

Original Article

Seroprevalence Investigation of Newcastle Disease in Rural Poultry of the Northern Provinces (Golestan, Gilan, and Mazandaran) of Iran

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ABSTRACT

Rural poultry farming is common in the Northern provinces. Similar to commercial poultry, rural poultry is susceptible to most infectious diseases. In addition, by increasing the density of poultry farming, the probability of disease incidences has been increased. Newcastle disease is the most highly infectious disease which is endemic in Iran and causes outbreaks among commercial and rural poultry every year. The present study aimed to investigate the prevalence and virus circulation of Newcastle disease among rural poultry in Northern provinces of Iran. In the current study, 70 villages in 3 provinces (20, 30, and 20 villages in Mazandaran, Golestan, and Gilan, respectively) and a total of 1,374 birds (600, 400, and 374 birds in Mazandaran, Golestan, and Gilan, respectively) were sampled. Each village was regarded as an epidemiological unit. In the present study, birds of 67 (96%) villages were positive (presence of antibodies against Newcastle disease virus), including 28 (93.3%), 19 (95%), and 20 (100%) villages in Golestan, Mazandaran, and Gilan, respectively. Moreover, out of 1,374 birds, 616 (45%) of them were seropositive against Newcastle disease virus with 242 (41%), 159 (39.8%), and 211 (56%) samples in Mazandaran, Golestan, and Gilan, respectively. According to the results of the current study, the seroprevalence rate was reported to be high in both villages and birds. Such a high seroprevalence rate was indicative of the continuous exposure of the rural poultry to Newcastle virus and high virus circulation rate in the mentioned provinces which could result in the dissemination of the disease to commercial farms. Consequently, the implementation of proper control and care programs (e.g., vaccination of native poultry) can facilitate the reduction of Newcastle disease prevalence.

Keywords: Seroprevalence, Newcastle disease, Poultry, Northern provinces, HI

Une enquête sur la séroprévalence de la maladie de Newcastle chez des volailles rurales dans les provinces du nord de l'Iran (Golestân, Gilân et Mâzandarân)

Résumé: L'aviculture rurale est courante dans les provinces du nord. Semblable à la volaille commerciale, la volaille rurale est sensible à la plupart des maladies infectieuses. De plus, en augmentant la densité de l'élevage de volaille, la probabilité de l'apparition de maladies est augmentée. Le Newcastle est l'une des maladies les plus hautement infectieuses et fortement endémique en Iran provoquant chaque année des épidémies chez les volailles commerciales et rurales. Cette étude visait à étudier la prévalence et la circulation du virus de la maladie de Newcastle chez les volailles rurales dans les provinces du nord de l'Iran. A cet effet, 70 villages répartis sur les 3 provinces (20, 30 et 20 villages de Mâzandarân, Golestân et Gilân, respectivement) et un total de 1,374 oiseaux (600, 400 et 374 oiseaux à Mâzandarân, Golestân et Gilân, respectivement) ont été

échantillonnés. Chaque village était considéré comme une unité épidémiologique. Dans la présente étude, les oiseaux de 67 (96%) villages étaient positifs (présence d'anticorps dirigés contre le virus de la maladie de Newcastle), dont 28 (93,3%), 19 (95%) et 20 (100%) des villages de Golestân, de Māzandarān et de Gīlān, respectivement. De plus, sur 1,374 oiseaux, 616 (45%) d'entre eux étaient séropositifs pour le virus de la maladie de Newcastle avec 242 (41%), 159 (39,8%) et 211 (56,4%) échantillons à Māzandarān, Golestân et Gīlān, respectivement. Selon les résultats de notre étude, le taux de séroprévalence était élevé chez les oiseaux des différents villages étudiés. Un taux de séroprévalence aussi élevé est révélateur de l'exposition continue de la volaille rurale au virus de Newcastle et du taux élevé de circulation du virus dans les provinces susmentionnées, ce qui pourrait entraîner la dissémination de la maladie dans les fermes commerciales. Par conséquent, la mise en œuvre de programmes de contrôle et de soins appropriés (par exemple, la vaccination des volailles indigènes) pourrait faciliter la réduction de la prévalence de la maladie de Newcastle.

Mots-clés: Séroprévalence, Maladie de Newcastle, Volaille, Provinces du nord, HI

INTRODUCTION

Rural poultry farming constitutes a large portion of villagers' income in the Middle Eastern countries. Moreover, poultry products (e.g., meat and egg) which are the main source of protein are produced through poultry farming in these countries. In Iran, rural poultry farming is popular and a part of people's income and their way of producing protein in villages. Based on the country statistics, almost 46 million pieces of rural poultry are produced annually (Abraham-O.J 2014). A large part of the production takes place in North of Iran, including Mazandaran, Golestan, and Gilan provinces, respectively. These provinces due to their mild climate and green pasture are suitable for poultry farming in both rural and industrial regions. Only a few houses, especially in rural parts of these regions, can be found empty from poultry or other birds. Similar to commercial poultry, rural poultry are also susceptible to the most infectious diseases (Awan et al., 1994). In addition, by increasing the density and extent of poultry farming, the probability of disease occurrence has been increased. Newcastle disease is one of the best-known diseases. It is a highly contagious viral disease caused by paramyxovirus type 1 (Wang et al., 2008). This virus could infect more than 200 bird species (Rezaeianzadeh et al., 2011). Newcastle disease is currently one of the most prominent poultry diseases

from economic point of view considering that it results in a widespread loss within a short period of time (Awan et al., 1994; Alexander D. J., Senne D. A., 2008). Newcastle disease virus is endemic in Iran and causes scattered outbreaks in commercial and rural poultry in the country every year. Since rural poultry are a source for maintaining, spreading, and transferring the virus to commercial poultry, the present study aimed to investigate the seroprevalence of Newcastle virus among unvaccinated rural poultry in the Northern provinces of Iran.

MATERIAL AND METHODS

The Studied Area. Geographical areas studied in the present study were Northern provinces including Golestan, Mazandaran and Gilan provinces of 58 167 sq. Km totally. (Figure 1). The mentioned provinces were surrounded between Caspian Sea and Elburz mountain range. They extend to Caspian Sea from North, to Turkmenistan and the Northern Khorasan province from east and northeast, to Armenia and Ardabil province from west and Northwest, and to Alborz Mountains from the south. Climate of Northern provinces is mild and humid. The area includes many rivers, wetlands and swamps (Iran's Provinces Atlas of Astronomy, M., 2004). In various parts of mentioned provinces, there are local daily live bird markets to

trade poultries, so that there is a continues moving of birds among different regions.



Figure 1. the studied area to review Newcastle prevalence (Golestan, Gilan, and Mazandaran provinces) in 2014-15 (The provinces of Northern Iran show with green color).

Furthermore, because of climate conditions, there is a yearly migration of different kind of birds into these provinces (Waterfalls of Iran., 2010). In addition almost 48.98 % of meat breeder farms and 19.85 % of meat chickens farms of the country are located in the Northern provinces (table 1).

Table 1. The frequency and relative frequency of broiler breeder farms (Broiler Chicken and hens in Northern provinces in 1394 year

province	Number of broiler farming	Hens laying	Broiler breeder
Mazandaran	2335 (11.6%)	30 (1.8%)	208 (30.14%)
Gilan	770 (3.84%)	1 (0.06%)	77 (11.16%)
Golestan	885 (4.4%)	19 (1.17%)	53 (7.68%)
total	3990 (19.87%)	50 (0.31%)	338 (47.98%)
total in country	20078	1623	690

Study design. In the present study the Population of target was rural poultries in Golestan, Gilan, and Mazandaran provinces respectively. The study was conducted cross- section ally and from the beginning of 2014 to the end of 2015 in the population of target.

The required number of villages for sampling was chosen, in the form that according to 70 percent prevalence and with 95 percent of confidence, at least one positive serum village could be found in each province (Onapa et al.,2006). In addition, the number of the required birds in order to sample serum

determination in each village was chosen, in a way that by considering Seroprevalence equal or more than 25 percent, and with 95 percent confidence, at least one positive bird could be identified (Salihu et al.,2012). Sampling was done in villages where there was no vaccination history against Newcastle virus at least in a year and a half before the study, according to declaration of veterinary organization. The villages were selected randomly.

The laboratory test. One mL of blood was collected from the wing vein of each bird using a 2.5 mL syringe. To facilitate serum separation, the Blood samples were left at room temperature for an hour. After transferring the samples to the laboratory, the serums were separated, collected in 1.5 ml micro-, and then, the recorded serum samples, stored at -20 °C freezer up to the time of testing. On serums samples, hem agglutination inhibition (HI) test was conducted for evaluation of serum response of immune system of bird in the possible exposure to the Newcastle viruses. HI test was done according to OIE instruction using 4-unit anti- gen made by Razi institute. Titer 3 or higher was considered as positive based on log2 (Alexander., 2008; Allan et al., 1974; OIE et al., 2009). Villages having one positive serum bird were considered as positive. In addition, towns having at least one positive serum village were considered as positive.

Data Analysis. To analyze the data for quantitative data description, Mean, standard deviation, and distribution of serum titers were considered. In addition, to compare the rate of infection among various provinces, Chi-square test, at the significance level of $p < 0.05$ was used as statistical significance level. Data analysis was done using SPSS software, version 22.

RESULTS

In total, 70 villages in 3 provinces (20 villages in Mazandaran, 20 villages in Golestan and 30 villages in Gilan Province respectively) consist of 1374 birds (600 birds in Mazandaran, 400 birds in Golestan, 374 birds

in Gilan Province respectively) were sampled. Out of 70 sampled villages, 67 villages (96%), and out of 1374 birds, 616 (45%) were positive serum. All the towns studied in the three provinces had at least one infected village. Frequencies of villages and obtained samples for each separate province have been represented in table 2.

A) Mazandaran Province. Out of 20 villages, 19 villages (95 %) were seropositive. Moreover, out of 600 obtained samples of birds, 246 samples (41%) were positive serum. The highest rate of seroprevalence among the villages of Mazandaran province was 80%. meanwhile just one village were found negative The highest frequency of seropositive titer in the province was titer 4 that included 70 positive serum samples out of 246 samples (28.45%). Frequency of the obtained samples and the related titer for each village has been shown in table 3.

B) Golestan Province. Out of 30 sampled villages, 28 villages (93.3 %) were seropositive. Moreover, out of 400 serum samples, 159 samples (39.8%) were positive. The highest rate of Seroprevalence among the villages of Golestan province also was 80%. Moreover, only one village were found negative in this province.. The highest frequency of positive serum titer in the province was titer 4 that included 43 positive serum samples out of 159 samples (27.04%). Frequency of the obtained samples and the related titer for each village has been shown in table 4.

C) Gilan Province. In this province, all 20 sampled villages (100%) were positive serum and no village remained free from Newcastle disease virus infection. In addition, out of 374 total taken serum samples, 211 samples (56%) were positive. The highest rate of

seroprevalence was 94.4%, meanwhile the lowest rate of was 18.7%. The highest frequency of positive serum titer in the province was titer 5 that included 47 positive serum samples out of 211 samples (22.27%). Frequency of each serum titer has been presented in table 5. Considering the villages as a statistic unit, there is no significant difference in the rate of the villages that were found positive of infection among these three provinces ($P>0.05$). However the number of birds that were found positive in Gilan province was significantly higher than two other provinces, Mazandaran and Golestan respectively ($P>0.05$). No significant difference was observed in number of birds that were found positive between Mazandaran and Golestan ($P>0.05$).

DISCUSSION

The north of Iran has several important roles in epidemiology of Newcastle disease viruses. For migratory birds, these areas are main places for wintering. Given that these birds are as a reservoir and host of Newcastle viruses therefore the northern regions are should be considered as the main entering root of the viruses into the country. Regardless of how the virus enter the country according to the size and density of rural birds which are kept free in the place so that the north of the country could be consider as a place for increasing proliferation of the Newcastle viruses. If the dynamics of trade and transport of domestic birds with other regions of the country be considered as well as the importance and effect of dense proliferation of Newcastle viruses in the north to other parts of the country could be better understood. Perhaps the presence of High density of industrial

Table 2. Frequency and relative frequency of town Villages and bird sampling and seropositive (HI) for Newcastle disease in the northern provinces of Iran in 1394

Number	province	Number of city taken sample	The number of seropositive	Sampled villages	Village of seropositive	Number of birds sampled	The number of seropositive birds
1	Mazandaran	15	15(100%)	20	19(95%)	600	246(41%)
2	Gilan	16	16(100%)	20	20(100%)	374	211(56%)
3	Golestan	12	12(100%)	30	28(93%)	400	159(40%)
	total	15	15(100%)	20	19(95%)	600	246(41%)

poultry farm in the north regions has the biggest role in the epidemiology of Newcastle viruses. Almost thirty percent of bird's meat is produced in the north; furthermore about sixty percent of day old chicks are produced in these provinces and exported to other part

viral disease in this region could have a decisive effect on the outbreak and widespread Newcastle virus not only in north of the country but also across the country. This claim can be proved only by showing the phylogenetic relationship between viruses of regions of

Table 3. The frequency of samples taken (the percentage of seropositive and serum titration (HI) for Newcastle disease in Mazandaran province with separation each villages in 1393-94 year

No.	village	Taken samples	Number of bird										Positive serum	Percentage seropositive		
			Titer 0	Titer 1	Titer 2	Titer 3	Titer 4	Titer 5	Titer 6	Titer 7	Titer 8	Titer 9			Titer 10	
1	Ebrahimabad	15	8					1	5		1				7	%46
2	Eskardin	40	29		1	3	1	4	2						10	%25
3	Anbarsar	10	6		1	1	1	1							3	%30
4	Bereshi	10	6			1	3								4	%40
5	Cheftsar	2	2												0	%0
6	Hajiabad	25	11		2	5	6	1							12	%48
7	Khairood	10	3			2	3	1			1				7	%70
8	Dehmian	50	28		2	10	8	2							20	%40
9	Rostamkola	50	12			1	5	10	6	5	7	3	1		38	%76/6
10	Zarinabadsoufla	10	4					1	2	2	1				6	%60
11	Zorijan	8	3					1	2	2	1				5	%50
12	Abbasa	50	35		2	7	6								13	%26
13	firozabad	50	21				10	8	5	6					29	%58
14	Kelodeh	50	29		2	7	5	4	1	2					19	%38
15	Geramjan	50	36		3	6	5								11	%22
16	Momenabad	50	21			5	7	11	4	2					29	%58
17	Narenjben	10	5			1	2	1		1					5	%50
18	Nodeh	10	2			4	4								8	%78
19	Nimchahee	50	35		2	8	3		2						13	%26
20	Hashtikeh	50	43		1			4	2		1				7	%14
total		600	338	0	16	62	70	54	24	22	10	3	1		246	%41

of the country. According these, any disease occurrence and outbreak in industrial poultry has directly great consequences on the other part of Iran. According to the results of the study, the rate of Seroprevalence is high at the levels of both villages and birds. Such a rate of Seroprevalence is indicative of multiple exposures of rural poultry to Newcastle virus and continues circulation of the virus in the mentioned provinces. Several factors contribute to the high rate of prevalence including lack of vaccination, unavailable cold chain facilities multi-age of birds, very poor knowledge about ND improper dwellings of rural birds, insufficient feeding, unethical disposal of dead birds in premises and ignorance to other basic farming utilities (Khan et al., 2011). Most of these factors have been occurred in the Northern provinces. West regions of the north have more density breeding poultry due to favorable environment conditions. Therefore any occurrence of

the country. Shoushtari and colleagues have shown this phylogenic connection so that the virus isolated in the provinces of Tehran, Alborz, Hamedan province is very close to the viruses isolated from mazandaran province.

In a study conducted by Mamina et al (2007) in Madagascar and Mami , it was shown that the Newcastle virus was reason for losses in 44% of native poultries that had died in 12 months, from 1999 until 2000 (Maminiaina et al.,2007). In another study conducted by Gutiris in 2000 in Mexico, the rate of Newcastle prevalence among native poultries was 2.2 % (Gutierrez et al., 2000) .In Iran and other countries, various studies have been done regarding Seroprevalence of Newcastle among native poultries. In a study done by Rezaeean Zadeh et al (2011) on native poultries of Fars province, 69.1 % villages were positive serum. In the mentioned study, out of 1050 native poultries serum, 61.9 percent were announced to

be positive by HI serology test. Of course, they were all negative in RT-PCR test (Rezaeianzadeh et al., 2011). Although the rate of Seroprevalence in Fars province was high, it was lower than the rate in Northern provinces. In the study conducted by Hadi Pour et al

Ichroyumata, the rates of Seroprevalence among the native poultries were 47 percent and 23 percent, respectively (Kashem et al., 2011; Ichiro et al., 2010). In addition, in a study conducted by Abrahamou et al (2004) on 250 serum samples of native chickens in

Table 4. The frequency of samples taken (the percentage of seropositive and serum titration (HI) for Newcastle disease in Golestan province with separation each villages in 1393-94year

No.	village	Taken samples	Number of bird										Positive serum	Percentage seropositive	
			Titer 0	Titer 1	Titer 2	Titer 3	Titer 4	Titer 5	Titer 6	Titer 7	Titer 8	Titer 9			Titer 10
1		15	11			1		1	2					4	%26/6
2	Araztaghan	15	5	1	3				1	4	1			6	%40
3	Ashormohammadi	12	4		2	2	4							2	%16/67
4	Bazgir	15	4				1	2	6	1	1			11	%68/75
5	Basirabad	15	7			1			1	2	2	2		8	%53/3
6	Blochabadgogjeh	5	2			1	1	1						3	%60
7	Pichek mahhaleh	18	10				1	2	3	1	1			8	%44/4
8	Chaiee boein	10	5			3	2							5	%50
9	Chakaratabahlakeh	15	11					3	1					4	%26/7
10	chahardeh	10	7		2	1								1	%10
11	Ramian	15	12	2	1									0	%0
12	Sabzevariha	10	4		1		4	1						5	%50
13	Sartappeh	12	5			2	5							7	%58/3
14	Salagh	15	7					1	1	3	2	1		9	%60
15	Soltanabad	15	10					2	2	1				5	%33/3
16	Soltanali	15	11		2	1		1						2	%13/3
17	Sozesh	15	5			1	2	5	2					10	%66/7
18	Ghafarhaji	15	7			2	3	3						8	%53/3
19	Ghan germeh	15	10			3	2							5	%33/3
20	Ghorbanpeikar	15	11				1		3					4	%26/7
21	Ghare ghashli	15	10			1	1	2	1					5	%33/3
22	Kemeler	15	5			2	2	2	3	1				10	%66/6
23	Kivetmahalleh	15	12			1		1	1					3	%20
24	kohnehabadkalabad	15	11				1	3						4	%26/7
25	Gorjiabad	15	5			1	2	3	1	2	1			10	%66/6
26	Golha	15	9	1	1	3	1							4	%26/7
27	Gogjeh	13	5		1	2	5							7	%53/8
28	Mirzaali	5	4		1									0	%0
29	Nosratabad	5	2			1	1	1						3	%60
30	Nodekatol	15	9				1		1	2	1	1		6	%40
		400	220	4	14	29	43	31	29	17	9	4		159	

(2009) on native poultries of five villages around Mahar Lou Lake in Fars province that is a habitat for migratory birds, 37.5 % of samples were positive serum (Hadipour et al., 2009). This result strengthens the probability of transmission of the disease from migratory birds to domestic fowl. In the studies conducted in Pakitan by Kashim and in Bangladesh by

various areas of Nigeria, 23.6 % of the serum samples were positive (Abraham et al.,2014). In another study done by Courtecuisse et al (1990) on native poultries in Niger, 63 percent of the serum samples were positive (Courtecuisse et al., 1990). The above- mentioned studies show that the rate of prevalence of the disease in various countries and regions is different. Various

Table 5. The frequency of samples taken, the percentage of seropositive and serum titration (HI) for Newcastle disease in Gilan province with separation each villages in 1393-94 year

No.	village	Taken samples	Number of bird											Positive serum	Percentage seropositive	
			Titer 0	Titer 1	Titer 2	Titer 3	Titer 4	Titer 5	Titer 6	Titer 7	Titer 8	Titer 9	Titer 10			
1	Ardejan	20	14				3	1				2			6	%30
2	Ashkermidan	21	10				1	3	4	3					11	%52/3
3	Ayeshgah paein	17	7		1	1		1	2	1	1	3			9	%52/9
4	Bazgleh malak	20	2	1			1	5	7	4					17	%85
5	Balamahaleh sangachi	27	12		1	2	2	3	2	3	1	1			14	%51/8
6	Bialova	20	14			2	1	1	1	1					6	%30
7	Peshel	20	5		3	3	5	3		1					12	%60
8	Tazeh abad seghal mahleh	21	14				1	2	3				1		7	%33/3
9	Jamshid abad	18	9		2	2	1	1	2	1					9	%50
10	Dostkoh	21	7		1		3	3		2	4	1			4	%66/6
11	Divarsh	16	13				1	1		1					3	%18/8
12	Ziaratgah	15	3			2	4		4	2					12	%80/5
13	Abbasabad	18			1	1	3	3	3	7					17	%94/4
14	Karband salam	18	1			3	3	5	4	1		1			17	%94/4
15	Korm mahalleh	17	8			1	3	2		1	2				9	%52/9
16	Kohnesara	14	4			2	4	2		2					10	%71/4
17	Kiasar	17	9			1	3	1	2	1					8	%47
18	Lesbo	21	5				1	4	6	5					16	%76/1
19	Mileh sara	16	4			1		3		4	2	2			12	%75
20	Nefot	17	4		1	3	3	3		2	1				12	%70/6
Total		374	145	1	8	24	44	47	39	43	14	8	1		211	%56

factors play roles in spread and prevalence of the disease, some of them were studied in different researches. In Alexander's research (1973), the factor of distance and native poultries was considered as an important factor in spread and prevalence of Newcastle disease (Alexander, 2008). The same reason was investigated by Houk- Jones (1973) and it was declared as one of the prominent reasons of Newcastle disease prevalence in England from 1970 to 1971 (Hugh-Jones et al., 1973). In another study, Alexander et al knew the communication of farmers with each other as an important reason of the disease transmission to other areas. In the mentioned study, except workers, other factors like vehicles and migratory birds were mentioned as important factors of the disease spread (Alexander et al., 2003). In study of Gum et al (1999) in Sweden, the role of native birds, their products, and their displacement were recognized to be more important than the role of migratory birds in spread and of Newcastle disease (Gohm et al., 1999). The high rate of Newcastle prevalence among native poultries of Northern villages of the country is a potential threat for poultry industry in the mentioned provinces and even

for the country. As the greatest number of breeder chicken farms and incubation factories of Country are located in the mentioned provinces and the pollution of poultries can lead to incidence of outbreaks among commercial poultries.

According to the results of the present study, due to the high density of poultry production in Northern provinces on one hand, and the presence of native and migratory birds and numerous markets selling live poultry in the provinces, on the other hand, the rate of Newcastle prevalence and virus circulation in these areas are very high. Because of importance of native poultries that are considered as the source of the disease and are able to spread the disease to industrial farms, conduction of appropriate plans of care and disease control, including vaccination of native poultry is urgent. In addition, complementary studies to review epidemiology of the disease and to determine the risk factors as well as the condition of virus circulation among native poultries in order to provide control and prevention programs in native poultries are essential.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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