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OUTBREAK OF NEWCASTLE DISEASE IN BACKYARD POULTRY FARMS IN PEMAGATSHEL DISTRICT, EASTERN BHUTAN: CASE-CONTROL STUDY

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ABSTRACT: Newcastle Disease (ND) is an important disease of poultry and wild birds. Using a case control study design, we identified farm level risk factors for ND outbreaks in Pemagatshel district, Eastern Bhutan. Thirty households that experienced ND outbreak in 2016 were identified as case whilst those nearest households from same village that had not reported ND outbreaks in the same year were selected as controls. The data were collected using structured questionnaires, and univariable and multivariable logistic regression model were built to identify risk factors related to ND outbreaks. The odd of ND occurrences in the farms that mixed with the wild birds was 13.08 times (95% confidence interval: 2.96-57.78) higher than those farms that were not mixed with the wild birds. Significant higher odds (adjusted odd ratio: 10.66; 95%CI: 2.3-49.5) of the ND outbreaks were reported in the farms that were having larger flock size (>10) comparing to smaller farm (≤10 birds). Similarly, those farms that were located near the roads were 3.85 times (95% CI: 0.95-15.63) more likely to report ND outbreaks than those farms that were located far from the road. However, regular cleaning of the poultry sheds was found to be protective factors with those farms that practiced regular cleaning likely to be encountered 0.16 times (0.04-0.66) less outbreaks comparing to those farms that did not practice regular cleaning. Therefore, improving farm biosecurity and management practices with location of farms away from the road networks had potential to reduce the incidences of ND outbreaks in the future.

Keywords: Newcastle disease; case control study; risk factors

1. INTRODUCTION

Newcastle disease (ND) is one of the most important diseases of poultry and wild birds. The disease is known to affect approximately 241 species of birds (Madadgar et al. 2013) . Among the affected species of birds, chicken is considered to be one of the most important host (Rezaeianzadeh et al. 2011). When affected, the disease can result in devastating affects reaching 100% mortality and morbidity in unvaccinated chicken (Haque et al. 2010). In the last four decades, the disease had already caused four economically devastating panzootic, resulting in death of millions of poultry birds all across the world (Bello et al. 2018). From the total of 71 important animal diseases that were reported to World Organization of Animal Health (WOAH) during four years period (2006 to 2009), Newcastle disease was reported as 2nd most prevalent diseases by 56 countries from the 167 members countries of WOAH (Miller & Koch 2013). Due to its severe nature and its related consequences, Newcastle disease is considered as WOAH listed diseases by World Organization of Animal Health, requiring every member nation to report and notify the outbreaks of the disease (Aldous & Alexander 2001; Boynukara et al. 2013; OIE 2017). The disease is caused by Newcaslte Disease Virus (NDV), which is a single segmented negative sense RNA virus belonging to genus Avulavirus and family Paramyxoviridae (Abdisa & Tagesu 2017; Ashraf & Shah 2014). On the basis of the clinical and pathologic manifestations, five different forms of Newcastle are recognized -velogenic vscerotrophic ND, velogenic neutrotropic ND, Mesogenic ND, lentogenic ND, and asymptomatic enteric ND with velogenic vscerotrophic ND reported to be the severest form with 100% mortality (Bello et

al. 2018; Marks et al. 2014). The viruses are usually transmitted from one affected bird to another through respiratory aerosols, exposure to fecal and other excretions from infected birds, through newly introduced birds, selling and giving away sick birds and contacts with contaminated feed, water, equipment and clothing (Ashraf & Shah 2014). The virus is also considered to be mildly zoonotic and reported to cause mild conjunctivitis in human (Alexander 2000) and other species of mammals such as dogs and cats (Ashraf & Shah 2014).

ND was identified as one of the main constraints for the poultry farmers in Bhutan together with other infectious diseases (Alders 2003; Nidup & Tshering 2007). Since it first recorded report in 1998, sporadic outbreaks had been reported from many parts of the country (NCAH 2020). Between 1998 and 2019, a total of 43 outbreaks of ND were reported, averaging 2 outbreaks annually (NCAH 2020). In some of the outbreaks, the diseases had caused 100% mortality (personal field observation) causing the huge economics losses to the poultry farmers. As part of initiatives to combat ND, government had introduced thermostable I-2 NDV vaccine with support from Australian Centre for International Agriculture Research (ACIAR) (MoA 2001). The vaccine was recommended to use in the field after it was found to elicit sufficient immunity in both on-station and field trails (Raika 2007). Even now, there is continuous effort by the government to have more coverage through training of the poultry farmers and supply of vaccinated pullets from government farms. However, due to geographical terrain and scattered farms, not all poultry birds were vaccinated against the disease. In current scenario, more focus for the vaccination is given to the improved breeds leaving the indigenous chicken of rural pockets expose to diseases. Moreover, with changing of government policy from sustenance farming to commercial scale farming along with the provision of the subsidy package, there are many farmers taking up the poultry enterprise making the task more difficult resulting into frequent outbreaks.

Despite all the challenges and the frequent outbreaks, so far, no systematic studies had been conducted to understand about the disease. Therefore, we conducted a case-control study to identify the common risk factors leading to outbreaks of ND in Bhutanese farm managemental settings.

2. MATERIALS AND METHODS

2.1 Study area

The study was conducted in two villages (Chhimoong and Mecuri) under Pemagatshel district in eastern part of Bhutan (Figure 1). The district has a total area of 517.8 km ^{2} and 2547 households with a elevation that ranges from 1000m to 3500m above the sea level. Chhimoong gewog (sub-district) in which Chhimoong village is connected with 65km farm road from district headquarter. The sub-district has approximately 204 households, mostly depending on agriculture and livestock farming for living. Similarly, Mecuri village under Dungmaed gewog is about 85km from district headquarter, with people depending on agriculture and livestock for their livelihood.

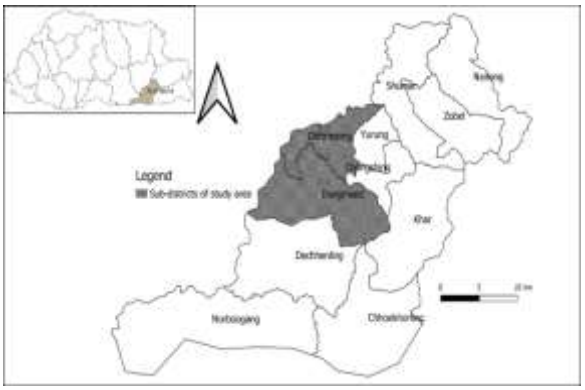

Figure 1: Map of Pemagatshel district (dzongkhag) showing two sub-districts (gewogs) in which the study was conducted

2.2 Study design

A farm level retrospective matched case-control study design was implemented to address the objectives of the study. In the village, a backyard farms that experienced ND outbreak in 2016 were selected as case whilst two farms in the same village that have not experienced any outbreaks were randomly selected as controls. Only those cases that were diagnosed during field investigations though history, clinical signs and post mortem, and later confirmed by rapid test and real time polymerase chain reaction (rT-PCR) at National Centre of Animal Health (NCAH) were included in the study. Initial information of the cases was gathered from the database maintained at Regional Livestock Development Centre (RLDC), Kanglung which were further verified during field visits with owners and livestock

officials of district and sub-districts. In case of controls, nearest households to the affected households that own a poultry farm but had not experienced any death or clinical signs due to ND selected as control.

2.3 Data collection

The data from both the cases and control farms were collected using same structured questionnaires. The questionnaires after designing were piloted with seven poultry farmers from Chhimoong village and necessary changes were incorporated to improve its applicability. The final survey questionnaires consisted of three parts. Part I consisted of socio-demographic characteristic of the respondents such as age, gender, education levels, occupation and number of family members of respondents. Part II consist of managemental, husbandry and biosecurity practice of the farm such as types of breeds, flock size, location of farms, feeding and watering, regular cleaning, status of vaccination, availability of food dips, mixing with the wild birds, isolation of sick birds, personal hygiene and accessibility of farm to the visitors. Part III consisted of environmental variables that were observed in and around farm vicinity that were suspected/considered to be risk factors for ND (e.g., presences of bushes, tall trees around farms). Data were collected though the face- to-face interviews by the lead researcher from each case and randomly selected control households in the month of February and March, 2017. The survey interviews were conducted mostly with the respondent dealing with the poultry and from those that were more than 18 years of age. The respondents were clearly explained on the objective of the study and oral consent for the participations were obtained before the survey.

2.4 Data management and analysis

The questionnaire survey data collected from the farm owners were entered into a database developed in EpiInfo software version 7.2.3.1 (CDC 2019). The data were then extracted into Microsoft excel 2013 (Microsoft Excel, Redmon, WA, USA) and check for any errors before final analysis. Data were analyzed in STATA version 14.0 (Stata Corp LP, USA). Descriptive statistics were determined by calculating the percentage, frequency, means, medians and ranges. For logistic regression, those continuous variables were converted to categorical data using the median (e.g., farm size, distance of farm from road). With ND outbreak status (Case Vs control) as outcome variables, univariable and multivariable analysis were performed for all the socio-demographic characteristics, management, farm biosecurity, husbandry practices, and environmental factors. All those variables that have P value of $\leq .20$ and considered to be biologically plausible were included for the Multivariable analysis. A forward stepwise method of mixed-effect multiple logistic regression analysis was performed to identify risk factors for ND occurrence. Final model is selected based on the $P < .05$.

3.RESULTS & DISCUSSIONS

In this case-control study, a total of 30 cases and 60 controls farms were invited to participate. All thirty cases and sixty control households, individually matched with cases (1:2) on village, year and disease outbreak status were enrolled. The mean (SD) age (years) of the respondents in case and control farms were 46.2 (13.6) and 47.9 (13.6) years respectively. The respondents were mostly female in both cases (n=16, 53.3%) and control farms (n=31, 51.7%). Similarly, majority of respondents were farmers- cases (n=26, 86.7%) and control (n=59, 98.3%), with no education or education less than primary levels (Table 1). During the time of outbreaks of ND, every farm owns an average of 14 birds. (Range:1 to 52). The affected farms reported the death of 12 birds on average (Range: 0 to 52 birds) during ND outbreaks.

From the total of 48 variables that were fitted for risk factors analysis in the logistic regression model, nine variables were found to be associated with ND outbreaks, given the cutoff P value at $\leq .20$. The model showed that cases were significantly more likely to have ND outbreaks when flock size of the farms were more than 10 birds (P=.154), location of the farms were near to road (P=.209) and poultry farms located near to owner's house (P =.154). Similarly, likelihood of more outbreaks was observed in the farms that keep all age of bird together, poultry birds are accessible to wild birds and poultry birds of nearby households, feeding of kitchen waste and keeping of the working farm equipment near the poultry shed. However, good managemental practices such as regular removal of litters from

Table 1: Socio-demographic characteristic of cases and control farms enrolled in the case-control study of risk factors for Newcastle disease outbreaks in Pemagatshel district

Characteristics	Case (N1=30) n1(%)	Control (N2=60) n2 (%)
Age (years)		
<20	0(0.0)	0(0.0)
20-29	3 (10.0)	8(13.3)
30-39	3(10.0)	5(8.3)
40-49	9(30.0)	19(31.7)
50-59	9(30.0)	13(21.7)
60-69	3(10.0)	13(21.7)
≥70	2(6.7)	2(3.3)
Mean (SD)	46.2 (13.6)	47.9 (13.6)
Gender		
Female	16 (53.3)	31 (51.7)
Male	14 (46.7)	29 (48.3)
Education level		
Non-educated (≤ primary level)	25 (83.3)	47 (78.3)
Educated (> primary level)	5 (16.7)	13 (21.7)
Occupation		
Farmer	26 (86.7)	59(98.3)
Business	1 (3.3)	0 (0.0)
Government job	2 (6.7)	0 (0.0)
Student	1 (3.3)	1(1.7)
Sub-District		
Chhimoong	15 (50.0)	30 (50.0)
Dungmead	15 (50.0)	30(50.0)

the poultry shed were found to be a protective factor for ND outbreaks (Table 2).

The multivariable logistic regression model showed that mixing of the poultry birds with the wild birds was important risk factors in the occurrences of ND outbreak in the two-study area. Many studies had similar findings in which wild birds were reported to be important risk factors during the ND outbreaks in which they act as sources of NDV virus for domestic poultry (East et al. 2006; Marks et al. 2014; Ravishankar et al. 2022). At least 241 species of birds were reported to be infected with NDV, making wild/migratory birds important reservoir of the virus (Aldous et al. 2010). Although, the NDV virus isolated from feral birds are said to be of low virulence (Alexander 2000), the virulent form of virus has been reported sporadically (Perttula 2010).The wild birds like cormorants, pigeons and psittacine birds were reported to be commonly infected with virulent virus and serve as source for the domestic poultry .(Ravishankar et al. 2022). These birds were abundantly found in Bhutan and can mix with domestic poultry birds since most of the poultry farms are in rural areas of Bhutan where poultry is reared in locally constructed sheds/ceiling of house in which there are no biosecurity measures. In two villages of our study areas, most of the poultry birds were found to be kept in the house ceiling during night or small traditional local shed made of bamboos. During the day time, the poultry birds were let to freely scavenge in the field where they search for their own food and water (Tenzin et al. 2017).

Flock size of farm was found to be another important risk factor in the occurrences of the ND outbreaks in the study area. It was observed that the farmers owing the larger flock size are more likely to get the infection than the farms owing the smaller flock. In the farms where there is a greater number of poultry birds, the chances or frequency of the direct contact between the infected and healthy birds increases which helps in the spread of infection from one bird to another. Larger flock also covers a considerable space in search of food and water (Tenzin et al. 2017). Similarly, significantly higher odds (adjusted odd ratio: 10.7, 95% CI: 2.3-49.5) of ND outbreaks were observed in farm that are having larger flock size (>10 poultry birds) comparing to smaller farm (≤10 poultry birds). In the farms where there is a greater number of poultry birds, the chances or frequency

Table 2: Univariable logistic regression analyses of risk factors associated with Newcastle disease outbreaks in Pemagatshel district

Variables and categories	*b*	SE	OR (95%CI)	P value
Flock size of the farm				
Small (≤10 birds)	0		1	
Large (>10 birds)	0.657	0.461	1.9(0.8-4.8)	0.154
Keep bird of different age together				
No	0		1	
Yes	1.366	0.669	3.9(1.1-14.6)	0.041
Accessibility of wild birds to poultry				
No	0		1	
Yes	1.075	0.47	2.9(1.2-7.4)	0.022
Regular removal of litters from shed				
No	0		1	
Yes	-0.646	0.457	0.5(0.2-1.3)	0.158
Feeding of kitchen waste to poultry birds				
No	0		1	
Yes	0.71	0.457	2.0(0.8-5.0)	0.121
Mixing poultry birds with poultry birds from other households				
No	0		1	
Yes	0.939	0.469	2.6(1.0-6.4)	0.045
Keep farm equipment near poultry shed				
No	0		1	
Yes	0.956	0.54	2.6(0.9-7.5)	0.077
Location of farm from road				
Far (≥500 meters)	0		1	
Near (<500 meters)	0.572	0.455	1.8(0.7-4.3)	0.209
Location of farm from owner's house				
Far (≥6 meters)	0		1	
Near (<6 meters)	0.657	0.461	1.9(0.8-4.8)	0.154

of the direct contact between the infected and healthy birds increases which helps in the spread of infection from one bird to another. Larger flock also covers a considerable space in search of food and water (Tenzin et al. 2017), which increases a risk of coming in contact with infected sources such as wild birds, inanimate objects or droppings from the infected birds. The odd of ND outbreak occurrences in the farm located near the road (500 meters) were 3.9 (1.0-15.6) times more than those poultry sheds/farms located far ((≥500 meters) from road. It may be due to the fact that the virus may be carried by inanimate objects like equipment and vehicles from the infected farms (Martin & Spradbrow 1991). In the village settings, most of the household utensils and equipment are brought from market or nearby towns. Poultry products are also traded by the local traders between the village and the towns which has the potential to carry and spread the NDV. Although both of our study villages are located in remote areas, both of these villages were connected with road with reports of frequent movement of vehicles to other places. Farmers also reported frequent purchase of the poultry products like meat and eggs. Moreover, one of our study villages is located on the highway between border town and inner part of district from which most of the traders move. The practices of regular cleaning of the litters were found to be protective factors (AOR=0.2, 95% CI=0.0-0.7, P=.010) for the ND outbreaks. In the epidemiology and transmission of the diseases, the shedding of the virus in the feces from the infected birds and its survivability plays a very important role. It is reported that the Newcastle diseases virus can survive for about three months at 20 to 30° (Lancaster 1966) providing the favorable environment for the virus. Any healthy birds that come in contact with the infected feces or litter materials will pick up the infection. The litters materials containing virus can also be transmitted by rodents, insects, dogs and other scavenging animals to other uninfected farms.

Table 3. Multivariable logistic regression analyses of risk factors associated with Newcastle disease outbreaks in Pemagatshel district

Variable and categories	Adjusted OR (95% CI)	P Value
Accessibility of wild birds to poultry		
No	1	
Yes	13.08 (2.96-57.78)	0.001
Flock size of the farm		
Small (≤10 birds)	1	
Large (>10 birds)	10.66 (2.3-49.50)	0.003
Location of the farm from road		
Far (≥500 meters)	1	
Near (<500 meters)	3.85 (0.95-15.63)	0.059
Regular removal of litters from shed		
No	1	
Yes	0.16(0.04-0.66)	0.012

Likelihood ratio test: 36.08; Chi square: <0.001

The probability of mechanical spreading of the virus through inanimate objects like boots, farm equipment, clothing is also increases (Ullah et al. 2004).

4. CONCLUSIONS & RECOMMENDATION

In order to prevent the outbreaks for the ND in backyards farms, particularly in rural areas of Bhutan, there is need for improving the managemental and biosecurity practices by poultry owners. The litters from the poultry shed need to be clean regularly and barriers need to be constructed to prevent the entry of wild birds into the poultry farms. Since it is difficult to adopt such good management and biosecurity practice in the traditional ways of raising the chickens, awareness needs to be given to farmers on benefits of raising chickens in the proper shed. In the rural setting, where access to markets is limited, and knowledge of farmers are low, owning a farm with smaller flock size will be more beneficial in terms of less disease outbreaks. Moreover, in order to reduce the incidences of ND outbreaks, farms should be constructed away from the road.

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