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Avian influenza in ostriches: epidemiological investigation in the Western Cape Province of South Africa

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Summary

An H5N2 avian influenza virus was isolated from ostriches in the Eastern Cape Province of South Africa in July 2004. During a subsequent national survey to determine the possible presence of the disease in other areas of South Africa, specific antibodies against H5 avian influenza were detected by the haemagglutination inhibition test in ostrich sera collected in the Western Cape Province. However, the sampling strategy used in the initial survey was developed to accommodate practical constraints but did not truly reflect the prevalence of the sero-reactor entities. A follow-up survey was therefore conducted from March to May 2005 to obtain more representative samples. None of the 15 126 serum samples collected from ostriches aged 0-4 months was positive, while 2.4% of the 14 664 serum samples collected from slaughter ostriches aged 5-14 months and 3.66% of the 8 791 samples collected from breeder ostriches aged over 14 months were positive. Fourteen farms were inspected in particular to identify relevant risk factors and risk mitigation procedures that could minimise the spread of the disease. It was concluded that biosecurity measures should be improved on ostrich farms, in particular to minimise possible contact between ostriches and wild birds.

Keywords

Avian influenza, Epidemiology, Ostriches, Sero-surveillance, South Africa, Viruses.

Influenza aviaria negli struzzi: indagine epidemiologica nella Western Cape Province, Sud Africa

Riassunto

Nel Luglio 2004 è stato isolato un virus influenzale H5N2 da struzzi nella Eastern Cape Province, Sudafrica. Nel corso di una successiva indagine condotta a livello nazionale al fine di determinare la possibile presenza della malattia in altre aree del Paese, furono individuati anticorpi specifici per virus H5N2, mediante test di inibizione dell'emoagglutinazione, in sieri di struzzi prelevati nella Western Cape Province. Tuttavia, la strategia di campionamento usata nella fase iniziale dell'indagine dava la precedenza a fattori di ordine pratico senza riflettere del tutto fedelmente la prevalenza degli animali sierologicamente reattivi. Pertanto venne condotta una successiva indagine, nel periodo fra Marzo e Maggio 2005, al fine di ottenere campioni più rappresentativi. Nessuno dei 15.126 sieri prelevati a struzzi dell'età compresa fra 0 e 4 mesi risultò positivo, mentre risultarono positivi il 2.4% dei 14.664 sieri raccolti da struzzi macellati in età fra 5 e 14 mesi e il 3.66% di 8.791 campioni prelevati da struzzi riproduttori di età superiore a 14 mesi. Vennero controllati altri allevamenti, in particolare al fine di identificare rilevanti fattori di rischio e procedure atte a ridurli, per ridurre al minimo una diffusione della malattia. Si giunse alla conclusione che negli allevamenti di struzzi debbano essere migliorate le misure di biosicurezza, in particolare per ridurre al minimo possibili contatti fra struzzi e uccelli selvatici.

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Parole chiave
Epidemiologia, Influenza aviaria, Siero-sorveglianza, Sud Africa, Struzzi, Virus.

Introduction

Until July 2004 when the H5N2 subtype of highly pathogenic avian influenza (HPAI) virus was isolated from ostriches (*Struthio camelus*) on a farm in the Eastern Cape Province, this virus subtype had not been reported in domestic poultry in South Africa. The first isolations of influenza viruses from ostriches prior to 2004 were viruses of the H7N1 subtype, obtained during the 1991 outbreak in the Western Cape Province, but these were of low pathogenicity in chickens. During this outbreak, young ostriches aged between 5 days and 14 months were most affected, while very few adult ostriches developed clinical signs. Chicks under one month of age died peracutely with mortality rates often exceeding 80%. Young ostriches between the age of 2 and 8 months had mortality rates that ranged between 15 and 60% (1). Since then, H6N8 (1998) and H10N1 (2001) avian influenza (AI) virus subtypes have been diagnosed in ostriches in the Western Cape. Both of these were classified as low pathogenic avian influenza (LPAI) subtypes (A.J. Olivier, personal communication). During 1998, a H6N8 virus was isolated from a wild Egyptian goose *(Alopochen aegyptiacus*) from the locality where this specific virus subtype was also isolated from an ostrich. Similarly, a virus of the H5N2 subtype was isolated from a wild Egyptian goose in the Western Cape Province in 2004, two weeks prior to the outbreak in ostriches in the Eastern Cape. Unfortunately the H5N2 virus isolated from the Egyptian goose in 2004 was lost and no further classification studies could be conducted (A.J. Olivier, personal communication). These findings emphasise the critical need for further investigation into the role of wild bird populations in the epidemiology of AI.

During the national AI survey conducted in 2004, 50 of the 463 farms tested in the Western Cape Province were serologically positive for H5 notifiable avian influenza (NAI) using the haemagglutination inhibition (HI) test. No clinical symptom or mortality associated with AI was detected on these farms. Despite intensive sampling, followed by antigen detection by polymerase chain reaction (PCR) or virus isolation, only negative results were obtained. Considering that the sampling strategy may influence negative results, a revised sampling frame was designed and adapted to the field situation and management practices on farms. A follow-up survey was therefore conducted from March to May 2005 to obtain more suitably representative samples of ostriches in the Province. No clinical cases were detected but interesting epidemiological deductions could be made from the improved sampling strategy employed. In accordance with previous studies, AI viruses are present in some of the wild aquatic bird populations in the Oudtshoorn area and several farms were inspected to determine the degree of exposure of ostriches to wild birds (7) (A.J. Olivier, personal communication).

Materials and methods

Study population

Field samples were collected from a representative number of ostriches reared on all the ostrich farms in the Western Cape Province over two periods, from August 2004 to February 2005 and from March to May 2005. The number of serum samples collected totalled 17 675 from 463 farms during the first survey and 38 581 from 761 farms during the second survey. Cloacal swabs were also taken and these totalled 228 pools (5:1) during the first survey and 3 189 pools (5:1) during the second survey.

In addition, 14 farms in particular were inspected until the end of the survey to examine any possible differences in management practices that could contribute to an increased or decreased risk of infection. Eleven of these farms had a high number of sero-reactors, while the remaining three farms tested sero-negative although they were all surrounded by sero-positive farms.

Study design

The basic epidemiological formula for the detection of disease was used for the calculation of sample sizes during both surveys:

$$n = \left[1 - (1-\alpha)^{\frac{1}{D}}\right]\left[N - \frac{D-1}{2}\right]$$

(3), where:

α = 1 – confidence level

D = estimated minimum number of diseased animals in the group

N = population size.

At the beginning of the survey in 2004, the assumption was made that the disease would spread rapidly amongst ostriches, as is usually the case in poultry. It was assumed that if the disease were present in an ostrich population, the population would have a minimum expected prevalence of 20%. This was compatible with the World Organisation for Animal Health (OIE) guidelines for the surveillance of NAI in an establishment (6) albeit based on a surveillance strategy to detect the presence of disease in poultry establishments. Using the above formula with 95% confidence intervals, a sample size of 14 ostriches was selected, which was increased to 16 since this was more convenient for the diagnostic laboratory procedures. As the survey progressed, it was evident that the disease did not spread rapidly among ostriches and the minimum expected prevalence was adjusted to 10%, and 30 samples were then collected per farm. On farms where sero-reactors were found, the sampling size was increased to 60, which corresponded to a minimum expected prevalence of 5%. Individually identified birds were tested repeatedly. During the 2004 survey, the slaughter birds (aged 5 to 14 months) were targeted as these were assumed more likely to be subject to exposure to risk factors as they were housed close together during the pre-slaughter period and therefore reared in less extensive conditions than breeding birds. However, this assumption limited the epidemiological deductions that could be made from the results and an epidemiology unit had to be redefined for the second survey in 2005.

Following consultation with local and international experts, the second survey in 2005 was therefore redesigned to detect virus and/or antigen within an epidemiological unit at a prevalence of at least 5% and antibody for H5 NAI at a prevalence of 10%. An epidemiological unit was defined as a group of ostriches that is managed separately from other groups on the farm and thus with a risk profile that differs from the other groups. According to this definition, three possible epidemiology units could be identified on a farm, as follows: 0- to 4-month-old chicks (normally held in intensive rearing systems), 5- to 14-month-old slaughter birds (mostly intensive feedlot situations) and breeders (extensive range situations). A stratified random sampling approach was adopted with the three above groups constituting the strata. Random samples were taken from all the inhabited camps on a property and weighted according to group size in a specific camp. To ensure the correct sampling size for each farm, the individual farms were previously visited by officials to obtain a camp-by-camp census captured on a specifically designed census form. The data was gathered, compiled and filed at a central epidemiology centre and presented on a spreadsheet that was calibrated to calculate the sample sizes required from each camp on the farm. A print-out of the

sampling frame was provided to the sampling teams thereafter. This translated to approximately 60 samples (cloacal swabs) per epidemiology group (180 per farm) for virus detection and approximately 30 serum samples per epidemiology group (90 per farm) for serology.

Sampling procedures

Blood was collected from the jugular vein in 7 ml yellow stopper gel tubes, containing clot activator. The sera were immediately separated by centrifugation before transport by courier to the relevant diagnostic laboratory: the Provincial Veterinary Laboratory in Stellenbosch, the Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI) or the Allerton Provincial Laboratory, depending on available capacity of the laboratories on a specific day.

Cloacal swabs were collected and grouped in five swabs pooled in 5 ml of phosphate buffered saline (PBS) in cryotubes, without addition of antibiotics. The swab samples were despatched on ice within 12 h of collection by courier to the PCR Laboratory of the Onderstepoort Veterinary Institute.

Analytical procedures

The sera were tested for HI antibodies. The HI test was conducted in accordance with the procedure described in the 5th edition of the *Manual of diagnostic tests and vaccines for terrestrial animals* (5). The sera were adsorbed in advance with packed chicken red blood cells to remove any interfering factors. The HA antigen used was a strain of H5N2 virus isolated from infected ostriches in the Eastern Cape Province in 2004. The HA titre of this antigen varied from log 2^4 to log 2^5 between individual tests. Two-fold-dilution of serum was added with 4 units of HA antigen and 1% chicken red blood cell suspension was added to the wells. A HI titre exceeding 1/16 (log 2^4) was judged as positive.

The extracts of swabs were tested for the presence of antigen using the reverse transcriptase polymerase chain reaction (RT-PCR) assay according to the Starick method, which uses primers to the matrix gene (9).

Data analysis

The data was entered into a Microsoft Access© database to record all relevant epidemiological information and a Microsoft Excel© spreadsheet for statistical analysis. The statistical analysis included methods to determine central tendency (e.g. mean) and methods of spread (e.g. range).

Results

Table I summarises the results of the first (August 2004 to February 2005) and second (March to May 2005) surveys and Table II depicts the additional epidemiological information obtained during the second survey.

All of the 14 farms inspected for epidemiological analysis were using concrete watering troughs (similar to those used in cattle and sheep farming) and vehicle tyres for feed holders. One of the three sero-negative farms was almost free from contact between ostriches and wild birds (including waterfowl and terrestrial birds) but on the other two farms a high degree of contact with waterfowl was observed. The biosecurity measures on the latter two farms differed from others in which water troughs and feed holders had been cleaned at least once a week.

Discussion

In the second survey, there were two main reasons to explain why a greater number of farms were analysed. The first depended on farming practices that are characteristic of the ostrich industry. Some farms had no ostriches during the first survey and were consequently omitted from testing. The second was geographic location. This might be

Table I
Comparison between results from the first survey on avian influenza (August 2004 to February 2005) and second survey (March to May 2005)

Epidemiological parameters	First survey	Second survey
Number of farms tested	463	761
Number of farms positive for HI antibody[a]	50	124
Number of farms positive for antigen/virus (RT-PCR) detection[b]	0	0
Mean on-farm sero-prevalence	16.08%	7.82%
Range of on-farm sero-prevalence	0%-60%	2-42.55%
Number of serum samples	17 675	38 581
Rate of positive serum samples	7.39%	1.68%
Mean HI titres	log 2^5	log 2^5
Range of titres	log 2^{4-11}	log 2^{4-11}
Number of swab pools	228	3 189
Number of swab pools positive for antigen	0	0

a) antibody detection to H5N2 using the HI test
b) detection of H5N2 by PCR

more important since some farmers use different land areas under the same name and registration number. In the second survey, these farms were considered as separate units in order to obtain more precise geographic locations.

The higher number of antibody-positive farms (16.29% of farms tested in the 2005 survey versus 10.80% of farms tested in the 2004 survey) could raise some concerns regarding the evolution of the disease in the area between August 2004 and May 2005. However, there are several facts that prove the contrary. The average on-farm sero-prevalence decreased from 16.08% to 7.82% and the total percentage of positive samples decreased from 7.39% to 1.68%. This, together with the fact that no virus could be detected by PCR and no ostrich chicks aged 4 months or younger tested antibody-positive, suggest that the increased number of farms with antibodies could rather be attributed to the increased number of farms tested

Table II
Sero-prevalence of avian influenza in the different epidemiological units during the survey (March to May 2005)

Epidemiological parameters	Chicks (0-4 months)	Slaughter birds (5-14 months)	Breeders (>14 months)
Number of samples tested	15 126	14 664	8 791
Rate of positive serum samples for HI antibody*	0%	2.40%	3.66%
Mean on-farm sero-prevalence	0%	9.57%	11.43%
Range of on-farm sero-prevalence	0%	0-42.86%	0-85.71%

* antibody detection to H5N2 using the HI test

and the improved sampling strategy employed. A relatively low rate of total positive samples (7.39% and 1.68%, respectively) and a low number of sero-prevalent farms suggest that the disease was not spreading rapidly among farms and even among ostriches. Experience from the 1991 outbreak with H7N1 virus showed that mortality in young birds reached 80%. Comparing the present data with the 1991 outbreak, the virus responsible for the H5 antibodies in the Western Cape Province could be classified as a virus of lower pathogenicity for ostriches.

The average sero-prevalence amongst breeder birds (11.43%) is higher than the average for slaughter birds (9.57%). This could indicate an increased exposure to risk factors in the extensive environment among the breeder birds but also a history of previous exposure as it is uncertain how long detectable antibody titres remain in adult ostriches after viral challenge. On certain farms, ostriches are normally kept under extremely extensive conditions and the birds are paired into separate camps during the breeding season from May to January. Human contact and interventions are restricted to a minimum, resulting in the undisturbed access of wild birds to feed and water in these camps. Chicks of less than 4 to 5 months of age are normally reared in a less extensive environment and are frequently visited by humans, creating an unsuitable and disturbing environment for wild birds. This might explain why none of the 15 126 chicks tested demonstrated detectable antibodies. The Karoo area of the Western Cape Province, where the majority of sero-reactor farms were detected, is a semi-arid region with sparse natural grazing and an average rainfall of 228 mm. The majority of reactor farms are located along rivers and in riverine areas where ostrich farmers also established irrigated lucerne pastures for ostriches and sheep. Wild migratory birds and other waterfowl are abundant in these areas and accumulate in vast numbers on the ostrich farms. Previous studies confirm that these birds are the most probable source of viral infections (7) (A.J. Olivier, personal communication). They tend to graze with ostriches on irrigated pastures and concentrate in great numbers around the watering troughs and feeders where contamination occurs through faecal matter. The exposure of ostriches to viral challenge could be minimised by applying biosecurity measures aimed at reduced contact between wild birds and ostriches. The most important procedure is the regular cleaning of watering and feeding troughs with a viricidal agent. It seems that such a management practice reduces the viral load in the environment and subsequently prevents the infection of ostriches. Other management practices, such as the utilisation of water and feeding troughs that do not attract wild bird species, could also be employed. The concrete troughs and vehicle tyres that are mostly used on ostrich farms create an ideal environment for social gatherings of migratory birds. Smaller water troughs that are elevated from the ground that have sharp edges as well as self-feeders will discourage wild birds from making use of these resources. Terrestrial birds, e.g. hadeda (*Bostrychia hagedash*), African sacred ibis (*Threskiornis aethiopicus*), various dove species, etc., also have extensive contact with the ostriches and the possible role of these birds in the spread of the disease needs to be investigated.

Conclusions

The serological findings and intensive epidemiological investigations indicate that there was no active H5 NAI virus circulating for at least four months prior to the second survey. The slow spread of the H5 AI virus, estimated from the sero-prevalence study, also suggests that the virus that affected the Karoo region of the Western Cape

was not highly transmissible between ostriches. It is generally accepted that wild waterfowl play a major role in the global dissemination of AI and therefore also pose a constant threat of infecting ostriches with these viruses, given their close contact (2, 4, 8). Although vaccination of ostriches in South Africa is prohibited and thus has not been implemented as part of a regular health management procedure, a means to limit virus excretion needs to be considered in view of a possible seasonal viral challenge from migratory birds. Biosecurity measures should be improved on all ostrich farms and should be aimed specifically at minimising contact between ostriches and wild birds.

The epidemiological investigations conducted in 2004 and 2005 also indicate that ostriches do not respond to AI virus infection in the same way as chickens. Chickens and turkeys are domesticated birds that are reared in intensive conditions which is not at all compatible with the management practices applied on ostrich farms. The sensitivity of ostriches to AI viruses may also be different in comparison with the common domesticated poultry species. Further research on the epidemiology and pathogenicity of AI in ostriches is urgently needed, including research on the role of wild waterfowl and terrestrial birds in transmitting the virus to ostriches reared under different managerial practices.

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