vaccinated birds are more prone to the disease (Alexander, 1997). It is caused by Avian Paramyxovirus serotype 1 (APMV-1) of the genus Rubulavirus belonging to the family Paramyxoviridae (Rima *et al*., 1995; Alkhalaf, 2009; Hafez, 2011). There are three important pathotypes of ND virus, Velogenic, Mesogenic and Lentogenic.

In a developing country like Pakistan significantly important factors are associated with the epidemics of this contagious and lethal disease and result in eggs and meat shortage, poultry industry's destability and WTO restrictions. Knowledge and understanding of the epidemiological profile of this highly fatal malady is to go down the bedrock to asses and address the basic needs of rural poultry population of a locale, as it enables us and other international agencies like OIE/FAO/WHO etc to develop strategies not only for control but eradication of this trans-boundary disease.

No detailed epidemiological studies have been conducted regarding ND in rural poultry. This project was therefore, designed to determine the epidemiology of ND in rural poultry. The results are hoped to help in control of the disease in rural poultry. This study project was not a naive prima fascia of parochial approach but facts presumably have association of global implications. Study area is an important geographical hub of commercial layers and broilers as well as rural poultry population.

MATERIALS AND METHODS

Gojra and Samundri are located at 31.15^o AN 72.68^o AE and 30.48^o AN and 71.52^o AE, respectively. Gojra 50 km from Faisalabad, is tehsil headquarter of Toba Tek Singh district, while Samundri 45 km from Faisalabad, is

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tehsil headquarter of Faisalabad district. Both are interconnected by 28 km long Gojra Samundri road. Each tehsil comprises of 28 union councils and 5-6 villages in every union council. Eight villages among these were selected randomly for epidemiological study of Newcastle disease (ND) according to the 1 stage cluster formula:

$$\text{No of clusters to be sampled (g)} = \frac{(1.96)^2 \text{ nVc} + \text{Pexp (1-Pexp)}}{\text{nd}^2}$$

A questionnaire was designed and data regarding factors and mortality pattern were collected from all the families rearing poultry birds in the selected villages. From all the outbreaks in the study period postmortems were conducted and samples (liver, spleen, brain & lungs) were collected from the ND infected birds and placed in the ice chamber of refrigerator. The frozen organs were cut into very fine pieces and thoroughly ground in to a thin paste in a sterilized glass pestle and mortar, to which a small quantity of sterilized sea sand was added. A 10-15% sample of crushed tissues was ultimately prepared by adding phosphate buffer containing antibiotics. The suspension was centrifuged at 3000 rpm for 30 min. The supernatant was sucked in a 1 mL disposable syringe and stored in the ice chamber (Arshad *et al.,* 1988).

Fertile eggs were obtained from Poultry Husbandry Department of University of Agriculture Faisalabad and incubated at 37.5°C and 60-70% RH. The eggs were candled on 9th day and 10 day old embryos were used for virus isolation (Arshad *et al.,* 1988). Pooled organs suspensions collected in disposable syringes were allowed to thaw and 0.2 mL of each was inoculated in to the allantoic cavities of embryonated eggs. The inoculated eggs were incubated at 37.5°C and 60-70% RH. Candling was done at 24 h intervals and the embryos found dead 24 h post-inoculation were discarded and material was re inoculated in to fresh embryos. The embryos dying between 24-96 h and those alive after 96 h were chilled at 4°C (Arshad *et al.,* 1994).

The allantoic fluid, about 5 mL in quantity was aspirated from every embryonated egg in to a 10 mL pipette and poured in to a sterilized ampoule at – 20°C (Arshad *et al.,* 1994).

The allantoic fluid was tested for the presence of haemagglutinins through haemagglutination test (HA) using 1% chicken erythrocytes. The samples giving positive results by HA were further confirmed for Newcastle disease virus by haemagglutination inhibition (HAI) test using positive antiserum against Newcastle disease virus (Allan, 1978).

Newcastle disease virus isolates were further processed for pathogenecity test following the criteria described by Allan (1978). The virus isolates were characterized on the basis of intracerebral pathogenecity index in day old chicks. Intracerebral pathogenecity index values of those isolates were recorded (Akram *et al.,* 2000).

The prevalence of NDV antibodies was recorded through collection of the randomly selected eggs from the non vaccinated but ND survivor birds of the families present in the selected villages, (on the principle of one egg from one hen) and processed for anti NDV-HAI antibodies determination through yolk content. The birds showing detectable levels of antibodies were considered exposed to NDV. Epidemiological data were analyzed as described by Thursfiel (2005) and Martin *et al.* (1977).

RESULTS

Epidemiological study: The survey conducted through Questionnaires revealed that 57% of the families reared chickens. All chickens were Aseel 4%, Golden 31%, Fayomi 30% and Desi or indigenous chickens of Pakistan 35%. Forty percent birds were hatched by broody hens and 60% were got by the paddlers. In total the eight villages comprised around 8767 chickens reared by 908 families, 2074 (24%) were adult male, 5168 (59%) were adult female (above 75 days approximately) and 1525 (17%) were chicks (both male & female) below 75 days approximately. About 12% families of birds were reared (Table I).

About 12% families have limited knowledge of ND and poultry management while 88% had very poor knowledge of disease and its management. In 8% of the families the birds were kept by men and in 92% families women were responsible for the management of the birds. The flock size was on average 10 birds per family. Only 4% of the farmers vaccinated their chickens, whereas 96% never vaccinated their birds. Proper dwellings were available for 26%, while 67% had temporary seasonal arrangement in very small wooden cages or dark and dirty congested houses and 7% had trees or perches to stay at night. About 67% birds were getting their feed from kitchen residues, 27% were offered grains and commercial feeds were given to 6% birds only. Participatory appraisal based data were compiled through questionnaires for spatial and temporal prevalence of ND in 2008. Out of eight selected villages, disease outbreaks were reported from four villages. From total 457 families rearing rural birds in 4 villages, 301 (65.8%) families having poultry birds were affected by this disease and annul mortality of these outbreaks was reported as 54% (Table II).

Factors contributing prevalence of the disease: From the selected villages a participatory appraisal based data were compiled through questionnaires for spatial and temporal prevalence of ND in 2008-2009 and importance of various factors associated with the spread of this disease were analyzed.

Vaccination housing and feeding: Mortality was relatively higher among those families where ND vaccination was not practiced as compare to the families regularly vaccinating their birds against ND. During housing, maximum mortality was seen among the farmers keeping their birds in dark, congested and dirty houses at night. Mortality was less among the farmers rearing their birds in proper dwellings.

Table I: Flock profile at the beginning of the study

Village	Total Families	Adult Male Birds	Adult Female Birds	Chicks	Total Birds
1	113	176	798	189	1163
2	103	237	645	194	1076
3	83	225	540	207	972
4	115	244	534	176	954
5	86	223	408	153	784
6	134	269	594	162	1025
7	175	455	1163	289	1907
8	99	245	486	155	886
Total	908	2074	5168	1525	8767
Average	10Birds/family	24%	59%	17%	-

Table II: Reported mortality by ND during the year 2008

Village	Total Families	Affected Families	%of affected families	Total Mortality	% of Mortality	January 08-December 08
1	113	79	69	543	47	January 08
3	83	62	74	561	57	December 08
5	86	60	66	345	44	July 08
7	175	100	72	1153	60	June 08
Total	457	301	65.8	2602	54	

Although birds roosted on trees or perches were also infected but mortality was less. In the feeding trial, mortality was less among the families providing their birds a balanced diet in the form of commercial feed as compare to compare to scavengers or the farmers rearing their birds on kitchen residues and grains.

Disease awareness: Families having limited knowledge of disease used different measures to save their flocks from disease problems and as a result their attempts were successful to some extent while birds of ignorant to disease were victim of ND.

Role of paddler's birds: Paddlers and their cages or birds presented for sales were a permanent source of infection. Outbreaks were reported among the families mixing new birds from paddlers in the already present birds of different age groups as compare to families rearing birds from broody hens in the houses.

Weather effects: Extremities of summer and winter lead towards lower immune status of the birds surviving in un-favorable conditions like lack of shelter, inadequate food and water supply. Outbreaks were reported in harsh climatic conditions of extreme hot and cold seasons.

Mortality profile during outbreaks: In the selected area two ND outbreaks occurred within six months. One outbreak occurred in village No.4 and second in village No.7. The areas of outbreak were visited immediately. In village 4, disease started on 5th January 2009 in a foul and foggy weather in a family keeping non vaccinated birds in very unhygienic conditions in a dark and dirty over crowded house. The family threw the dead birds on wastage heaps, from where stray dogs, scavenging poultry birds and crows spread the disease in whole village. The disease remained active for 15 days and 66% families lost their precious adult birds and chicks. Total mortality was 65%, including 76% chicks, 55% adult male birds and 65.5% adult female birds. Survivor hens dropped the egg production.

Second outbreak occurred in village 7 on 15th May 2009, among the birds of two families who purchased the chicks of more than one month age from peddlers few days earlier and mixed them with the already present stock. All the birds were non-vaccinated, caged in foul and dirty houses and feeding on fusty kitchen residues in a very hot season. With in next 10 days 68% families of village were severely infected loosing 49% adult male birds, 52% adult female birds and 80% chicks. From these two outbreaks total mortality shared by chicks was 21.7%, adult male birds 21.4% and adult female birds 56.8%, while total mortality among chicks was 78%, adult male birds 51% and adult female birds 56%. During these two outbreaks birds of 67% of the families were infected (Table II & III).

Marked depression, greenish diarrhea, head tremor and twisting, occasional paralysis and lateral recumbence were the major clinical signs. Mortality was first noticed on day 3rd post infection and continued until day 8th post challenge. Most of the dead birds showed severe pathological lesions with hemorrhages throughout the intestinal tract. About 88% of the dead birds showed lesions in the caecal tonsils with button-like ulcers (44%). More than 90% of the birds had hemorrhages in the proventriculus, whereas less than 10% of the birds had congestion in the lungs and trachea. Spleenomegaly was frequently observed.

There were no significant differences in mortality rates due to ND between male and female birds. In these birds, mortality due to diseases other than ND also took place but severity was not significant.

Isolation and characterization of ND virus: From all the 40 samples collected from these two outbreaks, after inoculation on embryonated eggs, 36 samples killed the embryo and after performance of HA test and HAI test 31 samples were found positive for NDV (Table-IV). Pathotyping conducted on the basis of MDT (51 & 54) (Table-V) and ICPI (Table-VI) values (1.70, 1.74, 1.52 & 1.59) revealed the causative organism virulent.

Antibodies prevalence: The prevalence of NDV antibodies was recorded through collection of the randomly selected eggs from the nonvaccinated birds (on the principle of one egg from one hen) and processed for anti NDV-HAI antibodies determination. Yolk content of each egg was diluted with the normal saline as 1:4 ratios and subjected for HAI titration as described by Sajid (2001). The relatively high level of antibodies against ND in unvaccinated birds was observed during the study, (33% in egg laying hens) indicated a high prevalence of NDV infections in village chickens. Villages where outbreaks occurred during this year gave higher levels of antibodies 4 (49%) and 7 (59%) as compare to villages, where outbreaks occurred during last year 1 (31%), 3 (33%), 5 (19%), 7 (59%) or no outbreak took place 2 (23%), 6 (12%) 8 (15%) (Table VI). Table VII further provides an overview of the antibody level in un-

Table III: Mortality profile during outbreaks

Area	Families		Birds				Mortality			
Village No.	Total families	Infected families	Male birds	Femalebirds	Chicks	Total	Male birds	Femalebirds	Chicks	Total
4	115	76	244	534	176	954	136 (55%)	350 (65%)	134 (76%)	620 (65%)
7	175	119	455	1163	289	1907	244 (49%)	606 (52%)	232 (80%)	1062 (55%)
Total 4+7	290	195	699	1697	465	2861	360 (51%)	956 (56%)	366 (78%)	1682 (58.8%)
%age4+7	-	67%	-	-			51%	56%	78%	58.8%

Table IV: NDV isolation and identification

Outbreak	Sample	Emb. Death	HA +iv	Titre 32	Titre 64	Titre 128	Titre 256	Titre 512	Titre 1024	HI +iv
1	24	21	18	-	-	4	10	4	-	18
2	16	15	13	-	-	3	5	3	2	13
Total	40	36	31	-	-	7	15	7	2	31

Table V: Mean Death Time

ND Isolate	Replicate	Hour 24	Hour 36	Hour 48	Hour 60	Hour 72	Hour 84	Hour 96	MDT	MDT
1	1	-	2	7	2	1	-	-	50	51
	2	-	2	5	3	2	-	-	53	
	3	-	3	3	4	2	-	-	53	
	4	-	3	6	3	-	-	-	48	
2	1	-	2	4	3	3	-	-	55	54
	2	-	4	4	3	2	-	-	49	
	3	-	2	4	4	2	-	-	54	
	4	-	2	2	5	2	1	-	58	

Table VI: Intra cerebral pathogenicity index

Sample	Group	Observation Days								Survival	Mortality	ICPI
		1	2	3	4	5	6	7	8	-	-	-
1	1-1	-	3	4	2	1	-	-	-	0	10	1.70
-	1-2	1	3	5	1	-	-	-	-	0	10	1.74
2	2-1	-	1	5	4	-	-	-	-	0	10	1.52
-	2-2	1	3	3	3	-	-	-	-	0	10	1.59

Table VII: Prevalence of ND antibodies

Source	Distribution of birds on the basis of anti ND-HAI antibodies										
Village No. s	Total samples	Titre (0)	Titre (32)	Titre (64)	Titre (128)	Titre (256)	Titre (512)	Titre (1024)	Total +iv	%(+iv)	%-iv
1	52	36	2	6	5	3	-	-	16	31	69
2	43	33	2	5	3	-	-	-	10	23	77
3	40	27	1	3	5	4	-	-	13	33	67
4	39	20	-	5	5	6	2	1	19	49	51
5	48	39	2	2	3	2	-	-	9	19	81
6	50	44	2	3	1	-	-	-	6	12	88
7	88	36	4	7	13	14	8	6	52	59	41
8	40	34	1	3	2	-	-	-	6	15	85
Total	400	269	14	34	37	29	10	7	131	33	67

vaccinated laying birds, during the study period. The mean HAI titers in the survivors just after the outbreaks were relatively high (>32). Villages where outbreaks were of previous years the mean HAI titers were relatively low (Table VII).

A significant difference in the mean antibody level was found between the birds after outbreak and without outbreak ($p<0.0001$). Furthermore, there was a highly significant difference in the NDV antibody level between the recent outbreaks and outbreaks of previous year.

DISCUSSION

It was observed that 57% of the families reared chickens. Forty percent birds were hatched by broody hens and 60% were got by the paddlers. This information was not in accordance with Barman (2001), whom reported that about 89.9% families of Bangladesh reared rural birds. Reasons might be the developed commercial poultry of Pakistan or presence of a sufficient quantity of commercial layer and broiler population in the vicinity, to fulfill routine

requirements.

Only 12% families have limited Knowledge of ND and poultry management, while 88% have very poor knowledge of ND and management of birds. In 8% of the families the birds were kept by men and in 92% families women were responsible to keep the birds. These findings were in accordance with a survey conducted by (Yasmin *et al*., 1989) regarding knowledge about poultry rearing among 100 poultry rearers in 10 villages. This showed that about 17% of the farmers had very limited, 70% medium and 13% had better knowledge of poultry rearing. Afzal (1997) reported that raising poultry is traditionally women's work, which not only provides eggs and meat for family consumption but also meat for guests.

The flock size was on average 10 birds per family. This is in accordance with Barman (2001), who reported 13 birds per family. Structure of typical traditional village poultry comprises of 5-8% adult males, 16-30% adult females and remaining more than 50% growers of both sexes at different age groups (Janviriyasopak *et al*., 1989; Cumming, 1992; Gunaratne *et al*., 1992). Of the total 67% birds got their feed from kitchen residues, 27% were offered grains while commercial feeds were given to 6% birds only. The scavenging system is practiced by the majority of the farmers, where household refusal and picking from the surroundings is the main source of feed for the poultry birds. This scavenging system contributes over 85% of the rural poultry production (Huque *et al*., 1999).

Proper dwellings were available for 26% birds, 67% birds had temporary seasonal arrangement and 7% had trees or perches to stay and rest at night. Accordingly, Javed (2003) reported that fewer chicken was found in flocks given no housing facility (24.3±1.02) than those given part time housing facility (29.4±0.85). Awan *et al*. (1994) also reported similar pattern of housing or confinement of chicken at night in smaller household groups, either in houses, under large airy basket or on trees.

Very interesting observations were reported by Huchzermeyer (1993) on the role of housing in the epidemiology of ND in villages. He believed that chickens that were housed at night were more susceptible to infection that occurred by close contact of sick and healthy birds in unhygienic and dirty environment of houses, in the absence of solar radiation. When the birds roosted in trees, there was less spread. As sick birds could not reach branches to rest and remained segregated. It was reported that brooding hens and hens with groups of chicks that were kept segregated could also escape infection. Present study also revealed that housing had very important role in the spread of ND in rural poultry birds, as the ratio of the disease was high among the families keeping their birds in dark, congested and dirty houses.

Only 4% of the farmers regularly vaccinated against ND, while 96.0% of the farmers did not use vaccine, mainly due to lack of proper knowledge, multi-age of their flocks, lack of cold chain facilities and poverty as reported by Spradbrow (1993/94). Some farmers believe that after introducing vaccines mortality rises. Often farmers do not vaccinate until an ND outbreak occurs, which may influence the mortality after vaccination (Musiime, 1992).

Forty ND suspected samples taken from two outbreaks, were inoculated in fertile chicken eggs for the isolation of ND virus. From 38 samples embryonic death occurred. But 31 samples were HA positive and all these 31 samples confirmed the presence of NDV after HAI activity with specific hyperimmune serum raised against ND. Two out of these samples were subsequently characterized and found to be velogenic. Martin (1992) reported that velogenic NDV was responsible for the majority of ND outbreaks in village poultry. Also in Nigeria, Adu *et al.* (1985) reported that velogenic strains of NDV were prevalent in traditionally managed poultry. Huque *et al.* (1999) reported the mortality of scavenging poultry birds from 45-85%, while in present study same level of mortality was observed in two outbreaks. These findings were also in accordance to Chowdhury *et al*. (1982), where 662 samples from 19 districts were collected from birds suspected to suffer from ND. Out of these, 67.4% yielded NDV. In total 150 isolates were taken for characterization and 120 (80%) found to be velogenic by MDT.

The mortality due to ND in young birds was sign`ificantly higher than in old birds. Ezeokoli *et al.* (1984) concluded that in backyard management systems birds around 16-24 weeks of age had the highest risk of NDV infection. Some studies have suggested that the sex of the birds may influence the morbidity and mortality by ND virus. Kutubuddin (1973) conducted pathological investigations at the poultry farm at Bangladesh Agricultural University. He found that male birds were more severely affected by NDV than female birds. However, in the present study, there was no statistically significant difference between the mortality due to ND between male and female birds in either unvaccinated or vaccinated birds, however mortality % was higher in chicks. Seasonal influence on the incidence rate and severity of ND has been reported by many authors. Mishra (1992) found the number of ND outbreaks to be higher in the summer compared to other seasons. Nguygen (1992) reported that ND outbreaks were more common during the winter season in Vietnam and Musiime (1992) also concluded that ND infections were more common during cold and dry periods. In this study, the outbreaks in rural birds occurred through out the year. But frequency was higher in severe months of hot and cold weather.

Relatively high level of antibodies against ND in unvaccinated birds observed during the study, (33% in egg laying hens) indicated a high prevalence of NDV infections in village chickens. The birds showing detectable levels of antibodies were considered exposed, while those having undetectable level of antibody titer against ND were considered as non-vaccinated. Similar studies to detect and quantify HAI antibodies against NDV and other

Paramyxovirus have been reported (Allan & Gough, 1974; Sajid, 2001). This result is supported by Bell and Moulodi (1988), who found 5-83% (average 35%) seropositive village chickens in Morrocco.

The presence of antibodies against ND in un-vaccinated egg laying adult hens was likely to be caused by exposure to NDV. Thitissak *et al*. (1989) reported, that mean HAI titres rose steadily as the age of birds increased, peaking in unvaccinated birds of 3 years of age. Schmidt and Schmidt (1955) considered HAI titre 32 and above to be protective against NDV. The present study showed, that mean HAI titres in the adult birds were within the protective level. However, Villegas *et al*. (1977) found that it was difficult to evaluate vaccination programmes adequately under field conditions, because there was not always consistent relationship between HAI titres, challenge and mortality.

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