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Sero-surveillance and risk factors for avian influenza and Newcastle disease virus in backyard poultry in Oman

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ABSTRACT

Avian Influenza (AI) and Newcastle disease (ND) are the most important reportable poultry diseases worldwide. Low pathogenic AI (H9N2) and ND viruses are known to have been circulating in the Middle East, including in Oman, for many decades. However, detailed information on the occurrence of these pathogens is almost completely lacking in Oman. As backyard poultry are not vaccinated against either virus in Oman, this sector is likely to be the most affected poultry production sector for both diseases. Here, in the first survey of AI and ND viruses in backyard poultry in Oman, we report high flock-level seroprevalences of both viruses. Serum and oropharyngeal swabs were taken from 2350 birds in 243 backyard flocks from all regions and governorates of Oman. Information was recorded on location, type of bird and housing type for each sampled farm. Individual bird serum samples were tested using commercial indirect antibody detection ELISA kits. Pooled oropharyngeal samples from each flock were inoculated onto FTA cards and tested by RT-PCR. Samples came from chickens (90.5%), turkeys (2.1%), ducks (6.2%), guinea fowl (0.8%) and geese (0.4%). The bird-level seroprevalence of antibody to AI and ND viruses was 37.5% and 42.1% respectively, and at the flock level it was 84% and 90% respectively. There were statistically significant differences between some different regions of Oman in the seroprevalence of both viruses. Flock-level NDV seropositivity in chickens was significantly associated with AIV seropositivity, and marginally negatively associated with flock size. AIV seropositivity in chickens was marginally negatively associated with altitude. All oropharyngeal samples were negative for both viruses by RT-PCR, consistent with a short duration of infection. This study demonstrates that eight or nine out of ten backyard poultry flocks in Oman are exposed to AI and ND viruses, and may present a risk for infection for the commercial poultry sector in Oman, or wild birds which could carry infection further afield.

1. Introduction

Avian influenza (AI) and Newcastle disease (ND) are the most important reportable poultry diseases worldwide (Malik et al., 2004; Wang et al., 2008). Both are highly contagious viral diseases affecting a wide range of bird species. Avian influenza and Newcastle disease are caused by influenza A virus (AIV) and Newcastle disease virus (NDV) respectively. The AIV belong to the *Orthomyxoviridae* family while the NDV is one of the avian paramyxovirus serotype-1 (APMV-1) viruses of the *Paramyxoviridae* family (Swayne and King, 2003). Infection of birds with AIV or NDV can be of varying clinical severity, ranging from 100% fatality to a silent infection (Swayne and King, 2003; Wang et al., 2008). In addition, both have zoonotic potential. In the case of AIV, this is especially true for viruses with haemagglutinin surface antigens H5, H7 and H9 (Alexander, 2007; Anon, 2010). High mortality in birds is caused by highly pathogenic avian influenza (HPAI) and velogenic Newcastle disease (vND) viruses; therefore the occurrence of vND viruses, and all H5 and H7 strains are notifiable to the World Organisation for Animal Health (Swayne and King, 2003; Lee et al., 2005). Genotypes of NDV are serologically similar, therefore low pathogenic genotypes are used for vaccination to control those that are highly pathogenic (Miller et al., 2007). Conversely, the AIV genotypes differ greatly in their immunogenicity; there is no cross-protection between viruses that differ in their haemagglutinin (HA) surface glycoprotein (Anon, 2010).

Migratory birds and the trade in live birds are believed to be the main sources of transmission of those two diseases globally,

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although for AIV, backyard birds, particularly water fowl (duck and geese), are important risk factors for transmission within endemic areas (Swayne and King, 2003). Backyard poultry is the main poultry production sector that suffers from the two diseases, particularly in developing countries (Alexander, 2001). This is due to the low level of biosecurity measures and the low rate or lack of vaccination. For example, ND was believed to be the causative agent of around 90% of backyard poultry deaths in Nepal during 1992 (Alexander, 2001). Newcastle disease has circulated in the Middle East since the middle of the last century (Mase et al., 2002), and low pathogenic avian influenza (LPAI) H9N2 has been detected in most of the Middle East region for around 20 years (Fusaro et al., 2011). Despite the importance of backyard poultry in the Middle East, there is relatively little published information on the prevalence of AIV or NDV in most countries. There is a particular paucity of information from Oman, with almost no published studies of avian respiratory viruses of any species, except on infectious bronchitis virus (IBV) and avian metapneumovirus (Al-Shekaili et al., 2015). This is despite Oman's geographic situation, between the horn of Africa and southern Asia, and its importance as a site for migrating wild birds. Most poultry production in Oman is carried out in commercial farms, which vaccinate against NDV and LPAI H9N2 (albeit at a lower intensity). Despite this, clinical cases of both Newcastle disease and LPAI are still observed annually in backyard birds. The backyard flocks are bred for household consumption and vaccination against either disease is not practiced within this sector. To date, the HPAI has not been detected in poultry or wild birds in Oman.

Given the paucity of information on the prevalence of AIV or NDV in Oman, this study aimed to determine and map the serological prevalence of both diseases in the backyard poultry sector, and to identify flock-level risk factors for AIV and NDV.

2. Materials and methods

2.1. Sampling method

Serum and oropharyngeal swabs were taken from 2350 birds of 243 backyard flocks from all regions and governorates of Oman, between mid-June and the end of September 2012. The total backyard poultry population was estimated by the Ministry of Agriculture and Fisheries/Department of Rural Women Development to be approximately 10,000 poultry flocks with a median size of 50 birds per flock. The vast majority of the sampled farms raised local chickens; however turkeys, guinea fowl, duck and geese were also present at some farms.

The required sample size was based on detecting expected AIV and NDV seroprevalences of 30% and 70% respectively with 95% confidence and 5% precision (Thrusfield, 2005). A two-stage cluster sampling method was used (Thrusfield, 2005), with a between cluster variance of 0.7 estimated from a study of AI in poultry flocks in Jordan (Al-Natour and Abo-Shehada, 2005). The desired number of flocks to be sampled was then stratified by region according to estimates of the number of poultry farms, number of poultry, number of people and number of backyard poultry present in each region.

Local veterinarians and animal production engineers in each region helped in selecting farms that met the sampling criteria within their territories. Criteria for inclusion were that they are backyard poultry (i.e. chickens that are not reared commercially as broilers, layers and breeders, and poultry of any other species), the minimum distance between two farms should exceed one kilometre, and there should not be more than two farms from one village. Where only one bird species was present, ten healthy adult (older than 3 months) birds were selected randomly and sampled, unless there were fewer than ten available, in which case all were sampled. A small number of premises had two or more species present. On some of these, only one species was made available by the owner and so just one was sampled as before. On others, a maximum of two species were sampled (up to a maximum of 10 birds each). In our analysis, these are treated as separate flocks on the same farm. On no farms did we sample three or more species, even if they were present.

Serum (1–2 ml) was collected from the wing vein of each bird using single-use only syringes and needles and stored in anticoagulant-free transport tubes. Oropharyngeal samples (for viral genomic identification) were taken using sterile wooden swabs. The samples were transported in a cool box with ice and cotton wool. Serum was extracted from blood samples and stored at −20 °C until tested by Enzyme-linked Immunosorbent Assay (ELISA). The oropharyngeal swabs of each flock/species were pooled in 1.5 ml distilled water and then 80–100 μl was pipetted onto the centre of circles of Flinders Technology Associates (FTA) cards (Sigma–Aldrich, Dorset, UK) using a sterile pipette. The cards were left for one hour to dry at room temperature (22 °C). Later, the FTA cards were stored at 4 °C in sealed sample bags and transported to the University of Liverpool, UK, for processing and analysis.

Farm information such as the date of sampling, owner name, location of the farm (village, state, and region), species of birds in the farm, flock sizes, number and type of sampled birds, type of housing and source of water were recorded for each farm. The spatial coordinates of the location were recorded using a handheld GPS (Garmin GPS MAP 62s, USA) and the altitudes of farms were obtained by feeding the GPS-recorded farm latitude and longitude into the Google Maps Elevation API (https://developers.google.com/maps/documentation/elevation/).

2.2. Detection of AIV and NDV antibodies

Antibodies to the H nucleoprotein of AIV in chicken and turkey samples were detected by indirect ELISA using a commercial kit (BioChek Ltd., Gouda, Holland) as described by the manufacturer. Antibodies in other bird species were examined using the IDEXX Ab multispecies ELISA kit (IDEXX, USA). Similarly, antibodies to NDV in chicken and turkey samples were detected using an indirect ELISA kit (BioChek, Gouda, Holland); however, the duck and geese serum samples were not tested as the kits have not been validated in these species. According to the manufacturer, the sensitivity/specificity of the AI and ND kits were determined by the manufacturer as 100/98% and 100/99–100% respectively.

For both AIV and NDV, all steps were carried out at room temperature and the ELISA plate was adapted to room temperature for around 30 min before use. In brief, for the BioChek ELISA kit testing, the serum was diluted 1:500 by adding 1 μl of sample to 0.5 ml of sample diluent and mixed well by vortexing. After the addition of 100 μl positive and negative control samples to the indicated wells of the ELISA plate, 100 μl of diluted sample was added to the appropriate well of the plate. The plate was incubated at 22 °C for 30 min and then washed 4 times with 350 μl/well of wash buffer. After addition of 100 μl/well of conjugate reagent (Anti-chicken IgG labelled with the enzyme alkaline phosphatase), the plate was covered again and incubated at room temperature for 30 min and then washed, as described in the previous step, to remove unreacted conjugate. Next, 100 μl/well of substrate reagent was added and the plate was incubated for 15 min, after which substrate development was halted with stop solution (100 μl/well) (sodium hydroxide in diethanolamine buffer). Optical density (ODs) was determined by measurement of absorbance at 405 nm with a microplate reader. The colour intensity was directly related to the amount of antibody present in the sample. Based on the ODs the sample to positive (S/P) ratios were calculated and used to express the mean (S/P) ratio per group. Samples with antibody levels above the thresholds

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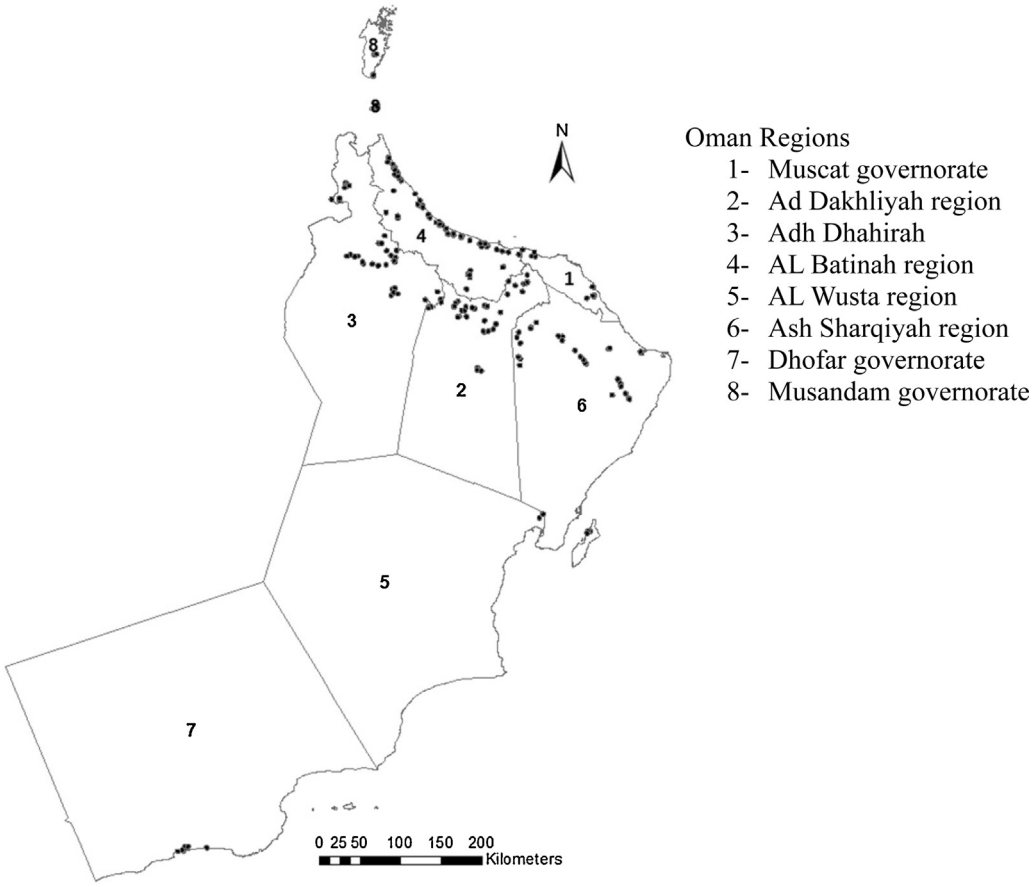

Fig. 1. Distribution of sampled farms from the eight regions and governorates of Oman.

Table 1
Reverse transcription polymerase chain reaction (RT-PCR) oligonucleotides used for avian influenza and Newcastle disease viruses Ribonucleic acid (RNA) detection in our study.

Virus		Oligo	Sequence (5′–3′)	Gene	Product size (bp)
AIV	Single step PCR	NP - F	AGRTACTGGGCHATAAGRAC	NP	–
		NP - R	ATTGTCTCCGAAGAAATAAG		
NDV	Single step PCR	MSF-1R	GACCGCTGACCACGAGGTTA	F	182
		MSF-2F	AGTCGGAGGATGTTGGCAGC		

defined by the kit manufacturer were classified as positive; all other samples were classed as negative.

2.3. RNA extraction from FTA cards and RT-PCR

RNA extraction was performed using a Qiagen, QIAamp Viral RNA Mini Kit (Qiagen Ltd., Germany) according to the manufacturer's instructions and RT-PCR was carried out as described below.

2.4. Elution of RNA from FTA cards

One circle from each FTA card was cut out using sterile scissors and forceps and placed in a bijou containing 800–1000 μl of TE buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0), vortexed and incubated at room temperature (RT) for 10 min (Abdelwhab et al., 2011). RNA was extracted from the supernatant.

2.5. RNA Extraction

One hundred and forty microlitre of supernatant was added to 560 μl of a viral lysis buffer (AVL) plus carrier RNA in an Eppendorf, vortexed and incubated at room temperature for 10 min. 560 μl of 100% ethanol was added, vortexed and pulse centrifuged. Then 630 μl of sample was transferred to a spin column, centrifuged at 8000 rpm for 1 min and the flow through discarded. This was repeated once more for the remaining sample. The column was then washed with a wash buffer 1 (AW1), a strong protein denaturant and centrifuged at 13,000 rpm for 1 min, followed by a final wash with wash buffer 2 (AW2) at 13,000 rpm for 3 min, discarding the flow through and a final spin at 13,000 rpm for 1 min. Finally the viral RNA was eluted from the membrane with RNase free water and stored at −20 °C until required.

2.6. Reverse transcriptase polymerase chain reaction (RT-PCR)

RT-PCR was performed on each of the AIV and NDV RNA extracts amplifying NP and F genes for these viruses respectively (Table 1). Both primers and cycle conditions were previously published (Banks et al., 2000; Aldous et al., 2003).

2.7. Statistical methods

Because the number of sampled flocks of birds other than chickens is very small (15 duck flocks, 1 flock of geese, 2 flocks of guinea

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Table 2
Number of flocks and birds of each poultry species present in farms visited in different regions of Oman in 2012.

Region	Number of chickens (flocks)	Number of ducks (flocks)	Number of turkeys (flocks)	Number of Guinea fowl (flocks)	Number of geese (flocks)	Percentage of birds other than chickens	Percentage of flocks other than chickens
Al Batinah Region	8259 (69)	556 (10)	133 (4)	100 (2)	5 (1)	8.8	19.8
Musandam Governorate	641 (11)	4 (1)	0	0	0	0.6	8.3
Ash Sharqiyah Region	4160 (35)	80 (1)	1 (1)	100 (1)	0	4.2	7.8
Ad Dakhliyah Region	4773 (37)	210 (4)	0	10 (1)	1 (1)	4.4	13.9
Adh Dhahirah Region	10516 (47)	0	30 (1)	0	0	0.3	2.1
Dhofar Governorate	2605 (7)	0	0	7 (1)	0	0.28	12.5
Al Wusta Region	321 (8)	0	0	0	0	0	0
Muscat Governorate	1600 (11)	13 (1)	9 (1)	0	0	1.3	15.4
Total	32875 (225)	863 (17)	173 (7)	217 (5)	6 (2)	3.7	11.1

Table 3
Avian influenza virus (AIV) and Newcastle disease virus (NDV) serological results in different regions of Oman.

Region	Total No. Tested		AIV positive (%)		NDV positive (%)	
	Birds[a]	Flocks[a]	Birds	Flocks	Birds	Flocks
Al Batinah Region	792/689	82/71	298 (37.6)	68 (82.9)	256 (37.2)	64(90.1%)
Musandam Governorate	88/84	11/10	30 (34.1)	9 (81.8)	20 (23.8)	8 (80%)
Ash Sharqiyah Region	355/335	36/34	114 (32.3)	30 (88.2)	188 (561)	32 (94.1%)
Ad Dakhliyah Region	383/353	39/36	147 (38.4)	33 (84.6)	144 (40.8)	33 (91.7%)
Adh Dhahirah Region	461/461	47/47	175 (40)	38 (80.9)	276 (59.9)	46 (97.9)
Dhofar Governorate	70/70	7/7	8 (11.4)	5 (71.)	9 (12.9)	5 (71.4%)
Al Wusta Region	72/72	8/8	39(51.4)	8 (100.0)	15 (19.4)	5 (62.5%)
Muscat Governorate	129/119	13/12	72 (55.8)	13 (100.0)	46 (38.6)	11 (91.7%)
Total	2350/2262	243/226	881	204	953	203
Overall Percentage			37.5%	84%	42.1%	90%

[a] Total number of birds and flocks tested for AIV/NDV, 81/68 represents 81 flocks tested for AIV and 68 flocks tested for NDV.

fowl and 5 flocks of turkeys), the power to detect statistically meaningful effects in groups of birds other than chickens is low. The decision was hence taken to restrict formal statistical modelling to chicken flocks only, of which there were 220 in the data set, and only one per farm.

Variables deemed to have a possible association with the probability of a chicken flock being positive for NDV or AIV were as follows: Region (factor with eight levels: al Batinah, Musandam, Ash Sharqiyah, Ad Dakhliyah, Adh Dhahirah, Dhofar, Al Wusta, Muscat), Altitude in metres (continuous), Flock size (continuous) and Flock composition (chickens only, or chickens and other species present). For NDV, the sample AIV seropositivity result (continuous) was also included.

Exploratory analyses in the form of smoothed scatter plots were used together with Generalised Additive Models (GAMs) to assess the relationship between the logit-transformed prevalence and quantitative explanatory variables. GAMs (Wood, 2001) modify the general linear model (GLM) to allow the investigation of relationships between predictor and arbitrary smooth functions of explanatory variables, which are often non-linear.

Because the outcome of interest was the seroprevalence of NDV or AIV in chicken flocks, we fitted a binomial GLM with a logit link to model the relationship between the probability of being positive for NDV or AIV and explanatory variables, transformed where necessary, using quasi-likelihood to allow for potential over-dispersion. Since all explanatory variables are at the flock level, we have a single observation per flock (the number of birds positive, k, out of number of birds sampled, n) and so a GLM-based analysis is appropriate, with quasi-likelihood providing a pragmatic approach to allowing for the over-dispersion which may be evidenced as a result of dependence within farms. Initially we investigated, using graphical approaches and GAMs as described, whether the assumption of a linear relationship between logit-transformed seroprevalence and altitude, flock size and (in the case of the outcome NDV seroprevalence) AI sample prevalence, seemed reasonable. If the estimated degrees of freedom (edf) associated with the smoothed function of each continuous variable in the GAM was close to 1, a linear relationship was assumed to be appropriate (see, for example, Wood, 2001); if it was not, we considered transformations of the associated explanatory variable which might improve linearity. Following exploratory analysis, the binomial GLM including all potential explanatory variables, transformed or otherwise, was then fitted, and an analysis of deviance based upon the F test (appropriate because the dispersion parameter is estimated when quasi-likelihood is used, rather than being fixed at 1 in the traditional binomial GLM) was used to determine which variables should be retained in the model. Insignificant variables were removed by backwards selection. The removal of each successive variable was determined by a deviance reduction test as described, with a 5% critical value being used for comparison. Region was included as a fixed effect because the study regions include all the regions of interest. For the two explanatory variables, Region and Flock composition, Dhofar and chickens only were set as the baseline.

Generalised linear models were fitted using the routine glm in R (R Core Team, 2014), available at http://www.r-project.org) and GAMs were fitted using the mgcv package (Wood, 2001), also in R.

3. Results

3.1. Sampled flocks and serological finding

Fig. 1 shows the distribution of the farms that were sampled and Table 2 presents the number of flocks by region and composition of different bird types. A total of 2350 birds, from five backyard poultry species (chickens, turkeys, ducks, geese and guinea fowl) were sampled from 243 flocks on 238 farms. Eighty-nine percent (n = 211) of the sampled backyard farms had chickens only. The sampled flocks were chickens (90.5%), turkeys (2.1%), ducks (6.2%), guinea fowls (0.8%) and geese (0.4%). In term of bird numbers; chickens comprised approximately 96% of all poultry present at the visited farms (Table 2). Ducks were the second most common

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Table 4
Number of avian influenza virus (AIV) and Newcastle disease virus (NDV) positive birds and flocks in Oman backyard birds, by bird type.

Bird Species	Number of tested birds	Number (%) of AIV positive birds	Number (%) of NDV positive birds	Number of tested flocks	Number (%) of AIV positive flocks	Number (%) of NDV positive flocks
Chickens	2134	827 (38.8)	938 (44)	220	185 (84.4%)	199 (90.5)
Ducks	142	35(24.6)	NA	15	12 (80%)	NA
Turkeys	49	15 (30.6)	15 (30.6)	5	4 (80%)	4 (80)
Geese	5	2	NA	1	1	NA
Guinea Fowl	20	2 (10)	NA	2	2 (100%)	NA

NA – not applicable, as ducks, geese and guinea fowl were not tested for NDV.

Table 5
Analysis of deviance for the reduced model for Newcastle disease virus (NDV) prevalence in Oman backyard chicken flocks fitted using quasi likelihood methods.

Variable	Degrees of freedom	Deviance explained by term	Residual degrees of freedom	Residual deviance	*F*-statistic	*P* (F > f)
Null model (constant mean)			219	991.8		
Region	7	148.5	212	843.3	6.96	0.001
Flock size	1	10.10	211	833.2	3.32	0.07
AI percent positive	1	94.25	210	738.9	30.94	0.001

species found in the visited farms, forming nearly 2.5% of the total; the main duck species were Muscovy (*Cairinia moschata*) and mallard (*Anas platyrhyncos*). The other three poultry species accounted for less than two percent of the total birds. Only chickens were found in the farms visited in the Al Wusta region. Three regions (Musandam, Adh Dhahirah and Dhofar) had a small percentage of farms with turkey or guinea fowl hens. Al Batinah region had the highest proportions of birds and flocks that were not chickens, reaching nearly 9% and 20% of the total respectively. The mean flock sizes were 146 birds for chickens (standard deviation = 210), 24.7 for turkeys (SD = 26.5), 50.8 for ducks (SD = 32.8), 43.4 for guinea fowl (SD = 43.4) and 3.0 for geese (which were just present in two flocks).

While Dhofar governorate had the smallest number of chicken flocks, it had the greatest mean flock size, reaching 372 birds (standard deviation = 352.3). The second largest mean flock size was 224 birds at Adh Dhahirah region (standard deviation = 340.4). In other regions mean chicken flock sizes were fewer than 130 birds.

The most prevalent type of housing for the backyard birds is semi-closed houses (227/238; 95.3%), particularly in the northern regions. In the Dhofar governorate and Al Wusta region birds tend to be kept outdoors, scavenging during daylight hours.

The bird and flock-level ELISA results for AIV and NDV, by region, are shown in Table 3. The seroprevalence of antibodies to AIV and NDV in birds was 37.5% and 42.1% respectively. The flock-level seroprevalence was 84 (SD = 31.57) and 90% (SD = 31. 23), respectively. Mean within-flock seroprevalences were 37.6% and 43.4%, respectively.

The percentages of birds and flocks of each species that were positive for AIV and NDV are shown in Table 4. There was no significant difference between the percentages of flocks of different species serologically positive for either virus (Fisher Exact, $p > 0.5$). Differences among birds were not tested for, because of possible clustering within flocks

3.2. Risk factors for NDV positivity

Our initial exploration using scatterplots and GAMs suggested that the relationship between logit-transformed seroprevalence and altitude is closer to linear when the altitude variable is square root transformed (the GAM including a smoothed function of altitude had an edf of 2.77, while a smoothed function of the square root of altitude gave an edf close to 1). For all other terms, both scatter plots and estimated edfs suggest that their direct inclusion as parametric linear terms in a GLM is appropriate. We hence model the seroprevalence as a function of flock size, percentage of birds AI positive (for ND), the square-root transformed altitude, flock composition ('farm only has chickens' versus 'farm has chickens and other birds',) and region in a binomial GLM.

Over-dispersion in this first model was evidenced by the fact that the ratio of the residual deviance and degrees of freedom in the model is 3.54 (when no over-dispersion is present this ratio should be 1), validating our choice of a quasi-likelihood approach to model fitting. The only terms which reduced the residual deviance by a statistically significant amount were region ($F = 6.91$ for which we obtain $p < 0.001$), and the proportion of birds on the same premises positive for avian influenza ($F = 29.67$ and we again obtain $p < 0.001$). The effect of flock size is marginal ($F = 3.79$, which yields a p-value of 0.053). We fitted a further model including only these terms and the analysis of deviance table for this model is presented in Table 5.

The model coefficients, standard errors and 95% confidence intervals around parameter estimates are summarised in Table 6. There was statistically significant evidence of a regional effect. Taking the most southern province, Dhofar, as the baseline, there was evidence ($p < 0.05$) that chicken flocks in two of the regions (Ash Sharqiyah, and Al Dhahira) may have a higher seroprevalence of NDV antibodies than chicken flocks in Dhofar. Furthermore there was marginal evidence ($p < 0.1$) that flocks in one of the other regions (Ad Dakhliyah) are likely to have a higher proportion of chickens positive than those in Dhofar. There was no evidence of a consistent North–South gradient in the magnitude of these effects. NDV seropositive chicken flocks are significantly more likely to be AIV seropositive as well.

3.3. Risk factors for AIV seropositivity

A similar analysis to build a model describing the relationship between the proportion of chickens positive and flock-level explanatory variables was conducted. Exploratory analysis using scatterplots and GAMs suggested that the inclusion of flock size and altitude directly as parametric linear terms in a GLM was reasonable (from the GAM the edf associated with smoothed functions of each variable was close to 1 in both cases). Region and an indicator of whether or not multiple species were present on the same premises were again included as factors. Again the potential presence of over-dispersion suggested the need for a quasi-likelihood-based approach to model fitting, and a subsequent analysis of deviance to assess the contribution made by each of the variables to the model was again conducted with a series of *F* tests. From the full

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Table 6
Coefficients, standard errors and 95% confidence intervals on parameter estimates from reduced model for Newcastle disease virus (NDV) prevalence in backyard chicken flocks in Oman, Dhofar governorate was the reference for statistical comparison of different regions; flock size and avian influenza virus (AIV) seropositivity (number of positive birds/number of sampled birds) are continuous variables.

Variable	Coeff	SE(Coeff)	95% confidence interval	*t*-value	$P(T>\|t\|)$
Intercept	−1.828	0.639	(−3.307, −0.711)	−2.859	0.005
Dhofar	Ref.				
Al Wusta	−0.331	0.827	(−1.928, 1.411)	−0.401	0.689
Ash Sharqiyah	1.696	0.663	(0.523, 3.209)	2.559	0.011
Ad Dakhliyah	0.991	0.664	(−0.185, 2.505)	1.494	0.137
Al Dhahira	1.842	0.654	(0.691, 3.342)	2.818	0.005
Al Batinah	0.729	0.653	(−0.421, 2.228)	1. 117	0.265
Muscat	0.573	0.734	(−0.771, 2.188)	0.780	0.436
Musandam	0.180	0.781	(−1.295, 1.858)	0.231	0.818
Flock size	−0.0008	0.0004	(−0.002, 0.000)	−1.937	0.054
AI Positivity	0.015	0.0027	(0.009, 0.020)	5.440	0.001

model (including flock size, altitude, region and flock composition as explanatory variables), the presence of over-dispersion, confirming the need for a quasi-likelihood approach to fitting, was again evidenced by the fact that the ratio of the residual deviance and degrees of freedom in this model is 4.74. Variables which may be associated with the proportion of birds seropositive for AIV are region ($F = 1.98$ giving $p = 0.059$) and altitude ($F = 3.41$ with $p = 0.066$, which is again marginally significant). Fitting a second model, therefore, which included only these terms and again conducting analysis of deviance based upon a series of F tests, provides the output in Table 7.

The summary of this reduced model is given in Table 8. Again there was statistical evidence of a regional effect. Taking the most southerly province, Dhofar, as the baseline, there was evidence that flocks in all regions except Musandam have experienced higher proportions of birds seropositive for AIV than those in Dhofar. The effect in Musandam is marginal but also positive. There was no clear systematic North–South effect. There was a marginal negative effect of altitude on the seroprevalence of AIV.

3.4. AIV and NDV RT-PCR

All PCR results for genome detection of both pathogens from the FTA cards were negative. A positive control was included in all PCR reactions and it gave an expected band size on the agarose gel.

4. Discussion

This is the first investigation into the seroprevalence of AIV and NDV in Oman on a national scale. The results show a widespread exposure to both viruses in backyard poultry. Nearly 84% and 90% of the sampled flocks, and about 4 of 10 sampled birds, had serological evidence of previous infection with AIV and NDV respectively. The high prevalence of seropositive flocks reported here may be due to the circulation of low pathogenic viruses of both types producing mild or no clinical signs in infected birds.

The free ranging of backyard birds presents a high risk for AIV (Terregino et al., 2007) and NDV (Schelling et al., 1999) transmission between wild birds and poultry in both directions. Although the majority of the visited backyard farms rear their birds in wire-netted enclosures, there are often gaps or damages in poultry houses that may allow the entry of wild birds. As such, the chance of contact with wild birds is higher in the backyard poultry rearing system than those raised in commercial farms where the whole house is concealed against entry of wild birds.

Backyard poultry health status is important for the birds' owners, the nearby commercial poultry farms and human health authorities (Madsen et al., 2013a,b), as the diseases affecting the backyard flocks could spill-over to commercial poultry or people. There have been only a few previous studies investigating the epidemiology of AIV and/or NDV in Middle Eastern countries, and the majority of these studies targeted HPAI H5N1 in commercial poultry farms (Al-Natour and Abo-Shehada, 2005; Aamir et al., 2007; Banet-Noach et al., 2007; Al-Azemi et al., 2008; Monne et al., 2008; Fereidouni et al., 2010; Hafez et al., 2010; Ababneh et al., 2012; Arafa et al., 2012; El-Shesheny et al., 2012; Watanabe et al., 2012; Hassan et al., 2013; Madadgar et al., 2013). Ours is the one of the first studies to measure the seroprevalence of AIV and NDV in backyard poultry on a national scale in a Middle East country.

We found high (80–90%) flock-level seroprevalences of both AIV and NDV, suggesting that there is widespread exposure of backyard poultry in Oman to both viruses. Possible explanations include exposure to wild birds, introduction of new birds onto existing flocks, co-mixing with neighbouring poultry and feeding of uncooked poultry waste. Studies elsewhere have found lower flock-level seroprevalences. For example, in New Zealand, 20.8% (5/24) of backyard poultry flocks were found to be seropositive for avian influenza viruses (Zheng et al., 2010). In Maryland, USA, 23.1% (9/39) of backyard flocks were seropositive for AIV (Madsen et al., 2013a,b) and in Côte d'Ivoire the ND seropositive rate was 19.8% (Couacy-Hymann et al., 2012). Factors such as testing method, species and age of birds, climate condition, time of year, farming practices and migratory bird routes contribute to the difference between locations (Madsen et al., 2013a,b).

In contrast to the high flock-level seroprevalences, we detected much lower (~40%) within-flock seroprevalences of both viruses. Similar, low within-flock seroprevalences have been reported elsewhere in the Middle East. Similar to our study, Saadat et al. (2014) found bird-level seroprevalences of AIV (39%) and NDV (40.1%). A second study near the Caspian Sea in Iran, however, reported higher seroprevalences (~73%) of H9N2 seropositivity in backyard chickens (Hadipour, 2010). There are several possible explanations for

Table 7
Analysis of deviance for the reduced model for avian influenza virus (AIV) prevalence in Oman backyard chicken flocks fitted using quasi likelihood methods.

Variable	Degrees of freedom	Deviance explained by term	Residual degrees of freedom	Residual deviance	F-statistic	$P(F>f)$
Null model (constant mean)			219	1061.8		
Region	7	54.79	212	1007.0	1.99	0.057
Altitude	1	13.51	211	993.5	3.44	0.065

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Table 8
Coefficients, standard errors and 95% confidence intervals on parameter estimates from reduced model for avian influenza virus (AIV) seroprevalence in backyard chicken flocks in Oman. Dhofar governorate was the reference for statistical comparison of different regions; altitude (m) is a continuous variable.

Variable	Coeff	SE(Coeff)	95% confidence interval	*t*-value	*P*(T>|t|)
Intercept	−2.040	0.744	(−3.864, −0.789)	−2.74	0.007
Dhofar	Ref.				
Al Wusta	2.109	0.879	(0.530, 4.114)	2.387	0.017
Ash Sharqiyah	1.544	0.787	(0.185, 3.423)	1.964	0.051
Ad Dakhliyah	2.023	0.810	(0.610, 3.932)	2.496	0.013
Adh Dhahirah	1.922	0.790	(0.556, 3.806)	2.419	0.016
Al Batinah	1.699	0.761	(0.405, 3.545)	2.029	0.027
Muscat	2.432	0.837	(0.955, 4.382)	2.740	0.004
Musandam	1.545	0.872	(−0.034, 3.535)	1.682	0.078
Altitude	7.02 x e-4	3.94 x e-4	(−0.002, 0.000)	−1.913	0.077

why fewer than half of the birds in exposed flocks in our study were seropositive. First, in backyard poultry flocks in Oman, the density of birds is less than in commercial premises, with less contact between individuals and less sharing of air space; this may mean that there is less transmission between birds than that which occurs under more intensive production. Second, some exposed birds may deal with the respiratory infection with mucosal immunity with little or no seroconversion, while other surviving birds may have seroconverted strongly. The humoral antibody response is dependent on the host (e.g. age, immune status), agent (e.g. virulence, dosage, co-infections) and environment (e.g. stocking density, air quality, ventilation). Third, especially if the initial exposure was to a low dose of virus, immunity may wane over time and older, exposed, birds may become seronegative. Fourth, if the circulating viruses are highly pathogenic, many exposed birds may die, and be replaced by unexposed stock. Fifth, variation in the level of genetic resistance between different breeds and species of poultry has been reported (Kapczynski et al., 2013).

There was a marginally significant difference in the seroprevalence of AIV in terms of birds and chicken flocks between different regions of Oman (Table 7). The Muscat Governorate and Al Wusta region had the highest seroprevalences of AIV. The high AIV prevalence in Muscat Governorate may be attributable to the presence of many known risk factors for the disease, such as high population density (Gilbert et al., 2008; Moriguchi et al., 2013), presence of water bodies (Fournie et al., 2012), high road density (Ward et al., 2008), the presence of live bird markets (Kung et al., 2007) and the presence of a large number of wild birds (Senne et al., 2006). In Al Wusta region, the high AIV seroprevalence in terms of both birds and flocks may be attributable to the presence of the Barr Al Hikman wetland areas used for nesting of migratory birds annually (Terregino et al., 2007). The wetland birds such as gulls, terns, and waders are a major natural virus reservoir of AIV (Olsen et al., 2006). By contrast, the Dhofar governorate in the south of Oman showed the lowest seroprevalences of AIV and NDV in chickens. This may be due to the small number of backyard flocks in this region.

In this study, we investigated antibodies against AIV using universal AIV antibody detection ELISA kits; we found no evidence for an effect of flock size on the percentage of seropositivity within the flock. In contrast, a recent study of backyard poultry in Ethiopia found that positive flocks (defined as having any positive birds) were smaller than negative flocks (Chaka et al., 2013).

Previous studies focusing on H5N1 in Southeast Asia and the H7N1 outbreak affecting Italy during 1999–2000 (Mannelli et al., 2006; Gilbert et al., 2008; Busani et al., 2009) found a negative association between AIV infection and the altitude of the area. Our result is in agreement with these studies, showing marginal evidence for a negative association between AIV infection and the altitude of the farms.

Previous studies investigating the risk factors for both AI and ND found similar risks associated with both diseases, such as low biosecurity levels (East, 2007; Iglesias et al., 2011), presence of wild birds (Otim et al., 2007; Gilbert et al., 2008), poultry density (East et al., 2006; Fang et al., 2008) and agriculture activity in the area (Otim et al., 2007; Iglesias et al., 2011). Our study supports this assertion, finding association between AIV and NDV infection.

We found no evidence of very recent or current AIV or NDV infections in any of the flocks, as all the PCR results (for the detection of viral RNA) were negative. However, the RNA of two other viruses (IBV and aMPV) were detected on a number of the same FTA cards, confirming that the sampling, transportation and laboratory methods did not have any adverse effects on the RNA (Al-Shekaili et al., 2015). Previous studies have demonstrated the stability of AIV (Abdelwhab et al., 2011) and NDV (Awad et al., 2012) RNA on FTA cards. Similar findings were reported for NDV and AIV in New Zealand (Zheng et al., 2010; Dunowska et al., 2013) and H5N1 in Egypt (Kayali et al., 2011). Our negative results may be due to the low chance of finding pathogenic viruses in samples taken from clinically healthy birds, or due to the short duration of shedding of ND and AI viruses in comparison to IB virus (Sjaak de Wit et al., 2011). It is also possible that the snapshot sampling method that we used, with all samples taken during the hot summer months, may have reduced the likelihood of detecting either virus although the seasonality of transmission of these viruses in Oman is not known.

Against a background of a paucity of information on AIV and NDV prevalence and their risk factors in Oman and other Middle East countries, this study provides evidence of a high serological prevalence of NDV and AIV in backyard poultry flocks in Oman and provides some preliminary information about risk factors that may help to target disease control measures and/or avian flu contingency planning.

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References

Aamir, U., Wernery, U., Ilyushina, N., Webster, R., 2007. Characterization of avian H9N2 influenza viruses from United Arab Emirates 2000 to 2003. Virology 361, 45–55.

Ababneh, M.M., Dalab, A.E., Alsaad, S.R., Al-Zghoul, M.B., Al-Natour, M.Q., 2012. Molecular characterization of a recent Newcastle disease virus outbreak in Jordan. Res. Vet. Sci. 93, 1512–1514.

Abdelwhab, E.M., Lüschow, D., Harder, T.C., Hafez, H.M., 2011. The use of FTA® filter papers for diagnosis of avian influenza virus. J. Virol. Methods 174, 120–122.

Al-Azemi, A., Bahl, J., Al-Zenki, S., Al-Shayji, Y., Al-Amad, S., Chen, H., Guan, Y., Peiris, J.S., Smith, G.J., 2008. Avian influenza A virus (H5N1) outbreaks, Kuwait, 2007. Emerg. Infect. Dis. 14, 958–961.

Al-Natour, M.Q., Abo-Shehada, M.N., 2005. Sero-prevalence of avian influenza among broiler-breeder flocks in Jordan. Prev. Vet. Med. 70, 45–50.

Al-Shekaili, T., Baylis, M., Ganapathy, K., 2015. Molecular detection of infectious bronchitis and avian metapneumoviruses in Oman backyard poultry. Res. Vet. Sci. 99, 46–52.

Aldous, E.W., Mynn, J.K., Banks, J., Alexander, D.J., 2003. A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. Avian Pathol. 32, 239–256.

Alexander, D.J., 2001. Newcastle disease. Br. Poult. Sci. 42, 5–22.

Alexander, D.J., 2007. An overview of the epidemiology of avian influenza. Vaccine 25, 5637–5644.

Anon, 2010. Avian influenza, Chapter 10.4., Terrestrial Animal Health Code.

Arafa, A.S., Hagag, N., Erfan, A., Mady, W., El-Husseiny, M., Adel, A., Nasef, S., 2012. Complete genome characterization of avian influenza virus subtype H9N2 from a commercial quail flock in Egypt. Virus Genes 45, 283–294.

Awad, F., Forrester, A., Baylis, M., Ganapathy, K., 2012. Detection of multiple poultry respiratory pathogens from FTA® card. In: Leirz, M., Huffels-Redman, U., Kaleta, E.F., Heckman, J. (Eds.), VII International Symposium on Avian Corona-and Pneumovirus Infections. Druckerei Schroder, Germany, pp. 136–137.

Banet-Noach, C., Panshin, A., Golender, N., Simanov, L., Rozenblut, E., Pokamunski, S., Pirak, M., Tendler, Y., Garcia, M., Gelman, B., Pasternak, R., Perk, S., 2007. Genetic analysis of nonstructural genes (NS1 and NS2) of H9N2 and H5N1 viruses recently isolated in Israel. Virus Genes 34, 157–168.

Banks, J., Speidel, E., Harris, P., Alexander, D., 2000. Phylogenetic analysis of influenza A viruses of H9 haemagglutinin subtype. Avian Pathol. 29, 353–359.

Busani, L., Valsecchi, M.G., Rossi, E., Toson, M., Ferre, N., Pozza, M.D., Marangon, S., 2009. Risk factors for highly pathogenic H7N1 avian influenza virus infection in poultry during the 1999–2000 epidemic in Italy. Vet. J. 181, 171–177.

Chaka, H., Goutard, F., Roger, F., Bisschop, S.P.R., Thompson, P.N., 2013. Household-level risk factors for Newcastle disease seropositivity and incidence of Newcastle disease virus exposure in backyard chicken flocks in Eastern Shewa zone, Ethiopia. Prev. Vet. Med. 109, 312–320.

Couacy-Hymann, E., Kouakou, A.V., Kouame, C.K., Kouassi, A.L., Koffi, Y.M., Godji, P., Nana, P., Tarnagda, Z., Akoua-Koffi, C., 2012. Surveillance for avian influenza and Newcastle disease in backyard poultry flocks in Cote d'Ivoire, 2007–2009. Rev. Sci. Tech. 31, 821–828.

Dunowska, M., Zheng, T., Perrott, M.R., Christensen, N., 2013. A survey of avian paramyxovirus type 1 infections among backyard poultry in New Zealand. N. Z. Vet. Assoc. J. 61, 316–322.

East, I., Kite, V., Daniels, P., Garner, G., 2006. A cross-sectional survey of Australian chicken farms to identify risk factors associated with seropositivity to Newcastle-disease virus. Prev. Vet. Med. 77, 199–214.

East, I.J., 2007. Adoption of biosecurity practices in the Australian poultry industries. Aust. Vet. J. 85, 107–112.

El-Shesheny, R., Kayali, G., Kandeil, A., Cai, Z., Barakat, A.B., Ghanim, H., Ali, M.A., 2012. Antigenic diversity and cross-reactivity of avian influenza H5N1 viruses in Egypt between 2006 and 2011. J. Gen. Virol. 93, 2564–2574.

Fang, L.Q., de Vlas, S.J., Liang, S., Looman, C.W., Gong, P., Xu, B., Yan, L., Yang, H., Richardus, J.H., Cao, W.C., 2008. Environmental factors contributing to the spread of H5N1 avian influenza in mainland China. PloS One 3, e2268.

Fereidouni, S.R., Werner, O., Starick, E., Beer, M., Harder, T.C., Aghakhan, M., Modirrousta, H., Amini, H., Moghaddam, M.K., Bozorghmehrifard, M.H., Akhavizadegan, M.A., Gaidet, N., Newman, S.H., Hammoumi, S., Cattoli, G., Globig, A., Hoffmann, B., Sehati, M.E., Masoodi, S., Dodman, T., Hagemeijer, W., Mousakhani, S., Mettenleiter, T.C., 2010. Avian influenza virus monitoring in wintering waterbirds in Iran, 2003–2007. Virol. J. 7, 43.

Fournie, G., Guitian, J., Desvaux, S., Mangtani, P., Ly, S., Cong, V.C., San, S., Dung do, H., Holl, D., Pfeiffer, D.U., Vong, S., Ghani, A.C., 2012. Identifying live bird markets with the potential to act as reservoirs of avian influenza A (H5N1) virus: a survey in northern Vietnam and Cambodia. PloS One 7, e37986.

Fusaro, A., Monne, I., Salviato, A., Valastro, V., Schivo, A., Amarin, N.M., Gonzalez, C., Ismail, M.M., Al-Ankari, A.-R., Al-Blowi, M.H., Khan, O.A., Maken Ali, A.S., Hedayati, A., Garcia Garcia, J., Ziay, G.M., Shoushtari, A., Al Qahtani, K.N., Capua, I., Holmes, E.C., Cattoli, G., 2011. Phylogeography and Evolutionary History of Reassortant H9N2 Viruses with Potential Human Health Implications. J. Virol. 85, 8413–8421.

Gilbert, M., Xiao, X., Pfeiffer, D.U., Epprecht, M., Boles, S., Czarnecki, C., Chaitaweesub, P., Kalpravidh, W., Minh, P.Q., Otte, M.J., Martin, V., Slingenbergh, J., 2008. Mapping H5N1 highly pathogenic avian influenza risk in Southeast Asia. Proc. Natl. Acad. Sci. U. S. A. 105, 4769–4774.

Hadipour, M.M., 2010. Seroprevalence survey of H9N2 avian influenza virus in backyard chickens around the Caspian Sea in Iran. Braz. J. Poult. Sci. 12, 53–55.

Hafez, M.H., Arafa, A., Abdelwhab, E.M., Selim, A., Khoulosy, S.G., Hassan, M.K., Aly, M.M., 2010. Avian influenza H5N1 virus infections in vaccinated commercial and backyard poultry in Egypt. Poult. Sci. 89, 1609–1613.

Hassan, M.K., Jobre, Y., Arafa, A., Abdelwhab, E.M., Kilany, W.H., Khoulosy, S.G., Bakry, N.R., Baile, E., Ali, A., Ankers, P., Lubroth, J., 2013. Detection of A/H5N1 virus from asymptomatic native ducks in mid-summer in Egypt. Arch. Virol. 158, 1361–1365.

Iglesias, I., Sanchez-Vizcaino, J.M., Munoz, M.J., Martinez, M., de la Torre, A., 2011. Spatio-temporal model of avian influenza spread risk. Proc. Environ. Sci. 7, 104–109.

Kayali, G., El-Shesheny, R., Kutkat, M.A., Kandeil, A.M., Mostafa, A., Ducatez, M.F., McKenzie, P.P., Govorkova, E.A., Nasraa, M.H., Webster, R.G., Webby, R.J., Ali, M.A., 2011. Continuing threat of influenza (H5N1) virus circulation in Egypt. Emerg. Infect. Dis. J. 17, 2306–2308.

Kung, N.Y., Morris, R.S., Perkins, N.R., Sims, L.D., Ellis, T.M., Bissett, L., Chow, M., Shortridge, K.F., Guan, Y., Peiris, M.J., 2007. Risk for infection with highly pathogenic influenza A virus (H5N1) in chickens, Hong Kong, 2002. Emerg. Infect. Dis. 13, 412–418.

Lee, C.W., Swayne, D.E., Linares, J.A., Senne, D.A., Suarez, D.L., 2005. H5N2 avian influenza outbreak in Texas in 2004: the first highly pathogenic strain in the United States in 20 years? J. Virol. 79, 11412–11421.

Madadgar, O., Karimi, V., Nazaktabar, A., Kazemimanesh, M., Ghafari, M.M., Azimi Dezfouli, S.M., Hojjati, P., 2013. A study of Newcastle disease virus obtained from exotic caged birds in Tehran between 2009 and 2010. Avian Pathol. 42, 27–31.

Madsen, J.M., Zimmermann, N.G., Timmons, J., Tablante, N.L., 2013a. Prevalence and differentiation of diseases in Maryland backyard flocks. Avian Dis. 57, 587.

Madsen, J.M., Zimmermann, N.G., Timmons, J., Tablante, N.L., 2013b. Avian influenza seroprevalence and biosecurity risk factors in Maryland backyard poultry: a cross-sectional study. PloS One 8, e56851.

Malik, Y.S., Patnayak, D.P., Goyal, S.M., 2004. Detection of three avian respiratory viruses by single-tube multiplex reverse transcription-polymerase chain reaction assay. J. Vet. Diagn. Invest. 16, 244–248.

Mannelli, A., Ferre, N., Marangon, S., 2006. Analysis of the 1999–2000 highly pathogenic avian influenza (H7N1) epidemic in the main poultry-production area in northern Italy. Prev. Vet. Med. 73, 273–285.

Mase, M., Imai, K., Sanada, Y., Sanada, N., Yuasa, N., Imada, T., Tsukamoto, K., Yamaguchi, S., 2002. Phylogenetic analysis of Newcastle disease virus genotypes isolated in Japan. J. Clin. Microbiol. 40, 3826–3830.

Miller, P.J., King, D.J., Afonso, C.L., Suarez, D.L., 2007. Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. Vaccine 25, 7238–7246.

Monne, I., Fusaro, A., Al-Blowi, M.H., Ismail, M.M., Khan, O.A., Dauphin, G., Tripodi, A., Salviato, A., Marangon, S., Capua, I., Cattoli, G., 2008. Co-circulation of two sublineages of HPAI H5N1 virus in the Kingdom of Saudi Arabia with unique molecular signatures suggesting separate introductions into the commercial poultry and falconry sectors. J. Gen. Virol. 89, 2691–2697.

Moriguchi, S., Onuma, M., Goka, K., 2013. Potential risk map for avian influenza A virus invading Japan. Diversity Distrib. 19, 78–85.

Olsen, B., Munster, V.J., Wallensten, A., Waldenstrom, J., Osterhaus, A.D., Fouchier, R.A., 2006. Global patterns of influenza a virus in wild birds. Science 312, 384–388.

Otim, M.O., Kabagambe, E.K., Mukiibi, G.M., Christensen, H., Bisgaard, M., 2007. A study of risk factors associated with Newcastle disease epidemics in village free-range chickens in Uganda. Trop. Anim. Health Prod. 39, 27–35.

R Core Team (2014). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Saadat, Y., Ghafouri, S.A., Tehrani, F., Langeroudi, A.G., 2014. An active serological survey of antibodies to newcastle disease and avian influenza (H9N2) viruses in the unvaccinated backyard poultry in Bushehr province,Iran, 2012–2013. Asian Pac. J. Trop. Biomed. 4, S213–216.

Schelling, E., Thur, B., Griot, C., Audige, L., 1999. Epidemiological study of Newcastle disease in backyard poultry and wild bird populations in Switzerland. Avian Pathol. 28, 263–272.

Senne, D.A., Suarez, D.L., Stallnecht, D.E., Pedersen, J.C., Panigrahy, B., 2006. Ecology and epidemiology of avian influenza in North and South America. Dev. Biol. (Basel) 124, 37–44.

Sjaak de Wit, J.J., Cook, J.K., van der Heijden, H.M., 2011. Infectious bronchitis virus variants: a review of the history, current situation and control measures. Avian Pathol. 40, 223–235.

Swayne, D.E., King, D.J., 2003. Avian influenza and Newcastle disease. J. Am. Vet. Med. Assoc. 222, 1534–1540.

Terregino, C., De Nardi, R., Guberti, V., Scremin, M., Raffini, E., Martin, A.M., Cattoli, G., Bonfanti, L., Capua, I., 2007. Active surveillance for avian influenza viruses in wild birds and backyard flocks in Northern Italy during 2004 to 2006. Avian Pathol. 36, 337–344.

Thrusfield, M., 2005. Veterinary Epidemiology, 3rd ed. Blackwell Science Limited, Oxford, UK.

Wang, L.C., Pan, C.H., Severinghaus, L.L., Liu, L.Y., Chen, C.T., Pu, C.E., Huang, D., Lir, J.T., Chin, S.C., Cheng, M.C., Lee, S.H., Wang, C.H., 2008. Simultaneous detection and differentiation of Newcastle disease and avian influenza viruses using oligonucleotide microarrays. Vet. Microbiol. 127, 217–226.

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Ward, M.P., Maftei, D., Apostu, C., Suru, A., 2008. Environmental and anthropogenic risk factors for highly pathogenic avian influenza subtype H5N1 outbreaks in Romania, 2005–2006. Vet. Res. Commun. 32, 627–634.
Watanabe, Y., Ibrahim, M.S., Ellakany, H.F., Kawashita, N., Daidoji, T., Takagi, T., Yasunaga, T., Nakaya, T., Ikuta, K., 2012. Antigenic analysis of highly pathogenic avian influenza virus H5N1 sublineages co-circulating in Egypt. J. Gen. Virol. 93, 2215–2226.
Wood, S.N., 2001. mgcv: GAMs and generalized ridge regression for R. R News 1/2, 20–25.
Zheng, T., Adlam, B., Rawdon, T.G., Stanislawek, W.L., Cork, S.C., Hope, V., Buddle, B.M., Grimwood, K., Baker, M.G., O'Keefe, J.S., Huang, Q.S., 2010. A cross-sectional survey of influenza A infection, and management practices in small rural backyard poultry flocks in two regions of New Zealand. N. Z. Vet. J. 58, 74–80.

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