Risk factors for seropositivity to H5 avian influenza virus in ostrich farms in the Western Cape Province, South Africa

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Abstract

In a 2005 serological survey, carried out in response to an outbreak of H5N2 avian influenza (AI) in ostriches in the Eastern Cape Province, 16.3% of ostrich farms in the Western Cape Province of South Africa were found to be seropositive to H5 AI virus. We subsequently carried out a questionnaire-based census survey on all available registered Western Cape ostrich farms that still existed at the end of 2005 (367 farms, of which 82 were seropositive), in order to identify risk factors associated with farm-level seropositivity. A farm was classified as seropositive for H5 AI virus if one or more birds had tested positive (haemagglutination inhibition titre >1:16) in the 2005 survey, which had been designed to detect a minimum within-group seroprevalence of 10%. For each farm, risk factor information was collected using a questionnaire administered during a face-to-face interview with each farm owner or manager. Information was obtained on the ostrich population, movements of birds, environmental factors, management practices, and frequency of contact between ostriches and various wild bird species. Multiple logistic regression models were developed for the whole Western Cape Province and also for the two largest ostrich farming regions, “Klein Karoo” and “Southern Cape”. Seroprevalence differed between regions, being highest in the Klein Karoo (31.6%). In all three models, increased risk of farm-level H5 AI virus seropositivity was associated with increasing numbers of ostriches, excluding chicks, present on the farm. Increased risk of seropositivity was associated with reduced frequency of cleaning of feed troughs (<1×/week vs. >1×/week), both overall (odds ratio (OR) = 4.5; 95% confidence interval (CI): 1.5, 13.3) and in the Southern Cape (OR = 53.6; 95% CI: 3.3, 864), and with failure to clean and disinfect transport vehicles, both overall (OR = 2.3; 95% CI: 1.1, 4.8) and in the Klein Karoo (OR = 2.6; 95% CI: 1.1, 6.5). Increased risk of seropositivity was also associated with increasing frequency of contact of ostriches with certain wild bird species: overall with white storks (*Ciconia ciconia*), in the Southern Cape with gulls (*Larus* spp.), and in the Klein Karoo with Egyptian geese (*Alopochen aegyptiaca*).

Keywords: Avian influenza; Ostriches; Risk factors; Epidemiology; Survey; South Africa

1. Introduction

Avian influenza (AI) is caused by influenza A viruses which are enveloped, single-stranded RNA viruses of the family *Orthomyxoviridae*. Subtypes are defined by the antigenicity of two major surface proteins on the envelope, namely haemagglutinin (H) and neuraminidase (N). Sixteen H (H1–H16) and 9 N (N1–N9) subtypes have been recognised, translating into 144 possible combinations, with each virus containing 1 H and 1 N antigen in any combination. AI viruses are further classified in two pathotypes based on their ability to cause disease in chickens. Highly pathogenic avian influenza (HPAI) spreads rapidly, and may cause serious disease and result in high mortality rates (up to 100% within 48 h). Low pathogenic avian influenza (LPAI) can cause mild disease that may go undetected or show no symptoms at all in some bird species (OIE, 2007). HPAI viruses possess multiple basic amino acids (arginine and lysine) at the cleavage site of their haemagglutinin glycoprotein precursor (Wood et al., 1993), and/or have an intravenous pathogenicity index (IVPI) ≥1.2 in chickens. HPAI has only been associated with H5 and H7 subtypes and these are the only subtypes notifiable to the World Organisation for Animal Health (OIE) (Senne et al., 2006). It has been calculated that the impact of AI on the poultry industry has increased 100-fold, with 23 million birds affected between 1959 and 1998, and more than 200 million affected between 1999 and 2004 (Capua, 2006).

In July 2004 the H5N2 subtype of HPAI virus was isolated from ostriches (*Struthio camelus*) on a feedlot farm in the Eastern Cape Province of South Africa. Mortality was about 18%, but was limited to a few groups of birds affected by high population density, cold weather and secondary bacterial infections (Olivier, 2006). Several other ostrich flocks in the region showed seroconversion but remained healthy. The HPAI classification of the isolate was based on nucleotide sequencing, but the IVPI was low, and no clinical or serological evidence of AI viral activity was found in chickens present on the affected farms (Olivier, 2006). Following this outbreak a national AI survey was conducted until May 2005. During this survey 124 out of 761 ostrich farms in the Western Cape Province tested serologically positive to H5 AI. In response to these findings 15,945 cloacal swabs were tested by polymerase chain reaction (PCR) to detect the agent. All tests yielded negative results (Sinclair et al., 2006). However, exports were banned from August 2004 until September 2005. In July 2006 HPAI H5N2 was again detected in ostriches, this time in the Albertinia district (Southern Cape region) of the Western Cape Province. Once more exports were banned from July 2006 until November 2006, when conditional exports (only from serologically negative farms) were allowed.

Ostrich farming in South Africa is heavily reliant on the export of leather and fresh ostrich meat products to trade partners in the USA, Japan and Europe. The South African ostrich industry's world market share is approximately 60%, with approximately 4000 tonnes of meat being exported annually to the European Union. The main production systems and processing facilities are located within a limited semi-arid region (Klein Karoo) within the Western Cape Province, representing 70–80% of the South African industry (Olivier, 2006). A ban of meat exports may lead to a loss of at least R50 million (±US$ 6.5 million) per month during the peak season. The ostrich industry directly employs 20,000 people; a ban on the export of ostrich meat would therefore place the industry under stress and job losses would be inevitable.

Despite the risks posed by AI to the South African ostrich industry, very little is known about its epidemiology and there have been no published studies of associated risk factors. The objective of this study was to identify farm-level risk factors for seropositivity to H5 AI virus, with a view to mitigating these risk factors and thereby helping to ensure sustainable exports.

2. Materials and methods

2.1. Study background

The study was conducted in the Western Cape Province of South Africa, which for the purposes of this survey was divided into five regions: Klein Karoo, Karoo, Southern Cape, Agulhas, and West (Fig. 1). The majority of ostrich farms are situated in the Klein Karoo region, followed by the Southern Cape. The Klein Karoo is a semi-arid region, bordered by mountains to the south and the north, with some rain falling throughout the year but more often during autumn and winter. Oudtshoorn, the major town in the region and the centre of the ostrich industry, lies at an altitude of 314 m above sea level and receives an average annual rainfall of 239 mm. Average daily minimum and maximum temperatures are 5 and 19 °C respectively in winter (July) and 16 and 31 °C in summer (January), and average relative humidity is 58% (South African Weather Service, 2007, personal communication). Ostrich farms in this region tend to be clustered along rivers and irrigation canals. The Southern Cape region lies between mountains and the coast and has a non-seasonal rainfall pattern, with between 400 and 500 mm/year in the main ostrich farming areas. Day/night temperatures are less extreme than in the Klein Karoo, particularly on the coast, and relative humidity is higher. In both regions, ostrich chicks are typically reared under intensive conditions, whereas breeding birds are kept on extensive natural ranges. Slaughter birds up to about 14 months are kept under intensive feedlot conditions. Planted pastures, particularly lucerne (*Medicago sativa*), are also widely used for chicks and slaughter birds.

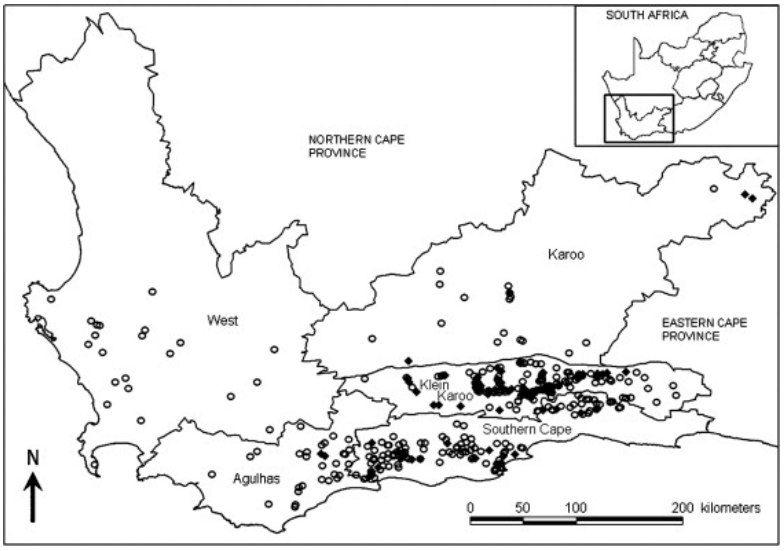

Fig. 1. Map of the Western Cape Province, South Africa, showing the 5 geographic regions and the location and H5 avian influenza serological status of the 367 ostrich farms surveyed. (♦) Farm seropositive for H5 avian influenza virus; (○) farm seronegative.

2.2. Study design

A census survey was performed, the sampling frame consisting of all registered ostrich farms that were present in the Western Cape Province of South Africa during the March–May 2005 AI surveillance period, and that were still registered at the end of 2005 (435 farms). Information on potential risk factors was collected by means of an interview-based structured questionnaire survey. Information on farm-level H5 AI virus seropositivity was obtained from the results of the 2005 AI serological survey (Sinclair et al., 2006).

2.3. Questionnaire

A structured questionnaire was designed to collect information on potential risk factors for farm-level seropositivity to H5 AI virus. Attempts were made to interview the owner or manager of every registered ostrich farm in the Western Cape Province. The questionnaire recorded the contact details of the owner, farm name, registration number and geographical location, as well as information on the ostrich population, movements of ostriches, environmental factors, management practices, and frequency of contact between ostriches and various wild bird species. For the latter, farmers were shown colour photographs of nine easily recognizable species or families of birds that were known to occur on ostrich farms and were asked to indicate the frequency with which each had been seen amongst the ostriches (never/seldom/frequently). Table 1 lists all the potential risk factors that were recorded as categorical variables. These were assessed in the questionnaire using closed-ended questions. The only continuous variable was the number of ostriches (excluding chicks) present on the farm, which was assessed using an open-ended question. The questions referred to conditions prevailing on the farms approximately 12–18 months previously, at the time of the H5N2 AI virus isolation in the Eastern Cape Province and immediately prior to the 2005 serological survey. The questionnaire was designed in consultation with experts in the ostrich industry, but was not subjected to pretesting or repeatability testing. Identical questionnaires were compiled in both English and Afrikaans and were used according to each owner or manager's preference. Administration of the questionnaire was done by animal health technicians who first underwent a training session. The responses to the questions were recorded on paper during a face-to-face interview with each farm owner or manager, lasting between 15 and 30 min.

Table 1.

Bivariable analysis of categorical risk factors for farm-level H5 avian influenza virus seropositivity in Western Cape ostrich farms

Risk factor and level	Number of farms tested	%Seropositive	*P*[a]
Region[b]			
Agulhas	30	6.7	<0.001
Karoo	20	20.0	

Risk factor and level	Number of farms tested	%Seropositive	*P*[a]
Klein Karoo	171	31.6	
Southern Cape	124	17.7	
West	22	0.0	
Type of birds present			
Slaughter birds only	64	20.3	0.633
Breeders only	15	13.3	
Both	276	23.9	
Chicks present on farm			
No	97	26.8	0.257
Yes	262	21.0	
Small water troughs			
No	287	21.3	0.283
Yes	77	27.3	
Medium water troughs			
No	41	26.8	0.551
Yes	324	21.9	
Large water troughs[b]			
No	298	21.1	0.194
Yes	66	28.8	
Frequency of cleaning water troughs			

Risk factor and level	Number of farms tested	%Seropositive	*P*[a]
>1×/week	55	23.6	0.941
1×/week	153	21.6	
<1×/week	156	22.4	
Method of cleaning water troughs			
Empty and refill	108	22.2	0.200
Scrub	167	15.6	
Type of feed troughs[b]			
Open feed troughs	270	19.3	0.050
Self-feeders	31	29.0	
Both	62	32.3	
Frequency of cleaning feed troughs[b]			
>1×/week	87	8.0	<0.001
1×/week	102	21.6	
<1×/week	146	30.8	
Method of cleaning feed troughs[b]			
Move only	101	32.7	0.035
Empty and refill	65	18.5	
Empty, refill and move	165	18.2	
None	9	11.1	
Frequency of visits by NCD vaccinators[b]			

Risk factor and level	Number of farms tested	%Seropositive	*P*[a]
1×/year or less	67	14.9	0.045
2×/year	124	18.5	
4×/year or more	171	28.1	
Lucerne pastures used as grazing for ostriches[b]			
No	58	12.1	0.040
Yes	75	24.4	
Backyard poultry present			
No	176	23.3	0.706
Yes	188	21.3	
Rented vehicle used			
No	187	20.9	0.406
Yes	123	25.2	
Vehicle cleaned and disinfected[b]			
No	78	30.8	0.085
Yes	219	20.5	
Open water sources present			
No	24	16.7	0.618
Yes	341	22.6	
Ostriches have access to open water sources			
No	209	23.9	0.375

Risk factor and level	Number of farms tested	%Seropositive	*P*[a]
Yes	156	19.9	
Open water sources used as water points			
No	250	24.0	0.278
Yes	115	18.3	
Movement of ostriches onto the farm			
Summer			
No	81	19.8	0.531
Yes	200	24.0	
Autumn[b]			
No	123	13.0	<0.001
Yes	158	30.4	
Winter			
No	188	20.7	0.290
Yes	93	26.9	
Spring			
No	126	26.2	0.253
Yes	155	20.0	
Wild birds seen amongst ostriches			
Egyptian goose (*Alopochen aegyptiaca*)			
Never	83	18.0	0.499

Risk factor and level	Number of farms tested	%Seropositive	*P*[a]
Seldom	120	22.5	
Frequently	162	24.7	
African sacred ibis[b] (*Threskiornis aethiopicus*)			
Never	159	20.1	0.002
Seldom	131	16.8	
Frequently	73	38.4	
Hadeda ibis[b] (*Bostrychia hagedash*)			
Never	63	19.0	0.106
Seldom	167	27.5	
Frequently	135	17.8	
Cattle egret (*Bubulcus ibis*)			
Never	157	21.0	0.787
Seldom	140	22.9	
Frequently	68	25.0	
Pigeons[b] (Columbidae)			
Never	51	11.8	0.083
Seldom	93	20.4	
Frequently	221	25.8	
Finches and sparrows (Ploceidae & Passeridae)			
Never	20	10.0	0.388
Seldom	59	20.3	

Risk factor and level	Number of farms tested	%Seropositive	*P*[a]
Frequently	286	23.8	
Gulls (*Larus* spp.)			
Never	323	22.0	0.946
Seldom	30	23.3	
Frequently	10	20.0	
White stork[b] (*Ciconia ciconia*)			
Never	218	21.1	0.164
Seldom	130	22.3	
Frequently	17	41.2	
Helmeted Guineafowl[b] (*Numida meleagris*)			
Never	71	31.0	0.037
Seldom	118	25.4	
Frequently	176	17.0	

[a] *P*-value for Fisher's exact test.
[b] Variable significant ($P < 0.20$) and therefore offered to the multiple logistic regression model.

2.4. Serological status of farms

The results of the 2005 AI serological survey (Sinclair et al., 2006) were used to classify the farms as either seropositive (one or more seropositive ostriches detected on the farm) or seronegative (no seropositive ostriches detected). Sufficient randomly selected samples had been collected from each epidemiological unit on each farm in order to detect a minimum within-group seroprevalence of 10%, using the standard formula for sample size to detect disease, taking into account group size (Dohoo et al., 2003). The three possible epidemiological units present on a farm were chicks (0–4 months old), slaughter birds (5–14 months old) and breeders. A farm was classified as seropositive if a positive bird was found in any of its epidemiological units. The sera were tested using the haemagglutination inhibition (HI) test according to standard procedures, including pre-treatment by adsorption

with chicken red blood cells (OIE, 2005), using antigen from the H5N2 AI virus isolated from ostriches in the Eastern Cape in 2004 (see Sinclair et al., 2006 for details). A HI titre exceeding 1:16 was regarded as positive. The sensitivity and specificity of this test in ostriches is unknown. The geographic location and H5 AI virus serological status of the farms that were interviewed are shown in Fig. 1.

2.5. Statistical analysis

The independent variables were tested for bivariable associations with the outcome variable (H5 AI virus seropositivity) using the Fisher exact test for categorical variables and simple logistic regression for continuous variables. All independent variables with $P < 0.2$ in the bivariable analysis, and with <15% missing values were included in the initial multiple logistic regression models. Before inclusion, collinearity amongst selected variables was investigated using pairwise Spearman's rank correlation. To determine whether the variable *number of ostriches on farm* should be modeled as a continuous or categorical predictor, it was categorised into quintiles and the log-odds of the outcome for each quintile was plotted against the midpoint of the quintile (Dohoo et al., 2003). In addition, the variable was centred and included in a model together with its quadratic term, and the significance of the quadratic term was assessed.

Firstly a model was developed for all areas combined. In this model, the “Klein Karoo” and “Karoo” regions were combined into a single region and “Agulhas” and “West” were combined (“Western Cape”). This was done on the basis of environmental and managemental similarities between these regions and in order to avoid having regions with very few or no seropositive farms. Three categories of *region* were thus created and it was modeled as a fixed effect. The model was developed by backward elimination by successively dropping the least significant predictor until all independent variables were significant in the model with $P_{lrtest} < 0.05$. All other independent variables, including those not initially selected for inclusion, were then individually retested by addition back into the model, and retained if $P_{lrtest} < 0.05$. Biologically meaningful one-way interactions amongst independent variables remaining in the model were also then tested by addition into the model.

In addition to the above combined model, separate multiple logistic regression models were developed in the way described above for each of the two largest ostrich farming regions, “Klein Karoo” and “Southern Cape”. This was done for the following reasons:

1. There were some bird species that came close to significance in the combined model, i.e. Egyptian geese ($P = 0.1$) and gulls ($P = 0.08$), and which, when forced in, either showed significant interaction with *region* (Egyptian geese, $P = 0.03$) or interaction could not be assessed (gulls) because they did not occur in some regions. The possible role of different wild bird species would therefore best be examined separately for different regions.

2. Significant interaction between *region* and *frequency of cleaning feed troughs* was also found. Because these were categorical variables each with three levels, inclusion of the resultant additional four interaction terms would further complicate interpretation of the combined model.

The fit of each logistic regression model was assessed using the Hosmer–Lemeshow goodness-of-fit test based on deciles of predicted probability of seropositivity. All analyses were done using STATA version 8.2 (Stata Corporation, College Station, TX, USA).

3. Results

The questionnaire was conducted on 84.4% (367/435) of the registered ostrich farms in the sampling frame. The owners of the remaining 68 farms could not be interviewed since they had either sold the farm, discontinued ostrich farming or were otherwise unavailable for the interview. Of these 367 farms, 82 (22.3%) were classified as seropositive (Sinclair et al., 2006), and this varied widely between regions (Table 1). Within-farm seroprevalence on seropositive farms ranged from 2.0% to 42.6%, with a mean of 7.8% (Sinclair et al., 2006). Of the 68 farms that could not be interviewed, 10 (14.7%) had been classified as seropositive. This proportion did not differ significantly from the proportion seropositive amongst the farms that were interviewed (Fisher's exact $P = 0.20$). The number of farms for which responses to each question were obtained, as well as the significance of the bivariable association between each predictor and farm-level H5 AI virus seropositivity, are shown in Table 1.

In the bivariable analyses, the number of ostriches, excluding chicks, present on the farm was positively associated with the risk of seropositivity ($P < 0.001$). There were also bivariable associations ($P < 0.20$) between a number of categorical risk factors and farm-level seropositivity (Table 1). These included the use of large water troughs, the types of feed troughs used and their method and frequency of cleaning, the frequency of visits by Newcastle disease (NCD) vaccinators, the use of lucerne pastures as grazing, the disinfection and cleaning of vehicles, the movement of ostriches onto the farm during the autumn, and the frequency of contact of ostriches with certain wild bird species. These variables, except for *vehicle cleaned and disinfected*, which had a high proportion of missing values, were therefore initially included into the multiple logistic regression models. There was no evidence of collinearity amongst the predictor variables, with |Spearman's rho| < 0.45 for all pairwise correlations. The plot of the log-odds of seropositivity for each quintile of *number of ostriches on farm* vs. the midpoint of each quintile showed a strictly monotonic increase in log-odds with each successively increasing quintile. The quadratic term of the centered variable was also not significant. Therefore, *number of ostriches on farm* was included in the logistic regression model as a continuous variable.

The final logistic regression model for all regions combined, excluding interaction terms, is shown in Table 2. The risk of seropositivity increased with increasing numbers of ostriches on the farm, with reduced frequency of cleaning of feed troughs, with failure to clean and disinfect vehicles and with frequent sightings of white storks amongst ostriches. The variable *vehicle cleaned and disinfected* was initially excluded from the model due to 19% missing values. However, when tested at the end for inclusion in the model, it was significant. Although its inclusion resulted in a reduction from 323 to 263 farms being included in the model, it did not materially change the coefficients for the other predictors; therefore it was retained in the final model.

Table 2.

Final logistic regression model for farm-level H5 avian influenza virus seropositivity in Western Cape ostrich farms—all areas combined (263 farms)

Variable and level	**OR**	**95% CI (OR)**	***P***
Region			
Klein Karoo and Karoo	1	–	–
Southern Cape	0.39	0.17, 0.90	0.027
Western Cape	0.11	0.02, 0.49	0.004
Number of birds on farm (excluding chicks)	–	–	<0.001
Frequency of cleaning feed troughs			
>1×/week	1	–	–
1×/week	4.02	1.33, 12.19	0.014
<1×/week	4.49	1.52, 13.30	0.007
Vehicle cleaned and disinfected			
Yes	1	–	–
No	2.28	1.09, 4.77	0.029
Wild birds seen amongst ostriches			
White storks (*Ciconia ciconia*)			
Never	1	–	–
Seldom	2.06	1.00, 4.27	0.050
Frequently	7.51	1.71, 32.93	0.007

Hosmer–Lemeshow goodness-of-fit test χ^2 = 4.36 (8 d.f.), P = 0.823.

For the Klein Karoo region, the final logistic regression model (Table 3) showed that increased risk of seropositivity was associated with larger numbers of ostriches on the farm, with failure to clean and disinfect vehicles and with frequent observation of Egyptian geese amongst the ostriches. Similar to the first model, the variable *vehicle cleaned and disinfected* was initially excluded due to 30% missing values. However, when tested at the end for inclusion in the model, it was significant. Although its inclusion resulted in a reduction from 163 to 115 farms being included in the model, it did not materially change the coefficients for the other predictors; therefore it was retained in the final model.

Table 3.

Final logistic regression model for farm-level H5 avian influenza virus seropositivity in Western Cape ostrich farms—Klein Karoo (115 farms)

Variable and level	**OR**	**95% CI (OR)**	***P***
Number of birds on farm (excluding chicks)	–	–	0.002
Vehicle cleaned and disinfected			
Yes	1	–	–
No	2.62	1.06, 6.54	0.037
Wild birds seen amongst ostriches			
Egyptian geese (*Alopochen aegyptiaca*)			
Never	1	–	–
Seldom	0.95	0.27, 3.30	0.937
Frequently	3.36	1.08, 10.47	0.036

Hosmer–Lemeshow goodness-of-fit test χ^2 = 8.42 (8 d.f.), P = 0.393.

For the Southern Cape region, the final logistic regression model (Table 4) showed that increased risk of seropositivity was associated with larger numbers of ostriches on the farm, with reduced frequency of cleaning of feed troughs and with frequent observation of gulls

amongst the ostriches. The presence of chicks on the same farm was associated with a reduced risk of seropositivity. Failure to clean and disinfect vehicles was narrowly excluded from this model (P = 0.1). No biologically meaningful one-way interactions were found to be statistically significant in either of the two regional models.

Table 4.

Final logistic regression model for farm-level H5 avian influenza virus seropositivity in Western Cape ostrich farms—Southern Cape (112 farms)

Variable and level	**OR**	**95% CI (OR)**	***P***
Number of birds on farm (excluding chicks)	–	–	0.013
Frequency of cleaning feed troughs			
>1×/week	1	–	–
1×/week	30.07	2.38, 379.4	0.009
<1×/week	53.60	3.32, 864.4	0.005
Chicks present on farm			
Yes	1	–	–
No	0.03	0.002, 0.43	0.010
Wild birds seen amongst ostriches			
Gulls (*Larus* spp.)			
Never	1	–	–
Seldom	6.61	1.39, 31.49	0.018
Frequently	20.63	0.93, 458.6	0.056

Hosmer–Lemeshow goodness-of-fit test χ^2 = 5.88 (8 d.f.), P = 0.660.

4. Discussion

In this study we have shown an association between certain risk factors and farm-level seropositivity to H5 AI virus in ostriches. However, the HI test used for the determination of the serological status of the farms, although validated for chickens, has not yet been validated for ostriches. Therefore, although the tests were performed according to OIE guidelines, the sensitivity and specificity of the test in ostriches are not known. It is possible that the presence of cross-reacting antibodies in ostrich serum may have produced false positives, resulting in overestimation of seroprevalence and a reduction in specificity of the farm-level test. In addition, serological testing had been done in order to detect a minimum within-farm seroprevalence of 10%, so farms with a lower prevalence of seropositive birds were more likely to have been missed, thus reducing the sensitivity of the farm-level test. However, it can be assumed that any misclassification of farm serological status would have been nondifferential, and that any bias in the estimated odds ratios would thus be towards the null (Copeland et al., 1977). This study may therefore have lacked sufficient power to identify certain risk factors for H5 seropositivity. The availability of validated tests for H5 and H7 AI subtypes in ostriches will greatly facilitate investigation of the epidemiology of notifiable AI viruses in this species.

The pathogenicity of AI viruses in ostriches is variable and apparently unrelated to pathotype, with subclinical infection or mild, non-specific clinical signs being common (Olivier, 2006). The failure of the 2005 survey to isolate virus from ostriches in the Western Cape suggests that the virus was no longer circulating in the ostrich population, or was present at a very low prevalence at the time of the survey. It was therefore not possible to determine definitively the viral subtype responsible for the titres. However, in 2004 a LPAI H5N2 virus had been isolated from an Egyptian goose in the Oudtshoorn district (Klein Karoo) and was found to be highly similar to the HPAI H5N2 virus isolated from ostriches in the Eastern Cape later that year (Olivier, 2006). In addition, the only subsequent isolations of AI viruses from ostriches in the Western Cape Province were a HPAI H5N2 virus in the Southern Cape region and a highly similar LPAI H5N2 virus in the Klein Karoo, both in 2006 and both sufficiently different from the 2004 isolates to suggest a separate introduction (M. Romito, 2008, personal communication). It is therefore likely that either a LPAI or a HPAI H5N2 virus had been responsible for the seroconversion in the Western Cape ostrich population during 2004 and had circulated either shortly before or shortly after the outbreak in the Eastern Cape. The importance of ostriches in the transmission or evolution of AI viruses is not known, although they may act as mixing vessels since phylogenetic analysis has shown that an outbreak strain of LPAI H6N2 virus in chickens likely arose by reassortment of LPAI H6N8 and H9N2 viruses isolated from ostriches (Abolnik et al., 2007).

Ostrich farms in the Klein Karoo and Karoo region showed the highest risk for seropositivity, followed by the Southern Cape, while the Western Cape region showed the lowest risk. It is possible that this may be due to geographic spread of the virus from the Eastern Cape Province westwards, but may also reflect differences in other, unmeasured risk factors between the regions. Apart from the inclusion of region as a risk factor in the combined model, no formal spatial analysis was done. However, spatial clustering of all farms and of seropositive farms is evident in Fig. 1. This may be largely due to the clustering of farms along rivers and irrigation canals, particularly in the Klein Karoo which is a semi-arid region.

The number of ostriches on the farm was consistently positively associated with the risk of seropositivity. This increased risk may partially be explained by a higher population density, resulting in more efficient viral transmission, and associated stress resulting in increased susceptibility to infection. However, even if individual birds' probability of infection was the same regardless of farm size, the risk of farm-level infection would increase with the number of birds. The frequency of visits by Newcastle disease vaccinators was significantly associated with seropositivity on a bivariable level, an association that disappeared in the multivariable analysis. This was probably due to confounding by size of farm, since the frequency of visits by vaccinators is largely a function of the number of birds on the farm. Nevertheless, the positive effect of the number of birds on the farm on the risk of seropositivity could still possibly have been related to greater frequency of potentially infectious contacts for large farms, e.g. via Newcastle disease vaccinators.

The reason for the strong negative association seen in the Southern Cape region between the presence of ostrich chicks on the farm and the risk of farm seropositivity, is not known, but may be due to confounding with other, unmeasured management factors. Chicks are normally reared in a more intensive environment, with more human intervention, creating a more disturbing and less suitable environment for wild birds, and it is possible that this effect may extend to the entire farm. None of the chicks tested during the 2005 survey were seropositive, indicating either that the chicks were hatched after the period of active viral circulation, or that chicks (due to different management practices or rearing environment) were not exposed to the risk factors.

The traditional ostrich-farming areas in the Western Cape Province report almost yearly outbreaks of LPAI in ostriches, which have been attributed to introduction by wild birds and certain climatic patterns (Abolnik et al., 2006). During the winter months lower temperatures and wetter conditions are more favourable for the spread of the disease. Stress associated with transport may increase viral shedding (should the newly introduced ostriches be infected) and/or weaken the birds' resistance to infection from the environment. When this occurs in favourable climatic conditions, the infection is more likely to spread. This is supported by the finding in the bivariable analysis that risk of seropositivity was higher when ostriches were moved onto the farm during autumn. Our study also found that failure to clean and disinfect the transport vehicles further contributed to the risk of seropositivity. This is consistent with the fact that transmission of AI viruses amongst domestic poultry is known to occur via movement of contaminated equipment, bird crates, fomites and vehicles and via contaminated organic material ([Capua and Marangon, 2006] and [Senne et al., 2006]).

Previous studies have showed that wild birds can act as reservoirs for viral infections in ostriches ([Alexander, 2000], [Pfitzer et al., 2000] and [Capua and Marangon, 2006]). In 1998 the presence of a H6 AI serotype was demonstrated in an Egyptian goose near Oudtshoorn (Klein Karoo region) shortly before an outbreak involving H6N8 in ostriches (Pfitzer et al., 2000) and in 2004 a H5N2 LPAI virus was isolated from an Egyptian goose in the Klein Karoo shortly before the H5N2 outbreak in the Eastern Cape (discussed above). Both instances suggest that the outbreaks in ostriches may have originated in wild waterfowl. Abolnik et al. (2006) showed by phylogenetic analysis that the AI viruses isolated from wild ducks in South Africa were most likely of Eurasian ancestry, and suggested that waders carry viruses from their breeding grounds and stopover sites, co-inhabited by

Eurasian migrants, to South Africa each year. Viruses shed into local wetlands via the faeces may then be ingested by sympatric indigenous waterfowl, which then become infected and act as reservoir hosts that move extensively throughout the country. Certain wild bird species, for example sacred ibis, storks, gulls and Egyptian geese, are encouraged to visit ostrich camps by open water sources and feeding points. Their numbers often exceed the ostrich numbers in a camp, especially where feed is provided *ad lib*. They congregate around the feeding troughs and watering points and contaminate them with their faeces. Should the wild birds be infected with an AI virus, failure to regularly clean feed troughs will increase the probability of the contamination reaching an infective level. In this study weekly, or less frequent, cleaning of feed troughs, compared to more frequent cleaning, had a strong positive association with the risk of farm-level seropositivity in the Southern Cape region, although this was not found to be significant in the Klein Karoo. This difference between the regions may be due to differences in the feeding habits of wild birds prevalent in the two regions and/or to climatic or management factors affecting the survival of AI virus in feed troughs. At the bivariable level, emptying and refilling the feed troughs, compared to just moving them, also reduced the risk of farm seropositivity. These findings, together with the association between the presence of certain wild bird species and the risk of seropositivity, is consistent with the theory that infection of ostriches may occur via faecal contamination of feed by wild birds.

The role of water sources is less clear in this study, with no significant associations being found between types of water troughs, method or frequency of cleaning of water troughs, or the presence or utilisation of open water sources. However, a large number of responses to the question regarding the method of cleaning of water troughs had to be discarded. Initially, “permanent chlorination” was offered as an additional response to this question, but a markedly greater prevalence of seropositivity was seen amongst farmers giving this response. Further investigation revealed that chlorination of drinking water was very rarely practised prior to the 2005 serological survey, and that institution of this practice was probably done by farmers who perceived their farms to be at risk, or in response to positive serological tests on their own or neighbouring farms. Management of water troughs should therefore not be excluded as a possible risk factor, since AI viral transmission in waterfowl populations is thought to occur by a faecal–oral route via contaminated water, and the virus can survive for extended periods of time in water (Brown et al., 2007).

The species of wild birds that appeared to increase the risk of farm-level seropositivity varied by region. The assessment of the abundance of individual species was difficult to standardise and was subjective, since it depended on the farmers’ observation and identification skills. Nevertheless, the various species included in the questionnaire were easily distinguishable, and for several species the tendency for increasing abundance to be positively associated with farm seropositivity made biological sense. In the Klein Karoo the positive association between frequent observation of Egyptian geese and farm-level H5 AI virus seropositivity is consistent with previous reports suggesting the involvement of this species as a reservoir ([Pfitzer et al., 2000] and [Olivier, 2006]). The crude association between the use of lucerne pastures as grazing for ostriches and the risk of farm seropositivity was probably a case of confounding due to a positive association between lucerne pastures and the abundance of certain wild bird species, namely Egyptian goose, African sacred ibis and white stork.

5. Conclusion

Increased risk of farm-level seropositivity to H5 avian influenza virus in Western Cape ostrich farms during 2005 was associated with increased numbers of ostriches on the farm, with infrequent cleaning of feed troughs, with failure to clean and disinfect transport vehicles, and with increased frequency of contact with certain wild bird species on the farm. Proper management of feeding (and possibly also watering) troughs in order to reduce faecal contamination by wild birds, as well as biosecurity measures to reduce mechanical transmission between premises, should reduce the risk of seropositivity. The validation of the HI test in ostriches is required in order to accurately monitor the occurrence of AI amongst ostriches and to further elucidate the epidemiology of AI in this species.

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