

Letter to the Editor

Newcastle disease as an emerging disease in peacocks of Tharparker, Pakistan

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Dear Editor,

Newcastle disease virus (NDV) also known as avian paramyxovirus serotype I (APMV-1) is an RNA virus belonging to the genus *Avulavirus* (family *Paramyxoviridae*) [1]. NDV strains can be categorized into three major classes: as velogenic (highly virulent) and can cause up to 100% mortality in an unvaccinated flock, mesogenic (intermediate virulence) or lentogenic (nonvirulent) which are used as a vaccine. NDV has an incubation phase of 2-5 days depending upon birds' immune status and age [2]. NDV has the ability to infect a wide variety of avian species. More than 200 species of birds have been estimated to be vulnerable to natural or experimental illness through this virus [3]. In most species of birds, the younger ones are more susceptible than adults [4].

Newcastle disease (ND) in domestic poultry is the matter of concern all over the world. The NDV is widely spread in Pakistan and frequent outbreaks are being repeatedly reported in wild captive, rural and commercial poultry flocks. Between 2012 and 2013, ND endemic was observed in peacocks in Tharparker desert, Sindh province of Pakistan, where approximately a population of 40,000 peacocks can be

found. There is no epidemiological study carried out on NDV epidemic in peacock in Tharparker. The present study was therefore designed to determine the role of epidemiological factors involved in the NDV outbreak.

The study was conducted in different villages of following areas in Tharparker (Sindh), Pakistan including Nagarparker, Islamkot, Kasboo, Naukot, Mithi, Mao and Veera Wah Umarnkot. A total number of 2,000 peacocks from eight villages of Tharparker of different age, sex and breed were randomly physically examined between July 2012 and September 2013 to find out the prevalence of disease and its possible causative factors. The epidemiological information was taken in a questionnaire comprising of sex, age, prevalence, feeding, weight, medication, topography and mating behaviour. To determine the statistical association between NDV infection and different parameters Chi-square statistical test was carried out using R 3.1.1 for Windows.

The results of current study showed that prevalence of ND in different areas of Tharparker, Sindh, Pakistan during this period was 19.7%.

Table 1. Different geographical factors in relation to prevalence of Newcastle disease in peacocks, Pakistan

Sr. No	Factor	Group	Total Peacocks Observed	Infected	Non-infected	Prevalence (%)	Statistical Analysis (Chi-square)
1	Year	2012	1000	300	700	30%	$\chi^2 = 147.017$ df = 1 p = .000
		2013	1000	86	914	8.6%	
2	Age	Group I (0.5-2 year)	173	54	119	31.2%	$\chi^2 = 25.053$ df = 2 p = .000
		Group II (2- 3.5 year)	1089	221	868	20.3%	
		Group III (>3.5 year)	738	111	627	15%	
3	Sex	Male	1054	223	831	21.2%	$\chi^2 = 4.936$ df = 1 p = .026
		Female	946	163	783	17.2%	
4	Feeding	Feed during disease	1476	152	1322	10.3%	$\chi^2 = 290.69$ df = 1 p = .000
		Don't feed during disease	526	234	292	44.5%	
5	Treatment	Non medicated	750	273	477	36.4%	$\chi^2 = 225.29$ df = 1 p = .000
		Medicated (Vaccination)	1250	113	1137	9%	
6	Topography	Stony	892	166	726	18.6%	$\chi^2 = .492$ df = 1 p = .483
		Sandy	1108	220	888	19.9%	
7	Weight	Group1 0.5 - 1.5 kg	494	163	331	33%	$\chi^2 = 116.474$ df = 3 p = .000
		Group 2 1.5 – 3kg	548	119	429	21.7%	
		Group 3 3 – 4.5 kg	801	102	699	12.7%	
		Group 4 > 4.5 kg	157	2	155	1.3%	
8	Mating	Mating	702	107	595	15.2%	$\chi^2 = 11.435$ df = 1 p = 0.001
		Non-mating	1298	279	1019	21.5%	
9	Village	Group1 Nagarparker	283	115	168	40.6%	$\chi^2 = 360.551$ df = 7 p = .000
		Group2 Islam kot	230	95	135	41.3%	
		Group3 Mao	351	90	261	25.6%	
		Group4 Kasboo	251	13	238	5.2%	
		Group5 Naukot	172	3	169	1.7%	
		Group6 Mithi	268	22	246	41.4%	
		Group7 Veera wah	111	46	65	8.2%	
		Group8 Umarkot	334	2	332	0.6%	

Chi Square test for independence was performed in order to test the effect of different factors on rate of infection. Results indicate infection rate depends on: year, age, sex, feeding, vaccination, weight, mating and village (p value < 0.05) whereas infection does not depend on topography (p > 0.05)

Statistical analysis of different parameters between NDV-infected and non-infected groups of peacocks was carried out. Results showed that year (2012 vs 2013), age (group I [0.5-2 years], group II [2-3.5 years], group III [$>$ 3.5 years]) (Supplementary Data), weight of animals, feeding behaviour (feeding during disease vs non-feeding during disease), mating behaviour (mating vs non-mating), different geographical distribution (different villages) and vaccination status (vaccinated vs non-vaccinated), were significantly associated with NDV infection while gender (male vs female) and habitat topography (stony vs sandy), apparently did not have any impact of NDV infection in Tharparker area (Table 1). The birds of younger age, male, non medicated, sandy area and located in the area of Nagaraparker/Islam Kot/Mithi were more infected in comparison to other factors.

The wide variation in year-wise prevalence of ND might be due to the delay of the monsoon rains in 2012 which caused unavailability of fresh water and increased the risk of disease spreading due to contaminated and infected water. The geographical distribution (village-wise) showed the highest infection in Islam kot, Mithi and Nagaraparker. The possible reason might be the lack of good quality conservation practices as well as unavailability of fresh water in the lake due to the delay of monsoon season in these villages. There is a stream of water in Nagaraparker which fills only during rainy season: it might be the major reservoir of NDV in the region. The disease sex-wise incidence showed a non-significant association which is in accordance with investigations carried out by Zeleke A *et al* [5]. The change in mating behaviour is also positively associated with infection status and a sudden drop in egg production was also observed in animals under the influence of NDV. The vaccination status clearly has an impact on viral infection, in fact animals subjected to vaccination showed lower disease ratio as compared to untreated ones. The possible reason behind the infection of vaccinated peacocks might be represented

by climate, because Tharparker has an extremely hot climate thus resulting in heat stress; moreover, the low monsoon rain, such as it was registered during the study period, resulted in a water shortage. Heat stress and water deprivation can lead to steroid production and results in immune suppression reducing the efficacy of the vaccine [6]. It is recommended that a thorough implementation of a suitable vaccination plan is carried out which may reduce the prevalence of ND in the future.

This is the first report showing the prevalence of NDV in peacocks in Tharparker region. Research on genetic nature of strains circulating in Pakistan is currently limited and further studies on NDV strains characterization circulation in Pakistan are warranted.

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Supplementary Tables**Supplementary table 1.** Pairwise comparisons in different age groups

Group	Group I (0.5-2 year)	Group II (2- 3.5 year)	Group III (>3.5 year)
Group I (0.5-2 year)	-	$\chi^2 = 9.816$ df = 1 P = 0.0017	$\chi^2 = 23.63$ df = 1 P = 0.0000016
Group II (2- 3.5 year)	-	-	$\chi^2 = 7.814$ df = 1 P = 0.0051

P value of less than 0.05 indicates a significant effect of the groups compared. All pair wise comparisons were performed while comparing each group with every other group with factors involving more than two levels. Almost all age, weight and village groups differ from each other. Infections are dependent upon groups.

Supplementary table 2. Pairwise comparisons of animals groups possessing different body weights

Group	Group I (0.5-1.5 Kg)	Group II (1.5-3 Kg)	Group III (3-4.5 Kg)	Group IV (> 4.5 Kg)
Group I (0.5-1.5 Kg)		$\chi^2 = 16.182$ df = 1 P = 5.7e-05	$\chi^2 = 75.8336$ df = 1 P = 2.2e-16	$\chi^2 = 61.6939$ df = 1 P = 4.012e-15
Group II (1.5-3 Kg)			$\chi^2 = 18.51$ df = 1 P = 1.69e-05	$\chi^2 = 34.4436$ df = 1 P = 4.388e-09
Group III (3-4.5 Kg)				$\chi^2 = 16.6507$ df = 1 P = 4.5e-05

Supplementary table 3. Village wise pairwise comparison

	Group1 Nagarparker	Group2 Islam kot	Group3 Mao	Group4 Kasboo	Group5 Naukot	Group6 Mithi	Group7 Veera wah	Group8 Umarkot
Group1 Nagarparker		$\chi^2 = 0.0039$ df=1 p=0.9499	$\chi^2 = 15.4234$ df=1 p=8.592e-05	$\chi^2 = 89.8269$ df=1 p=2.2e-16	$\chi^2 = 82.23$ df=1 p=2.2e-16	$\chi^2 = 75.7501$ df=1 p=2.2e-16	$\chi^2 = 0.001$ df=1 p=0.9742	$\chi^2 = 157.2102$ df=1 p=2.2e-16
Group2 Islam kot			$\chi^2 = 14.9942$ df=1 p=0.0001078	$\chi^2 = 87.8935$ df=1 p=2.2e-16	$\chi^2 = 81.4087$ df=1 p=2.2e-16	$\chi^2 = 73.5951$ df=1 p=2.2e-16	$\chi^2 = 0.0087$ df=1 p=0.9257	$\chi^2 = 155.6336$ df=1 p=2.2e-16
Group3 Mao				$\chi^2 = 41.7734$ df=1 p=1.025e-10	$\chi^2 = 43.4689$ df=1 p=4.308e-11	$\chi^2 = 29.9953$ df=1 p=4.331e-08	$\chi^2 = 9.3893$ df=1 p=0.002183	$\chi^2 = 90.1684$ df=1 p=2.2e-16
Group4 Kasboo					$\chi^2 = 2.4325$ df=1 p=0.1188	$\chi^2 = 1.4406$ df=1 p=0.23	$\chi^2 = 71.551$ df=1 p=2.2e-16	$\chi^2 = 10.271$ df=1 p=0.001351
Group5 Naukot						$\chi^2 = 7.0084$ df=1 p=0.008113	$\chi^2 = 71.5117$ df=1 p=2.2e-16	$\chi^2 = 0.5767$ df=1 p=0.4476
Group6 Mithi							$\chi^2 = 56.6425$ df=1 p=5.2e-14	$\chi^2 = 20.5528$ df=1 p=5.801e-06
Group7 Veera wah								$\chi^2 = 140.2062$ df=1 p=2.2e-16