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Polish Journal of Veterinary Sciences Vol. 24, No. 4 (2021), 497–503

DOI 10.24425/pjvs.2021.139974

Original article

Risk factors for outbreaks caused by variant strain of Newcastle disease on environmentally controlled broiler chicken farms in Lahore, Pakistan

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Abstract

Newcastle disease (ND) is a frequently reported disease in poultry among both vaccinated and non-vaccinated flocks in Pakistan. During 2011-2012 poultry industry in Punjab, mainly in Lahore region, faced fatal outbreaks of ND caused by a variant strain. An analytical study was conducted during outbreak period in Lahore region. A total of 114 environmentally controlled farms were selected with the help of convenient sampling method. A questionnaire was designed about the potential risk factors associated with the spread of ND outbreak. The bivariate relationships between ND status and independent variables were investigated by applying the Chi-square and Fisher's exact test. Multivariable logistic model was used to estimate the effect of each studied variable on the outcome by adjusting the other variables in the model.

The variables which showed an association with ND outbreaks at commercial poultry farms were improper method for dead birds disposal (OR=4.96; 95% CI 1.63-15.12), use of same feed transporting vehicle at multiple poultry farms (OR=4.92; 95% CI 1.58-15.33), farm to farm distance of less than 1 km (OR=9.32; 95% CI(1.19-73.12), number of sheds at one farm (OR=2.31; 95% CI 0.93-5.69), labor type (OR=2.72; 95% CI 0.83-8.88) and biosecurity (OR= 4.47; 95% CI 0.56-35.66).

Key words: Newcastle disease, retrospective, environmentally controlled house, biosecurity, vaccine

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Introduction

The poultry sector is one of the most structured and vibrant divisions of the agriculture industry with contribution of 35% to the livestock production of Pakistan. This sector has generated direct and indirect employment and source of income for about 1.5 million people (Chaudhry et al. 2015). The existing investment in the poultry industry is about Rs. 700.00 billion. The poultry sector has shown a vigorous growth of 8 percent annually, which reveals its distinctive potential (Pakistan Economic Survey 2020).

Newcastle disease virus (NDV) belongs to genus *Avulavirus* of family *Paramyxoviridea* (Mayo 2002) formerly known as *Avian Avulavirus 1*. Recently the International Committee on Taxonomy of viruses named it as *Avian Orthoavulavirus 1* (Kuhn et al. 2020, Ul-Rahman et al. 2021). NDV is a diverse group of single-stranded, negative sense, non-segmented, enveloped RNA viruses. NDV has a wide variety of host species and can infect various avian species as well as humans and animals. NDV has been established among 241 domestic and wild birds' species (Alexander 2000). ND affects gastrointestinal, respiratory and nervous systems of the bird. Depending upon the pathotype of virus involved in infection outcome of diseases varies from in-apparent to highly fatal consequences (Alexander 2000, East et al. 2006).

The main route of bird-to-bird transmission is inhalation of droplets and through ingestion of infected material. Aerosol transmission is perpetuated by suitable climatic factors such as temperature, humidity and high stoking density in poultry sheds (Alaxender 2000, Musa et al. 2010). Once chickens in a flock are infected, they become a source of infection for successive ND outbreaks (Wambura 2010).

Many outbreaks have been reported in vaccinated poultry flocks (Boven et al. 2008) and were frequently reported in non-vaccinated poultry. The yearly incidence of ND outbreaks in commercial and other domesticated poultry flock suggests that this disease alone can severely impede the production of both commercial and backyard poultry (Alexander 2001). It also has considerable influence on international trade of poultry and poultry products worldwide (Alexander 2001, Musa et al. 2010). ND is posing a huge risk and challenge to the international poultry industry (Alexander 2001). ND causes major damages to poultry by high mortality rate, hampering growth, decreased egg production and economically by confining exchange and by forcing producers to implement measures to anticipate the disease spread (Cornax et al. 2012).

ND is a major limitation to attain standard production levels in privately owned commercial enterprises (Sen et al. 1998). Outbreaks of virulent form of ND in different areas of Pakistan have been reported despite the biosecurity measures and vaccination. However, during the period of 2011-2012, a surge in number of outbreaks of ND was seen in commercial poultry industry (Ali et al. 2014). Especially, younger flocks of commercial broilers were one of the most affected groups of poultry. Change in vaccine type or vaccination strategy was not helpful to mitigate the disease. Under different vaccination programs various live or killed ND vaccine trials were attempted during the short lifespan of broiler flocks but no considerable solution could be recommended to the poultry farmers in this regard. A short and significant relief was noticed during start of 2012, but re-emergence of a new wave of ND was faced in the start of winter of 2012 (Siddique et al. 2013).

Rearing commercial broiler in environmentally controlled farms cost a huge investment in feeding, management and vaccination of chicken. Despite vaccination and biosecurity measures NDV finds its ways to enter the farms and cause huge mortality. Keeping in view the importance of poultry sector in the economy of Pakistan, and economical losses due to ND, current study was designed to epidemiologically investigate the outbreaks due to variant strain of ND in commercial chicken and to identify risk indicators associated with these outbreaks. The aim of this study was to investigate the risk factors associated with the outbreaks due to variant strain of Newcastle disease virus and recommendations for the control of ND.

Materials and Methods

Study Area

An analytical study was conducted in District Lahore. Lahore is laid between 31°15′- 31°45′ N and 74°01′- 74°39′ E, in its north and west Sheikhupura District, on the east Wagah, and on the south Kasur District is present. Ravi River also flows on the northern side.

Study design

ND outbreaks due to variant strain of NDV reported in Pakistan during 2011 and 2012 (Wajid et al. 2017). An analytical study was conducted in the study area to investigate the factors involved in outbreak of ND at commercial broiler farms. Selection of farms was based on convenient sampling method from different areas of Lahore and its peripheries from December 2012 to April 2013. Poultry farm managers were contacted for their consent to collect data. The purpose and objectives of study were elaborated to the farm mana-

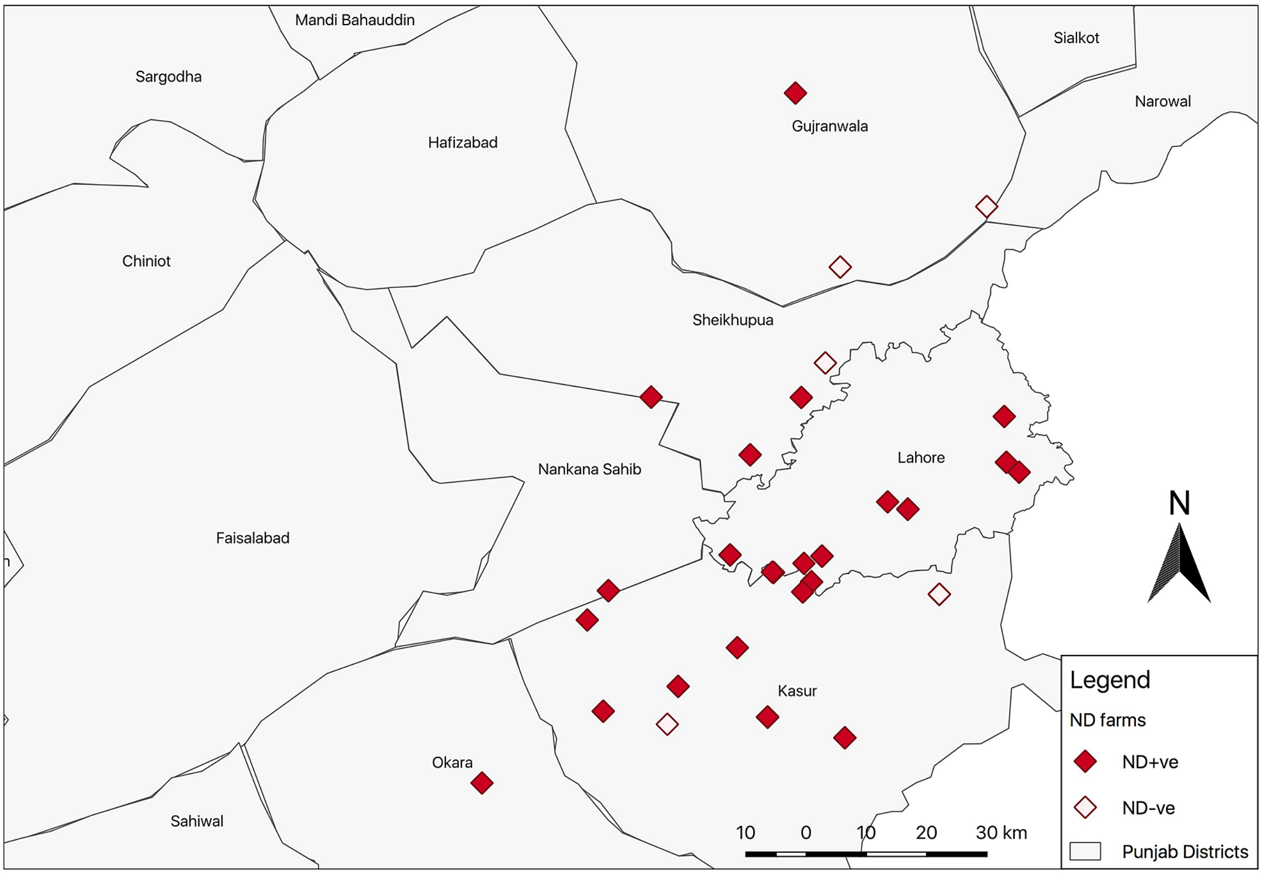

Fig. 1. Spatial distribution of Newcastle disease affected farms in study area.

gers but many of them refused to participate in the study. Among those who agreed to participate, they had a recent history of unexpected increased mortality due to ND at their farm. Most of the farm owners/managers relied on clinical symptoms or postmortem lesion by their technical person or consultant for diagnosis of ND. As majority of farmers did not confirm the presence of ND through laboratory tests, the criteria of selection of ND infected farms in current study was based on clinical picture and postmortem lesions.

A commercial broiler farm was considered to be affected by ND, when mortality rate due to the disease was equal to or greater than 10% and ND was confirmed by the veterinarian/technical person on the basis of clinical signs or postmortem lesions. The commercial broiler farm with mortality less than 10% and with no latest history of ND was regarded as unaffected by the ND. All the flocks at selected farms were vaccinated against ND, using vaccine of their choice.

The unit of interest was environmentally controlled broiler farm. The information and data were collected with a pre-structured questionnaire which included questions about the factors related to poultry husbandry practices, which were considered to influence the occurrence and spread of variant of NDV. These factors were compiled after reviewing the literature on respiratory diseases of poultry (Akhtar and Zahid 1995, East et al. 2006, Chaudhry et al. 2015) and getting suggestions from relevant practicing veterinarians. All the information was collected during personal visits of the poultry farms through face to face and telephonic interviews of farm manager/owner or from the farm record.

Statistical analysis

For all univariable and multivariable analysis, the dependent variable was ND affected or unaffected farms. Other variables included in the analysis are defined in Table 1. All analysis was carried out in R statistical software package (version 2.15.1.0) which effectively perform likelihood ratio test (Chaudhry et al. 2015). The bivariate relationships between ND status and independent variables were examined using the Chi-square and Fisher's exact test where appropriate (Woodward 2004). Multivariable logistic model was used to estimate the effect of each study variable on the outcome, adjusting for the other variables in the model (Kleinbaum et al. 1982). Variables with significant univariable relationship at $p<0.25$ were included in multivariable analysis. Multivariable model selection was performed by forward stepwise procedure. Models were selected on AIC (The Akaike Information Criteri-

Table 1. Univariable analysis of qualitative variables for analytical study of ND outbreaks among 114 commercial broiler farms near Lahore territory, December 2012-April 2013.

Sr. No	Factors	Variable level	OR	p-Value	95%CI
1	No. of sheds at one farm	One			
		> one shed	2.31	0.0699*	0.93-5.69
2	Flock size in one shed	≤ 30000			
		> 30000	1.6	0.33	0.59-4.88
3	Age of flock when affected	<14 days			
		>14 days	1.76	0.279	0.6-4.91
4	Farm to farm distance	<1 KM	9.32	0.034**	1.19-73.2
		>1 KM			
5	Labor type	Full Time			
		Temporary	2.7	0.0968*	0.83-8.88
6	Biosecurity of farm	Adequate			
		Inadequate	4.47	0.157	0.56-35.66
7	Written plan of biosecurity	Yes			
		No	1.35	0.589	0.46-3.96
8	Visitors allowed at farm other than farm labor	Yes	17.72	0.006**	2.27-138.5
		No			
9	Heat source	Coal / Wood	2.92	0.04757**	1.01-8.45
		Gas/electricity			
10	Feed storage	Proper			
		Improper	2.66	0.361	0.33-21.74
11	Feed transporting vehicle	Personal vehicle			
		Agency vehicle	4.92	0.0060**	1.58-15.33
12	Litter disposal method	Proper			
		Improper	1.55	0.69	0.18-13.17
13	Shed painted before new flock	Yes	0000	0.0657*	0.12-1.07
		No			
14	Dead birds disposal method	Proper			
		Improper	4.96	0.0048**	1.63-15.12
15	Use of antiparasitic drugs	Yes	0.64	0.448	0.2-2.02
		No			
16	Avian Influenza (AI)	Yes	13.22	0.0139*	1.69-103.4
		No			
17	IBD	Yes	1.98	0.529	0.24-16.47
		No			

* p-value <0.25, ** p-value <0.05, OR – Odds ratio CI – Confidence Interval

on is a mathematical method to compare different possible models to determine which model is best fit for our data) value (Dohoo et al. 2003). Variables were eliminated from the model on the basis of Wald Statistics p-value (p>0.05). A variable with p-value <0.05 was considered as significantly associated with occurrence of disease. After fitting the final model, logistic coefficients and their standard errors were used to compute odds ratio and corresponding 95% CIs.

Results

A total of 114 environmentally controlled sheds of commercial broilers were selected with the convenient sampling method and investigated during the study period. As all the chickens at selected farms were vaccinated against NDV, IB and AI following their own vaccination protocol so vaccination status was not analyzed as a variable. Total 96 poultry farms confirmed

Table 2. Multivariable analysis of variables significantly associated (p-value <0.05) with ND outbreaks at commercial broiler farms near Lahore territory, December 2012-April 2013.

Sr. No.	Factors	p-value	OR	95%CI
1	Dead birds disposal method	0.00482	4.96	1.63-15.12
2	Feed transporting vehicle	0.0060	4.92	1.58-15.33
3	Visitors allowed at farm other than farm labor	0.006	17.72	2.27-138.5
4	Avian Influenza(AI)	0.0139	13.22	1.69-103.4
5	Farm to farm distance	0.034	9.32	1.19-73.12
6	Heat source	0.047	2.92	1.01-8.48

p-value <0.05, OR – Odds ratio, CI – Confidence Interval

Table 3. Multivariable logistic forward stepwise model analysis for variables showed significant association with ND outbreaks commercial broiler farms near Lahore territory, December 2012-April 2013.

Sr. No.	Factors	p-value	OR	95%CI
1	Dead birds disposal method	0.007	4.96	1.63-15.12
2	No. of sheds at one farm	0.006	2.31	0.93-5.69
3	Labor type	0.038	2.72	0.83-8.88

p-value <0.05, p-value <0.05, OR – Odds ratio, CI – Confidence interval

the recent or ongoing ND outbreak (mortality >10% and ND confirmed by postmortem or lab test) while 18 poultry farms had no current status of ND also have mortality <10%. Maximum broiler farms with more than 10% mortality were present at the junction of both district Lahore and Kasur Fig.1.

A total of 17 variables analyzed are shown in the Table 1.

Out of the 17 variables analyzed, 6 variables were found to be associated with the ND outbreak (p<0.05) after univariable analysis, which are; dead birds disposal method, feed transporting vehicle, visitors allowed at farm other than farm labor, Avian Influenza, farm to farm distance, heat source, shed painted before new flock, number of sheds at one farm, labor type and biosecurity, showed an association with ND outbreaks at commercial poultry farm as compared to the farms with mortality less than 10% and with ND outbreak not confirmed at their farm (Table 2).

Final multivariable logistic regression using forward elimination is given in Table 3. Three variables remained in the model, which were; dead birds' disposal method, visitors allowed at farm other than farm labor, number of sheds at one farm and labor type at the farm. There was no co-linearity among these variables.

Some variables were excluded after screening of data due to zero cell value, which are; number of flocks affected at one farm, water quality at farm, shed washed with hot water each time prior to new flock, use of antifungal drugs and Hydro pericardium syndrome (HPS). Due to wide range of CI which may be due to small sample size, AI and Visitors allowed at farm other than farm labor were also excluded.

Discussion

Risk factors of ND have been well studied and documented worldwide (Akhtar and Zahid 1995). ND epidemics in any poultry flock could occur due to some mismanagement in poultry husbandry practices, followed by rapid mechanical spread to other flocks in that area. Once outbreak erupts the secondary spread is facilitated by different methods, e.g. first introduction of NDV in an area may have many paths like movement of migratory birds or through trade of backyard poultry, captive caged birds or game birds, which could spread the infection to the commercial poultry and then infection may spread from flock to flock. Most of the secondary spreads of ND in past years have occurred through uncontrolled movement of personals, vehicles and fomites (Alexander 1988). Secondary spreads may also be facilitated by equipment movement. Mechanical spread of NDV through scavenger animals, insects or rodents has been reported (Alexander 2000).

In our study, in univariable analysis improper disposal of dead birds was associated with spread of ND (OR=4.96, p<0.05). Carcasses of infected birds are a potential source of Newcastle disease virus (Musa et al. 2010). Disposal of dead birds is most important thing in preventing the transmission of infection. Most of the poultry farms threw their birds around the farm premises or did not bury them deep (Abbas et al. 2012, Chaudhry et al. 2015). Stray dogs and wild birds might be involved in the transmission of virus from one area to the other (Alexander 1988). Inappropriate farm management practices and inexperienced labor may contribute to Newcastle disease outbreaks or epidemics (Akhtar and Zahid 1995).

More than one sheds at a farm contributed to the spread of disease with an OR of 2.31 (p-value=0.006). When one shed on poultry farm was infected with ND, all the sheds at that time became infected with ND (Musa et al. 2010) as ND spreads through aerosol route and infected birds became the source of further outbreaks (Wambura 2010).

Biosecurity is the key measure to avoid or curtail the disease. Breach in biosecurity at any level could facilitate the transmission of pathogens (Chaudhry et al. 2015) like ND virus. Those farms where biosecurity was breached and visitors were allowed showed odds ratio of 17.72 (p>0.005). Untrained labor or daily wagers was also associated (OR=2.72, p>0.038) with outbreaks of ND at commercial poultry farms. In addition to this, during ND epidemic, farm owners were bearing heavy losses due to devastating effects of this fatal disease. They were trying every possible option and consulting as many poultry consultants as they can. Those consultants were visiting many infected and non-infected farms every day which was witnessed during data collection but due to some ethical limitation, could not explore further. More data would be required to establish a precise relationship with this variable as the confidence interval (CI) of this variable was too wide (2.27-138.5).

In univariable analysis, feed transporting vehicles also showed significant association (OR=4.92, p>0.006) with ND spread. Poultry feed-producing companies give incentives to the poultry farmers on feed purchase like free of cost delivery of the feed on the farm. Subsequently, agency vehicle travels miles of distance to transport feed on different farms on same day and become a source of infection from infected farms to the healthy farms (Leibler et al. 2010, Chaudhry et al. 2015). Vehicles for the feed transportation to commercial broiler farms revealed an involvement as a risk factor (Yunus et al. 2009). Lahore district has become the hub of poultry industry in Punjab to fulfill the meat requirement of this densely populated city and its surrounding cities. The dot map showed that affected farms were mostly present in the adjoining area of District Lahore and Kasur. A recent study on sero-prevalence of avian influenza also showed same kind of pattern (Hasni et al. 2021). Most of the areas in Lahore district have higher poultry farms concentration which have not maintained the ideal distance between poultry farms. If distance between poultry farms is less than 1 km the univariable analysis also showed association with ND (OR=9.32, p>0.034). It is already well documented that Newcastle disease outbreaks are perpetuated by the higher concentration of poultry farms (East et al. 2006). Between the farms having shorter distance, ND is also transmitted through air (Shankar 2008). In a recent study the minimum distance of 300 meter between poultry farms was set as criteria. Distance above 300 meters was supposed to be the protective factor. It was also endorsed that the farms located in closer vicinity facilitate the horizontal spread as well as the spread through stray dogs, wild bird, visitors, poultry trade connections and vehicles wandering in that area. Concentrated farms owned by different poultry producers in an area having different trading links for purchase of feed and sale of poultry birds results in an influx of farm workers, feed supply vehicles and other suppliers, which in turn become a risk factor for the spread of disease (Wiseman et al. 2018). Low pathogenic Avian Influenza (LPAI) infection was found as concurrent or co-infection with pathogenic NDV infection (OR=13.22, p>0.002) on many commercial broiler farms in the study area. Naturally, co-infection of NDV with LPAI or some other secondary pathogens results in severe disease consequences and higher mortality than NDV alone (Gowthman et al. 2019). It is also proved experimentally that co-infection with ND and LPAI resulted in more severe clinical consequences (Lee et al. 2013). In current study, analysis of LPAI as concurrent or co-infection with ND outbreak also showed a wide range of CI (1.69-103.4). More information on this will be needed to explore the actual association between ND outbreaks with AI. So AI was excluded in multivariable analysis.

Keeping in view all the findings it is suggested that the standard distance between the farms should be maintained, strict biosecurity practices should be adopted at the poultry farms, unauthorized visitors should not be allowed on the farm. Throwing dead birds should be avoided on farm premises and they must be buried deep. Feed transporting vehicles should be from reliable source with proper disinfection. Foot dip and vehicle dip areas at entrance of farm should be mandatory. Farm workers should be well trained about their work. Always use the vaccine prepared from the field virus and try to avoid the use of imported vaccines, proper protocol of vaccination should be followed.

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