### **Scientific Report**

# Seroepidemiology of Newcastle disease in domestic village chickens of plain areas of Isfahan province, central Iran

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#### **Summary**

Newcastle disease is one of the most important diseases of poultry. It usually causes a great loss in poultry industry and domestic village chickens. Since domestic chickens in villages are free ranging for food, the chance of their contact with wild birds that may act as reservoirs is high. To determine the role of domestic chickens in the epizootiology of Newcastle disease virus in villages of Isfahan province, 400 serum samples from chickens with no history of vaccination from four regions (Khomeinishahr, Zarinshahr, Falavarjan and Mobarekeh) were collected in summers of (1998) and (1999). Haemagglutination inhibition (HI) test was used for titration of antibodies against Newcastle disease virus. Chi-square and binomial tests were used for statistical analyses. 69.5 and 68.5% of the sera were positive in the two consecutive seasons (P>0.05). About 25% of 3–4 month- and 1–2-year-old chickens were negative for HI antibodies and thus were sensitive to the disease in each season. Significant correlation was observed between the HI antibody titers and the age of the chickens (P<0.01). A specific pattern of seroconversion was observed which was independent to the prevalence of the disease in industrial poultry flocks in each region and all regions studied. It was concluded that about 35% of the domestic village chickens are protected against virulent strains of Newcastle disease in summer. For protection of the remaining chickens, routine vaccination, especially in spring and summer is suggested.

Key words: Domestic village chickens, Seroepidemiology, Newcastle disease, Iran

### Introduction

Newcastle disease is one of the most important viral diseases of poultry in Iran. It is an endemic and sometimes epizootic disease in chickens. It causes a great loss in domestic village chickens which are one of the main sources of animal protein in developing countries and also in poultry industry (Spradbrow, 1994; Gutierrez-Ruiz *et al.*, 2000; Oakeley, 2000; Thekisoe *et al.*, 2004). This is, most probably, true in Iran too.

Wild and domesticated birds sometimes harbour the Newcastle disease virus (NDV) while showing no detectable clinical signs of the disease (Lancaster, 1964). These strains of the virus require several passages in chickens before becoming highly virulent for chickens (Alexander and Parsons, 1984). Recent findings suggest that virulent virus may emerge in poultry as a result of mutations in viruses of low virulence (Alexander, 2001). As village chickens have more contacts with these birds, they may play a role in amplifying the virulence of the field viruses. The persistence of V4 strain under conditions which simulated husbandry of village chickens in Malaysia is already reported by Samuel (1987). Therefore, village chickens are probably very important for survival of NDV in the environment and may also play an important role in the spread of the virus among industrial poultry flocks. This proved to be true in high mountainous altitudes and areas of

Shahrekord (Chahar-Mahal va Bakhtiari) province of Iran where specific pattern of seroconversion was observed which was independent to the prevalence of the disease in industrial poultry flocks. In the area studied, 42.3% of the chickens tested were seropositive and 71.1% were susceptible (HI titers of less or equal to 2 log base 2) to the infection (Bouzari and Mousavi, 2002).

To determine the presence of NDV, regardless of its pathotype, the pattern of seroconversion in domestic village chickens and their role in the spread of NDV, 400 domestic village chickens were examined for the presence of antibodies against the virus in two consecutive summers of (1998) and (1999) in plain areas of Isfahan province, central Iran.

## Materials and Methods

Four hundred domestic village chickens from four plain areas of Isfahan province including Zarinshahr, Mobarekeh, Falavarjan and Khomeinishahr, with no history of vaccination were randomly selected and their sera were examined for the presence of antibodies against NDV by haemagglutination inhibition (HI) test in two consecutive summers of (1998) and (1999). The age of the chickens was estimated approximately.

One to 1.5 ml of blood, after collection from brachial vein, was allowed to clot and after handling to the laboratory, kept at 37°C for 2–3 hrs. The serum was decanted, centrifuged and stored at -20°C till tested.

All sera were tested using micro HI test (Allan and Gough, 1974) with some modifications. Serial two-fold dilutions of sera were reacted with 4 haemagglutination (HA) units of B1 strain of NDV (Razi Institute, Karadj, Iran) for 20 min at room temperature. Chicken red blood cells (RBC) (1% suspension) were added and the test was read after an additional 45 min. The last dilution with complete inhibition of HA was recorded as the HI titer of the serum. Controls of positive serum of known titer, negative serum, RBC and serum itself were included. The results were expressed in log base 2 notations. Chi-square and binomial tests were used for statistical analyses.

# Results

The frequency of different HI titers in different areas in summers of (1998) and (1999) are shown in Tables 1 and 2. Totally, 50 chickens were examined in each area in each year and HI titers of up to 5 log base 2 were observed.

The frequency of chickens with positive HI titers in different areas and years are shown in Table 3. Significant differences between the positive and negative cases among different areas were observed (P<0.05). The distribution of positive cases in different areas in two consecutive summers was not the same (P<0.05). Significant differences of positive cases were observed among different areas studied (P < 0.05). The changes in positive cases in two consecutive summers in Falavarjan was significant (P<0.05), but this was not the case for other areas studied (P>0.05). In summer of (1998), except for Mobarekeh (P>0.05), the difference between positive and negative cases were significant in Zarinshahr (P<0.05), Falavarjan (P<0.01) and Khomeinishahr (P<0.01). In summer of (1999), the difference in Mobarekeh and Falavarjan was not significant (P>0.01), while it was significant in Khomeinishahr and Zarinshahr (P<0.01).

As 3 (log base 2 notation) is regarded as the minimum titer that will ensure protection against most strains of virulent NDV (Allan and Gough, 1974; Balla, 1986), chickens with HI antibodies  $\leq 2 \log base 2$  were considered as susceptible in exposure to field highly-pathogenic strains of the virus. Frequency of susceptible chickens in different areas and years studied are shown in Table 4. Susceptible chickens in 3-4month-old chickens had no significant difference in two consecutive summers (P>0.05). This was the same for 1-2-yearold chickens (P>0.05). In each summer, no differences were significant observed between the two age groups (P>0.05). Totally, no significant differences were observed between all chickens examined in two consecutive summers (P>0.05).

According to records of the headquarter of Veterinary Organization of Isfahan province, Newcastle disease was reported in all areas and seasons studied (Table 5).

### Discussion

Significant correlation was observed between HI antibody titers and age of the chickens (P<0.01) which is consistent to the

results reported by Samuel (1987). The higher antibody titers in a high percentage of the 1–2-year-old chickens in Zarinshahr, Falavarjan and Khomeinishahr indicate the more possibility of exposure with the field viruses.

Area	Titer <sup>a</sup>	3–4 N	Ionths	1-2	Years	Total	
	-	$\mathrm{F}^{\mathrm{b}}$	%	$F^{b}$	%	-	
Zarinshahr	0	4	8	C	-	50	
	1	9	18	-	-		
	2	6	12	-	-		
	3	14	28	-	-		
	4	11	22	-	-		
	5	6	12	-	-		
Mobarekeh	0	-	-	19	38	50	
	1	-	-	11	22		
	2	-	-	11	22		
	3	-	-	3	6		
	4	-	-	6	12		
Falavarjan	0	37	74	-	-	50	
·	1	13	26	-	-		
Khomeinishahr	0	-	-	3	6	50	
	1	-	-	6	12		
	2	-	-	5	10		
	3	-	-	10	20		
	4	-	-	20	40		
	5	-	-	6	12		

Table 1. Frequency of fit titers in university areas in summer of (199	Tab	ble 1	1:	Freq	uencv	of HI	titers	in differen	t areas i	n summer	of	(1998
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a = HI titers log base 2, b = Frequency, c = No cases

#### Table 2: Frequency of HI titers in different areas in summer of (1999)

Area	Titer <sup>a</sup>	3–4 Months		1-2	Years	Total
	-	$F^{b}$	%	F <sup>b</sup>	%	
Zarinshahr	0	3	6		-	50
	1	18	36	-	-	
	2	10	20	-	-	
	3	-	-	9	18	
	4	-	-	6	12	
	5	-	-	4	8	
Mobarekeh	0	30	60	-	-	50
	1	-	-	-	-	
	2	14	28	-	-	
	3	4	8	-	-	
	4	2	4	-	-	
Falavarjan	0	-	-	21	42	50
	1	-	-	9	18	
	2	-	-	8	16	
	3	-	-	12	24	
Khomeinishahr	0	7	14	-	-	50
	1	3	6	-	-	
	2	19	38	-	-	
	3	20	40	-	-	

a = HI titers log base 2, b = Frequency, c = No cases

Year	Za	Zarinshahr		Μ	Mobarekeh		Falavarjan			Khomeinishahr			Total
	F	Т	%	F	Т	%	F	Т	%	F	Т	%	
1998	46 <sup>a</sup>	50	92	31	50	62	13 <sup>*b</sup>	50	26	47 <sup>b</sup>	50	94	68.5
1999	47 <sup>b</sup>	50	94	20	50	40	$29^{*a}$	50	58	43 <sup>b</sup>	50	86	69.5

Table 3: Frequency of chickens with positive HI titers in different areas studied

\* = Significant difference between two consecutive summers (P<0.05), a = Significant difference between negative and positive cases (P<0.05), b = Significant difference between negative and positive cases (P<0.01), F = Frequency, T = Total

Table 4:	Frequency	of chickens	with	positive	HI	titers in	different	areas studied
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Area	3–4 Months†			1–2 Years†			3-4	4 Mont	hs‡	1–2 Years‡		
	F	Т	%	F	Т	%	F	Т	%	F	Т	%
Zarinshahr	19	50	38	-	-	-	31	31	100	0	19	-
Mobarekeh	-	-	-	41	50	82	44	50	88	-	-	-
Falavarjan	50	50	100	-	-	-	-	-	-	38	50	76
Khomeinishar	-	-	-	14	50	28	29	50	58	-	-	-
Total	69	100	69	55	100	55	104	131	79.4	38	69	55
Total all ages				124	200	62				142	200	71

 $\dagger$  = Serum samples collected in summer of (1998),  $\ddagger$  = Serum samples collected in summer of (1999), F = Frequency, T = Total

 Table 5: Frequency of Newcastle disease reports in industrial poultry flocks in the areas studied

Area		Summer of (1998)		Summer of (1999)				
	Cases	No. of death	Т	Cases	No. of death	Т		
Zarinshahr	2	1000	23100	2	1900	21700		
Mobarekeh	2	700	25000	2	1000	20000		
Falavarjan	2	500	22000	2	1500	42000		
Khomeinishahr	1	850	22000	1	1500	23000		

T = Total number of affected chickens

Table 6:	Frequency	of the	positive	and	susceptible	chickens	in	Isfahan	and	Shahrekord	(Chahar-
Mahal va	1 Bakhtiari)	provinc	ces								

Summer	Pos	sitive chicke	ns	Susceptible chickens					
	Frequency	Total	%	Frequency	Total	%			
Isfahan (1998)	137	200	68.5 <sup>a</sup>	124	200	62			
Isfahan (1999)	139	200	69.5 <sup>b</sup>	142	200	71			
Shahrekord (1999)*	44	104	42.3 <sup>a,b</sup>	74	104	71.1			

\* = Data extracted from Bouzari and Mousavi (2002); a, b = Data with the same letter have significant difference (P < 0.01)

Totally, 69.5% of chickens examined were seropositive in summer of (1998) and 68.5% were positive in summer of (1999) (P>0.05). This was significantly higher than the percentage (42.3%) reported from high altitudes and mountainous areas in Shahrekord (Chahar-Mahal va Bakhtiari) province of Iran (P<0.05) (Bouzari and Mousavi, 2002) (Table 6). On the other hand, the percentages of susceptible chickens in the two consecutive seasons were 71% and 62% which were not significantly different (P>0.05) in comparison to that of high altitudes and mountainous areas of Shahrekord (Chahar-Mahal va Bakhtiari) province of Iran (71.1%) (Bouzari and Mousavi, 2002). This indicates that although the total number of positive chickens in the plain areas is higher, the number of susceptible chickens is nearly similar (about 35%) to the number of chickens protected against virulent strains of NDV and thus, the consequences of exposure to virulent strains of the virus would be the same. The different geographical and climate situations may have little to do with the epidemiology of the Newcastle disease in domestic village chickens. For the protection of susceptible chickens, routine vaccination, especially in spring and summer is suggested. The determination of pathotypes of the viruses involved will help to have a better understanding of the epizootiology of the disease.

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