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Full Length Article

Seroprevalence of Newcastle disease virus in backyard chickens and herd-level risk factors of Newcastle disease in poultry farms in Oman

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ABSTRACT

Newcastle disease (ND) is an endemic disease in Oman's poultry industry and impacts negatively on food security. However, little is known regarding the potential risks of the disease in backyard poultry. The objectives of this study were to determine the seroprevalence of Newcastle disease virus (NDV) in backyard chickens and the herd-level risk factors in Oman. In total, 1383 serum samples were collected from chickens in 139 flocks from nine governorates. Information on associated risk factors was assessed using a semi-structured questionnaire. The samples were tested using commercial indirect ELISA kits.A logistic regression model was applied to assess the associated risk factors. The bird and flock-level NDV seroprevalence was 33.8% (95% Confidence Interval (CI): 12.8–38.6%) and 57.1% (95% CI: 35.7–71.4%), respectively. The highest seroprevalence of antibody to NDV at bird and flock levels was recorded in North Ash Sharqiyah (38.6%) and Al Buraimi (71.4%), respectively. Also, the lowest seroprevalence at bird and flock levels was recorded in Musandam (12.8%) and South Al Batinah (35.7%), respectively. A significant difference in NDV seroprevalence at flock and bird levels was only recorded in Ad Dakhliyah. Factors associated with higher seroprevalence to NDV included absence of a veterinarian in the farm (OR = 5.3; 95% CI: 2.1, 11.7), usage of dead ND vaccine (OR = 2.3; 95% CI: 1.2–4.2), employment of non-permanent staff (OR = 3.9; 95% CI: 1.5, 10.6) and free entry of visitors (OR = 6.2; 95% CI: 2.0, 20.3). In conclusion, the results of this study revealed a high exposure of backyard chickens to NDV and the identified risk factors could be vital in the prevention and control of the disease in Oman.

1. Introduction

Newcastle disease (ND) is a highly contagious viral disease affecting poultry and wild birds globally [1]. ND is regarded as an important reportable poultry disease and a major cause of economic loss in the poultry industry [1,2]. The causative agent of ND is the Newcastle disease virus (NDV), which is also known as Avian Paramyxovirus-1 (APMV-1) of the genus Avulavirus belonging to the family of Paramyxovirus serotypes [3]. According to the World Organisation for Animal Health, the virulent strains of NDV are responsible for ND infection in poultry [4,5]. NDV has the ability to infect over 200 species of birds, but the severity of disease produced is dependent on the affected host and the strain of virus [6]. In chickens, ND infections are manifested in varying severity ranging from high mortality to silent infection [1]. For instance, the velogenic ND virus induces high mortality reaching 100% in some cases, while other strains such as the mesogenic or lentogenic might elicit severe respiratory disease either by opportunistic infections or in adverse environmental conditions [6].

Generally, the major mean of prevention against the highly virulent ND is by vaccination, which is achievable with the low pathogenic genotypes attributed to the serological similarity between the NDV genotypes [5,7]. Despite vaccination, in Africa and Asia, endemicity of ND remains a significant problem with recent reports suggesting the fast spread of newly identified viruses of sub-genotype into the Middle East [8,9]. Furthermore, several studies have reported vital risk factors for the transmission of ND in poultry flocks in various countries. Accordingly, the role of migratory birds and trade in live birds have been reported as vital routes of ND transmission [10,11]. Most importantly, high seroprevalence of NDV was reported in backyard poultry and serving as a potential risk factor for ND transmission to commercial

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flocks [12,13]. This is often attributed to poor biosecurity measures and lack of proper vaccination program for backyard poultry [14].

Since in the 1950 s, NDV has been reported to be circulating in the Middle East. Smallholder farms represent the major poultry practice in the Middle East. In Oman, backyard chicken farms play a significant role as a source of food and income for the rural communities. Nevertheless, ND remains an important production limiting disease affecting the nation's poultry industry [15]. The geographical location of the Sultanate of Oman (between the horn of Africa and South East Asia) is suggestive to favor the transmission of NDV by means of the trajectory of migratory birds, as well as the growing trade between Oman and neighboring countries. A previous study in Oman reported a bird-level seroprevalence of 42% of antibodies to NDV in poultry birds (chickens, turkey, guinea fowl, turkey, and ducks) [15]. However, there is no current information regarding the occurrence of ND in backyard chickens, as well as the potential risk factors for the transmission of the disease. This is crucial to understand the current status and appropriate measures for the prevention and control of the disease in the country. Therefore, this study was designed to determine the seroprevalence of NDV antibodies and the herd-level risk factors associated with ND outbreaks in backyard chicken flocks in Oman.

2. Materials and methods

2.1. Study area and design

The study was conducted in the Sultanate of Oman located on the west coast of Gulf of Oman, approx. 21°00′N and 57°00′E. The sultanate is flanked by the Gulf of Oman, the Arabian Sea, and the Rub' al Khali (Empty Quarter) of Saudi Arabia. The inclusion criterion for the sampled flocks included the presence of backyard chickens without history of vaccination. For two or more farms to be selected from one region, a minimum distance of 1 km was required. The required sample size was estimated as 1300 poultry birds, based on the expected seroprevalence of 50%, 95% confidence interval, and 5% precision level [16]. A stratified sampling method was used to select the number of flocks to be sampled by considering the number of chickens, backyard farms, and the total number of chickens present in each region. In each farm, 10 healthy adult chickens were randomly sampled. The farm information collected included the date of sampling, farms' location, address, number of chickens sampled, and flock size. In total, 1383 serum samples were collected from chickens from 139 flocks distributed within the nine governorates of Oman from June 2016 to September 2016.

2.2. Sample collection and detection of NDV antibodies

Blood sample of 2 mL was collected from the wing vein of each bird using a single use only syringe and needle. The samples were transferred to free-transport tubes and transported in a cool-flask packed with ice and cotton wool. Following the extraction of serum from blood samples, storage was done at −20 °C and tested using Enzyme Linked Immunosorbent Assay (ELISA). The sensitivity and specificity of the indirect ELISA kit (ID Vet) was read from the manufacturer's information. All the reagents were adapted to room temperature (21 ± 5 °C) before use and homogenized afterward by inversion. The samples were pre-diluted at 1:500 in dilution buffer. In a pre-dilution plate, 245 µL of dilution buffer was added. Thereafter, 5 µL each of the positive and negative control samples was added to the labeled ELISA wells. A 5 µL of each sample, 90 µL of dilution buffer, and 10 µL were introduced to the appropriate well of the plate, covered and incubated at 21 °C for 30 min. The conjugate was prepared by diluting the concentrated conjugate (Anti-chicken IgG) in a dilution buffer, while the wells were emptied and washed three times with 300 µL of the wash solution. About 100 µL of the conjugate reagent was introduced to each well, covered and incubated as described previously. Again, the wells were emptied and washed 3 times with 300 µL to remove any unreacted conjugate. Finally, 100 µL of the substrate solution was added to each well, incubated at 21 °C for 15 min and followed by addition of 100 µL of stop solution to halt the reaction. The optical densities (ODs) was determined by quantifying the absorbance at 405 nm using a microplate reader. Also, the sample to positive ratio (s/p) was calculated and used to determine the mean ratio in each farm. Thereafter, the samples were classified into positive and negative based on the comparison of the absorbance between the samples and the thresholds defined by the kit's manufacturer.

2.3. Questionnaire survey

The risk factors associated with farms with recorded ND outbreaks were investigated by using a semi-structured questionnaire. The questionnaire was divided into five sections (A to E) containing items to obtain information on three broad areas. The first aspect was based on recorded ND outbreaks and the data collected included the date, number of birds affected, mortality rate, and confirmation of ND by a recognized veterinary diagnostic laboratory. Next to be assessed was the data relating to management risk factors such as vaccination, veterinarian services, biosecurity measures, and farm distance. The third section was based on farmers' practices during ND outbreaks such as reporting the disease to appropriate authorities, submission of samples, and stoppage of sales. A convenience sample size of 857 poultry farmers was reached to participate in this study. Farmers were briefed on the scope of the study and the confidentiality of all information provided. Those willing to participate were provided with a consent form prior to the administration of the questionnaire. The questionnaire was self-administered in paper format from July 2016 to August 2016.

2.4. Statistical analysis

All statistical analysis was carried out using SPSS 24 (Version 24.0, IBM Corp., and Chicago, IL, USA). The independent variable was dichotomous i.e. NDV seropositive or seronegative. As such, the data were organized for each governorate based on the flock size, number of sampled birds, and the dichotomous outcome. Descriptive statistics were computed for all the variables, while the Pearson Chi-square test was used to investigate the association between the seroprevalence at flock and bird levels in each governorate. Additionally, respondents from the survey data regarding reports of ND outbreaks were summarized using descriptive statistics. Median and mean were used to describe the categorical and continuous variables, respectively. A logistic analysis was applied to test the association between flocks with reported ND outbreaks and the considered risk factors. Factors were first considered in a univariate analysis and those with $P < 0.10$ were selected for the subsequent multivariate analysis using forward procedure. Odds ratios (ORs) and confidence interval (95%) were read from the parameter estimates and $P < .05$ was considered significant. The association between farmers' practices and recorded ND outbreaks was analyzed using Spearman's correlation coefficient.

3. Results

3.1. Descriptive analysis and NDV seroprevalence at bird and flock levels in backyard chickens

The mean flock size (number of birds to number of flocks) was 136. North Al Batinah and Al Buraimi regions had the highest (159.6) and lowest (77.1) mean flock size, respectively (Table 1). The overall seroprevalence of NDV at bird and flock level was 33.8% (95% CI: 12.8–38.6%) and 57.1% (95% CI: 35.7–71.4%), respectively. The highest NDV seroprevalence at bird level was recorded in North Ash Sharqiyah (38.6%) and lowest in Musandam (12.8%). At flock level, Al-Buraimi and South Al Batinah region had the highest and lowest

Table 1
Number of sampled flocks (n = 139) and birds (n = 1383) and their seroprevalence to NDV from nine governorates of Oman.

Flock-level seroprevalence						
Locations	No. of sampled flocks	NDV positive	No. of chickens	Mean flock size	No. of sampled chickens	NDV positive (%)
North Al Batinah	38	24 (63.2)	6225	159.6	381	143 (37.5)
Ad Dakhliyah	26	15 (57.6)	3620	139.7	260	91 (35.0)
Ad Dhahirah	19	7 (36.8)	2065	147.5	186	52 (27.9)
South Ash Sharqiyah	14	6 (42.8)	2050	146.4	137	44 (32.1)
South Al Batinah	14	5 (35.7)	1530	109.3	135	45 (33.3)
North Ash Sharqiyah	11	5 (45.5)	970	88.2	114	44 (38.6)
Al Buraimi	7	5 (71.4)	540	77.1	71	25 (35.2)
Muscat	6	3 (50.0)	720	120.0	60	19 (31.6)
Musandam	4	2 (50.0)	1170	292.5	39	5 (12.8)
Total (%)	139	72 (51.7)	18,890	136.0	1383	468 (33.8)
Mean ± SD	15 ± 10		2098 ± 1807		154 ± 108	

Table 2
Association between flock and bird levels NDV seroprevalence in each governorate of Oman.

	Birds		Flocks			
Locations	Total number	NDV positive (%)	Total number	NDV positive	χ^2 value	*P* value
North Al Batinah	6225	143 (37.5)	38	24 (63.2)	2.02	0.15
Ad Dakhliyah	3620	91 (35.0)	26	15 (57.6)	5.22	0.02
Ad Dhahirah	2065	52 (27.9)	19	7 (36.8)	0.66	0.42
South Ash Sharqiyah	2050	44 (32.1)	14	6 (42.8)	0.66	0.42
South Al Batinah	1530	45 (33.3)	14	5 (35.7)	0.32	1.00
North Ash Sharqiyah	970	44 (38.6)	11	5 (45.5)	0.20	0.75
Al Buraimi	540	25 (35.2)	7	5 (71.4)	3.53	0.21
Muscat	720	19 (31.6)	6	3 (50.0)	0.83	0.39
Musandam	1170	5 (12.8)	4	2 (50.0)	3.68	0.12
Total (%)	18,890	468 (33.8)	139	72 (51.7)		

χ^2 value = Pearson chi-square statistic.
P value < .05 is considered significantly different.

Table 3
Characteristics of farms based on farmers' reports (n = 500) and the percentage with recorded ND cases.

	ND cases (n = 202)	Not recorded (n = 136)	No information (n = 162)	Total (%)
Product				
Meat	134 (66.3)	90 (66.1)	76 (46.9)	300 (60.0)
Egg	42 (20.7)	38 (27.9)	54 (33.3)	134 (26.8)
Mix	26 (12.8)	8 (5.8)	32 (19.7)	66 (13.2)
Management system				
Local	68 (33.6)	34 (25.0)	88 (54.3)	190 (38.0)
Open	80 (39.0)	36 (26.4)	52 (32.1)	168 (33.6)
Close	54 (26.7)	66 (48.5)	22 (13.6)	142 (28.4)
Veterinarian				
Absent	178 (88.1)	94 (69.1)	158 (97.3)	430 (86.0)
Present	24 (11.9)	42 (30.9)	4 (2.7)	70 (14.0)
Capacity	832,020	1,212,100	2,227,670	

seroprevalence of 71.4% and 35.7%, respectively (Table 1). The relationship between NDV seroprevalence at flock and bird levels from each region is presented in Table 2. A significant association (*P* = 0.02) between the NDV seroprevalence at both levels was only recorded in Ad Dakhliyah.

3.2. Descriptive analysis and univariate analysis of risk factors associated with ND outbreaks

A response rate of 71% was obtained following the administration of the questionnaire. The proportions of respondents with or without recorded ND outbreaks in their farms were 40.4% and 27.2%, respectively. However, 32.4% had no such information at their disposal. As such, subsequent results were analyzed based on the farms with the required information on ND outbreaks. A higher proportion (54%) of the respondents practice the open system, whereas 38% and 28% were involved in backyard and close systems, respectively. Also, majority of the respondents produce chickens for meat purpose (60.0%) compared to egg (26.8%) and mixed products (13.2%) (Table 3).

At the univariate level, outbreaks of ND tended (*P* = .06) to be higher in farms producing chickens for egg products compared with farms engaged in meat or mixed production. The risk factors associated with the likelihood of ND outbreaks included the absence of veterinarian in the farm (Odds ratio; OR = 5.37, 95% CI 1.9–14.5), lack of vaccination program (OR = 8.0, 95% CI 1.5–10.5), non-employment of permanent staff (OR = 3.89, 95% CI 1.3–11.1), and non-restriction of visitors into farms (OR = 6.32, 95% CI 1.8–21.2). Also, an increased unit in the batch number of chickens was associated (*P* = .002) with farms reporting ND outbreaks (Table 4).

3.3. Multivariate analysis of factors associated with ND outbreaks and farmers' awareness and practices

Following multivariate analysis, the farms lacking the services of a veterinarian had five times increased odds of having ND outbreaks compared with farms with a veterinarian presence. The usage of dead vaccine (OR = 2.3, 95% CI 1.2–4.2) was associated with recorded ND cases compared with live and Notvac vaccine (OR = 1.0. Also, ND outbreaks were significantly higher in farms not restricting visitors' entry (OR = 6.4, 95% CI 2.0–20.3) and usage of temporary staff (OR = 3.9, 95% CI 1.5–10.6) compared to farms with the related biosecurity measures and employment of permanent staff (Table 4).

With respect to farmers' practices during ND outbreaks, only 16% of them indicated to regularly report the outbreaks in their farms to the appropriate authority. A higher proportion (57%) of the farmers affirmed to stop the sales of chickens during ND outbreaks, whereas < 30% of them either reported such disease outbreaks to a veterinarian or submitted samples to appropriate veterinary clinics. Overall, only 36.6% affirmed to be aware of the clinical signs of ND. There were significant positive correlations between farms with recorded ND outbreaks and the stoppage of sales of chicken products (r = 0.12, *P* = .03) and restricting production (r = 0.17, *P* = .002) (Table 5).

Table 4
Univariable and multivariable logistic regression models of factors associated with poultry farms (n = 338) with recorded outbreaks of ND in Oman.

	Univariate models			Multivariate models		
Item	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Products						
Meat	0.38	0.10–1.45	0.15			
Egg	2.71	0.94–7.79	0.06			
Mix	Ref					
Management system						
Open	0.65	0.18–2.33	0.50			
Local	0.55	0.23–1.17	0.11			
Close	Ref					
Veterinarian						
Absent	5.37	1.98–14.49	0.001	5.0	2.1–11.7	0.001
Present	Ref			Ref		
Vaccination						
Yes	8.04	1.45–10.54	0.005			
No	Ref					
Batch No.	1.62	1.20–2.18	0.002			
Vaccine type						
Live	0.37	0.03–4.15	0.42	0.97	0.53–1.77	0.93
Dead	1.07	0.10–11.8	0.95	2.28	1.24–4.21	< 0.01
Attenuated	1.79	0.09–36.1	0.70	4.84	0.87–26.8	0.07
Notvac	Ref			Ref		
Free entry of visitors						
Yes	6.32	1.87–21.24	0.003	6.4	2.0–20.3	< 0.01
No	Ref			Ref		
Farm distance						
100–500 m	0.49	0.19–1.27	0.14			
500 m–1 km	0.96	0.50–1.84	0.90			
Above 1 km	Ref					
Staff						
No	3.89	1.31–11.06	0.015	3.94	1.46–10.58	< 0.01
Yes	Ref			Ref		
Biosecurity						
Present	0.40	0.41–1.57	0.52			
Absent	Ref					
Disinfectant						
Yes	0.24	1	0.62			
No						

Ref = reference category.
OR = odds ratio.
$P < .05$ is significantly different.

4. Discussion

This study is the first attempt regarding the serosurveillance of NDV in backyard chickens and potential risk factors associated with the transmission of ND in Oman. The results herein revealed a high seroprevalence of NDV, with evidence of widespread infection and previous exposure to the pathogen. Accordingly, about 52% of the sampled flocks was seropositive for NDV. However, since the birds were apparently healthy, the reason might be the circulation of the low virulent and pathogenic strain of the virus. Maternal antibodies might still be present since the sampled chickens were between two to three week old [7]. However, the sampled flocks lacked any history of vaccination against NDV. Moreover, backyard poultry has been recognized to be highly predisposed to ND attributed to poor biosecurity measures [17,18]. Accordingly, the sampled flocks were characterized with inefficient facilities for adequate prevention against ND. The proportions of seronegative birds might be due to the difference in locations and the predominance of factors favoring the transmission of the disease.

The backyard system is an integral part of the poultry industry in Oman, thus the findings from this study are crucial in understanding the epidemiology of ND in the country. The high seroprevalence of NDV in backyard poultry has an implication on the maintenance of the virus and spread of the disease to commercial flocks. The present study recorded a high NDV seroprevalence (71.4%) at flock level, which is consistent with the findings from other authors [11–14]. The likely reasons for such finding could be the poor biosecurity, introduction of infected birds to existing flocks, lack of vaccination, close relationship with neighboring birds and, unhygienic feeding practices. The current study was conducted during the summer. As such, ND occurrence has been associated with seasonal patterns with peak level occurring during the hot dry season [19]. Moreover, in Oman, the ambient temperature is relatively high all year round which further supports ND occurrence in chickens as consequent of immunosuppression following heat stress [20]. In comparison with other studies, lower seroprevalence rates were reported in the studies conducted in Ivory Coast [21], Ethiopia [17] and Nigeria [22]. The difference might be related to species and age of birds, geographical locations, seasonal changes, diagnostic techniques, climatic conditions, and farming practices [12,19].

At bird level, the NDV seroprevalence in the present study was 33.8%, which is higher compared with the estimates reported in a recent study in West Indies, Trinidad (10%) [14] and Ivory Coast (22%) [23], in a seroprevalence survey of important viral pathogens affecting backyard chickens. Sharma et al. [24] in a similar study reported higher NDV seroprevalence of 66% compared with the estimate in our study. The difference might be due to lower proportions of backyard poultry to commercial farms, geographical locations, degree of previous exposure

Table 5
Responses of farmers regarding practices relating to ND outbreaks and correlation between the items.

Items	Frequency	%	Correlation with ND outbreaks	
			Spearman correlation coefficient	*P*-value
Do you report disease outbreaks in your farm to any authority				
No	168	83.2	0.065	0.23
Yes	34	16.8		
Do you report to a veterinarian				
No	166	82.2	−0.005	0.93
Yes	36	27.8		
Do you stop selling products during outbreaks				
No	86	42.6	0.118	0.03
Yes	116	57.4		
Restriction				
No	166	82.2	0.168	0.002
Yes	36	27.8		
Are you aware of clinical signs of ND				
No	128	63.4	−0.014	0.80
Yes	74	36.6		
Do you submit samples to appropriate centres?				
No	174	86.1	0.07	0.19
Yes	28	13.9		

to the pathogen, and efficacy of preventive and control measures. Additionally, pathogen-related factors such as the virulence, genetic resistance, and environmental factors could influence the epidemiological triad of NDV transmission [24]. However, our result is similar to the findings of other studies of Shekaili et al. [15] and Saadat et al. [25], which were both conducted in the Middle East, as well as a study carried out in Brazil [13]. Such finding might be related to the similar conditions in the geographical locations and predominance of backyard poultry.

Amongst all the regions, a significant association between NDV seroprevalence at flock and herd levels was only recorded in Ad Dakhliyah. This might be attributed to closely related risk factors influencing the exposure to the pathogen amongst the farms in the region. Nevertheless, the highest seroprevalences at flock and bird levels were recorded in Al Buraimi (71.4%) and North Sharqiyah (38.6%) respectively, which could be due to high population densities, number of poultry farms and the widespread of live markets in such areas [26]. In contrast, lowest NDV seroprevalences at flock and bird levels were observed in South Al Batinah (35.7%) and Musandam (12.8%) respectively, which could be related to the lower number of backyard flocks.

From the survey results, the presence of a veterinarian was demonstrated as a protective factor against ND outbreaks. Studies have shown that the presence of skilled personnel is crucial for effective prevention and control of ND [27,28]. A higher number of ND cases was reported by farmers lacking a vaccination program. The finding is not surprising as vaccination remains an essential mean of preventing ND occurrence [4,7]. Also, the wide distribution of backyard practice in Oman could be contributing, since farmers practicing such system rarely have a vaccination schedule [29]. Similarly, other identified risk factors for ND occurrence included free entry of visitors and employing the services of non-permanent staff. These practices promote contacts between personnel and on-farm facilities, thus facilitating the exposure to pathogens. Accordingly, ND can be transmitted mechanically through contaminated materials such as clothing, feed bags, human movements and visitors [12]. A similar result was reported by Andriamanivo et al. [30], as contact with animal health workers increased the odds of seropositivity for NDV amongst backyard chickens. Additionally, neighboring farms might be sharing one or more materials such as means of transportation or feed products that could aid in disease spread amongst farms as reported by other authors [17,28]. Also, the frequent changing of staff in the studied farms could further compromise any existing biosecurity measures. However, in the present study, no associations were found between farms with recorded ND outbreaks and management system, farm distances, and flock size. These results differ from the findings of other authors [13,31]. For instance, Marks et al. [13] reported decreased NDV seropositivity following increment in the distance between household poultry farms. Also, increasing unit of flock size was positively associated with NDV seroprevalence [21]. The disparity in the results might be related to the variation in management systems, study designs as well as the source of data collection.

The restriction and stoppage of sales of poultry products are vital measures taken to curtail the spread of ND outbreaks. Based on the respondents' data, these aforementioned practices were adequately adhered to, especially amongst farms with history of ND outbreaks. Furthermore, recent studies advocated for better quarantine, management of sick and dead birds, and improved technical efficiency, especially in backyard poultry systems [11,28]. However, most of the farmers in this study were little informed about the ND and they rarely submitted samples to appropriate centres during disease outbreaks. An effective reporting system is crucial for prompt dissemination of information between farmers and appropriate authorities regarding disease outbreaks [28]. Hence, these events depict the need for the improvement of Oman's poultry disease reporting system with emphasis on the education of farmers and appropriate practices to prevent and control the occurrence of ND.

5. Conclusions

Based on the paucity of information on the seroprevalence of NDV amongst backyard chickens in the Sultanate of Oman, this study provided vital data for better understanding of the epidemiology of ND in the country. The results herein revealed a high seroprevalence of NDV in backyard chickens, with a potential source of infection to commercial flocks. Factors such as the absence of vaccination program, veterinary services, and poor biosecurity measures relating to staff and people entering the farms could assist in reducing the exposure to NDV. Further investigations are recommended to identify the circulating virus genotypes and models of transmission for better understanding of ND epidemiology in backyard chickens in Oman.

Competing interests

None.

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