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Risk of Human Infections With Highly Pathogenic H5N2 and Low Pathogenic H7N1 Avian Influenza Strains During Outbreaks in Ostriches in South Africa

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Abstract

Background—Risk factors for human infection with highly pathogenic (HP) and low-pathogenic (LP) avian influenza (AI) H5N2 and H7N1 were investigated during outbreaks in ostriches in the Western Cape province, South Africa.

Methods—Serum surveys were conducted for veterinarians, farmworkers, and laboratory and abattoir workers involved in 2 AI outbreaks in the Western Cape province: (1) controlling and culling of 42 000 ostriches during (HPAI)H5N2 outbreaks in ostriches (2011) (n = 207); (2) movement control during (LPAI)H7N1 outbreaks in 2012 (n = 66). A third serosurvey was conducted on state veterinarians from across the country in 2012 tasked with disease control in

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general (n = 37). Antibodies to H5 and H7 were measured by means of hemagglutination inhibition and microneutralization assays, with microneutralization assay titers >40 considered positive.

Results—Two of 207 (1%) participants were seropositive for H5 and 4 of 207 (2%) for H7 in 2011, compared with 1 of 66 (1.5%) and 8 of 66 (13%) in 2012. Although individuals in all professions tested seropositive, abattoir workers (10 of 97; 10.3%) were significantly more at risk of influenza A(H7N1) infection ($P = .001$) than those in other professions (2 of 171;1.2%). Among state veterinarians, 4 of 37(11%) were seropositive for H7 and 1 of 37 (2.7%) for H5. Investigations of (LP)H7N1-associated fatalities in wild birds and quarantined exotic birds in Gauteng, AI outbreaks in poultry in KwaZulu-Natal, and ostriches in Western Cape province provide possible exposure events.

Conclusion—(LPAI)H7N1 strains pose a greater infection-risk than (HPAI)H5N2 strains to persons involved in control of outbreaks in infected birds, with ostrich abattoir workers at highest risk.

Keywords

Highly pathogenic; H5N2; Low Pathogenic H7N1; Avian Influenza; humans; ostriches; Africa

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Avian influenza (AI) viruses known to infect humans belong mainly to the H5 and H7 subtypes, although sporadic cases and death after H6, H9, and H10 infections have been reported [1–4]. To date highly pathogenic (HP) H5N1 and low pathogenic (LP) H7N9 AI viruses are associated with a high fatality rates in humans while most other H5 and H7 strains cause mild influenzalike illness or conjunctivitis [1]. In a 2003 (HPAI)H7N7 outbreak in the Netherlands, 89 human infections occurred, including a single fatality in a veterinarian [5]. The frequent reports of human infections and deaths due to (LPAI)H7N9 strains raise questions over transmissibility and pathogenicity of other H5 and H7 strains in humans [6–8].

In poultry, LPAI strains mostly cause subclinical infections restricted to the respiratory tract [9], whereas HPAI strains cause systemic infections with high mortality rates. Although (HPAI)H5N1 or (LPAI)H7N9 strains have never been detected in South Africa, outbreaks of (HPAI)H5N2, (LPAI)H7N1, and (LPAI)H7N7 occurred on several occasions in ostriches in the Western Cape province (WCP) [10–14]. H7 outbreaks were subclinical and detected through serosurveillance [10, 12, 15], whereas juvenile birds showed signs of depression, weakness, and death after (HPAI)H5N2 infection, displaying necrotizing hepatitis, splenitis, and airsacculitis at necropsy [16]. Surveys conducted in South Africa suggest that the ecology and epidemiology of infections in ostriches are related to contact between migratory water birds and ostrich flocks through the free-range production systems [17–19]. H5 and H7 AI strains have been detected in wild and migratory birds in a number of locations in South Africa [20].

The occurrence of outbreaks due to HPAI viruses are devastating to the ostrich industry owing to international trade restrictions and carefully controlled through vigorous surveillance, movement control, and culling of infected birds by veterinary services from the

provincial departments of agriculture, forestry, and fisheries [21]. In 2004, risk of infection to humans involved in control efforts was assessed through a serosurvey of an outbreak of (HPAI)H5N2 in the Eastern Cape province (L. B., unpublished data) when 26 000 ostriches were culled [22]. Less than 2% of 130 individuals involved seroconverted to H5 (1 veterinarian and 2 farmworkers), and none showed clinical signs (unpublished data, L. B.). Although the risk of severe disease due to AI exposure seems to be low, the high human immunodeficiency virus (HIV) seroprevalence in South Africa [23], raises concern over potential severe disease and prolonged shedding in immunocompromised individuals. HIV has been shown to be a risk factor for severe seasonal influenza infections in young HIV-positive adults in South Africa [24] but no data on disease severity of AI in HIV-positive individuals exist.

In February 2011, (HPAI)H5N2 outbreaks were confirmed by reverse-transcription polymerase chain reaction (RT-PCR) in ostriches in the Oudtshoorn district of the WCP, which resulted in slaughtering of 42 000 ostriches. The outbreak lasted until November 2011. Shortly thereafter, an outbreak of (LPAI) H7N1 was reported in Heidelberg District. Seropositive birds were identified during routine surveillance in December 2011, and acute cases confirmed by RT-PCR in May 2012. Movement control was implemented at the time of the (LPAI)H7N1 outbreak [25, 26].

To investigate the risk of infection to individuals exposed to (AI)H5 and H7 viruses detected in South Africa, we conducted a serosurvey in animal handlers and individuals involved in the control of AI in the country. This included veterinarians and laboratory workers involved in surveillance and outbreak control efforts, farmworkers on infected farms and abattoir workers involved in slaughter of ostriches exposed to the 2 outbreaks of (HPAI)H5N2 in August 2011 and (LPAI)H7N1 in May 2012, as well as veterinarians tasked with disease control across the country attending a conference for state veterinarians in August 2012.

Materials and Methods

Study Participants

Serum specimens were collected on 3 occasions from humans that may have been exposed to (HPAI)H5N2 or (LPAI)H7N1 after informed consent. The study was cleared by the University of the Witwatersrand (2011) and University of Pretoria (2012) ethics committees.

Survey 1—In August 2011, serum specimens were collected from 207 persons that had direct contact with ostriches through handling or culling of infected birds on farms that tested (HPAI)H5N2 positive. Questionnaires were completed to obtain demographic details and retrospective information on clinical symptoms after culling or handling of ostriches due for culling.

Survey 2—In August 2012, serum specimens were collected from 66 veterinarians, ostrich farmers, farmworkers, and laboratory workers during an AI stakeholders meeting on the ostrich research farm in Oudtshoorn organized by the WCP Department of Agriculture, as well as abattoir workers at provincial abattoirs involved in slaughter of ostriches after the

outbreaks. Study participants may have been exposed to AI during the H7N1 or previous H5N2 outbreaks.

Survey 3—As control group, serum specimens were collected from 38 veterinarians at a conference held in Pretoria, Gauteng province, in August 2012. The conference is mainly attended by state veterinarians from across the country, a proportion of whom had been involved in the control of the AI outbreaks. Inclusion criteria included any veterinarian involved in animal disease control, including outbreaks in poultry, livestock, and wildlife in South Africa. Limited demographic information was collected from each participant. Interviews were subsequently carried out with seropositive individuals to assess possible exposure events or illness.

Hemagglutination Inhibition Assay

The World Health Organization (WHO) influenza protocol for serum hemagglutination inhibition (HAI) was followed from the influenza surveillance manual [27] using both horse and turkey red blood cells (RBCs) in parallel to determine the optimum indicator cells for the H7N1 and H5N2 autologous strains.

Serum specimens were pretreated with Receptor Destroying Enzyme (Denka Seiken; lot 412061) overnight at 37°C and then heat-inactivated at 56°C for 30 minutes, followed by adsorption with RBCs. Samples were further diluted 1:10 in 0.85% saline and stored at −20°C until testing.

Serum specimens from 2011 were tested with HAI assays using both turkey (1%) and horse (0.75%) RBCs as indicator cells against the inactivated H7 and H5 antigens. For 2012, HAI titers obtained for control antisera against the antigens were as follows: H5N2, 64 for turkey and 16 for horse RBCs; H7N1, 512 for both; and H7N7, 256 for both. Owing to the low titers obtained for H5N2 antigen and antisera, we selected to continue using turkey RBCs only for HAI assays performed with the H5 antigen on serum specimens from 2012, whereas we used both turkey and horse RBCs as indicator cells for the H7 antigens. Because horse RBCs did not generally yield better titers than turkey RBCs and were more difficult to interpret, only results for turkey RBCs are reported.

HAI assays were performed in duplicate with horse and turkey RBCs, according to the WHO protocol [27], with reference antigens from the WHO 2012 HAI kits as well as autologous inactivated H5N2 and H7N1 antigens (Deltamune; AI diagnostic laboratory approved by the provincial departments of agriculture, forestry, and fisheries) specific to the 2011 and 2012 ostrich AI outbreaks. The same AI antigens and their respective antisera were used to conduct all assays: H5N2 (lot 291012; expiration 1 October 2013), H7N1 (lot 190712; expiration 31 August 2013), and H7N7 (lot 190712; expiration 31 August 2013) (Deltamune). Participant serum specimens from 2011 were tested by HAI assays against antigens of H5N2, H7N1, and influenza A(H1N1)pdm09 (the dominant seasonal influenza strain in 2011). The 2012 serum specimens were tested against inactivated H5N2, H7N1, H7N7, H3N2 (dominant seasonal strain in 2012), and A(H1N1)pdm09. Control reagents were included to monitor nonspecific agglutination and specific agglutination by test virus

antigens. The serum HAI titers were expressed as the reciprocal of the highest dilution of serum where hemagglutination was inhibited.

Serum Microneutralization Assay

Microneutralization (MN) assays (MNAs) were performed in duplicate, according to the WHO protocol for influenza surveillance [27], on all serum specimens with HAI titers >20 for H5N2 and H7N1 antigens after the 2011 H5 outbreak and on all serum specimens with titers >10 after the 2012 H7N1 outbreak. The following local virus isolates were used: AI A/H5N2/S2011/06_0005/2011 and AI A/H7N1/S2013/04_169/2013, both originally isolated from ostrich (obtained from the WCP Department for Veterinary Services). All MNA and passaging of AI viruses were done under enhanced biosafety level 3 conditions at the Centre for Viral Zoonoses, University of Pretoria.

Virus stocks were propagated in either the allantoic cavity of 10-day-old special pathogen-free embryonated hen's eggs or in Madin-Darby canine kidney (MDCK) cells. MDCK cells were seeded and maintained in Dulbecco's modifed Eagle medium (Lonza) containing 4.5 g/L glucose, with L-glutamine, without sodium pyruvate, 10% fetal bovine serum, 2 mmol/L L-glutamine, and antibiotics; incubated at 37°C and 5% carbon dioxide; and harvested 24–73 hours after inoculation. Cultures were clarified by centrifugation, aliquoted, and stored at −70°C. The titre of cultured virus was established using the WHO protocol [27] and the 50% tissue culture infectious dose established according to [28].

Freshly trypsinized MDCK cells were diluted in Dulbecco's modified Eagle medium at 3×10^5 cells/mL and 100 μL was seeded per well on 96-well high-binding tissue culture plates and incubated overnight at 37°C in a 5% carbon dioxide incubator, washed, and used for either virus titration or virus neutralization [27]. Each MNA included a virus control, cell control, and back titration on the virus. Other controls were known positive and negative serum specimens. MNA readout was performed by detection of residual virus after serum neutralization, using enzyme-linked immunosorbent assay to detect the viral nucleoprotein. The absorbance was measured at 492 nm with an automated plate spectrophotometer and neutralizing end point determined by 50% specific signal calculation. The end point titer was expressed as the reciprocal of the highest dilution of serum that showed 50% neutralization [28].

Statistical Analysis

Statistical analysis was carried out with Stata software (version 12). The Fisher exact test was used to compare frequencies of categorical variables, and 95% confidence intervals for the observed prevalence were obtained using the binomial distribution. Differences were considered statistically significant at $P < .05$.

Results

Serological Testing

HAI- and MNA-positive reactions for H5N2 and H7 are listed in Table 1. All HAI titers >20 were confirmed by MNA in 2011, and all serum specimen HAI titers >10 were tested by

MNA in 2012. Because H5 and H7 do not circulate in the general population and tests were performed 3–6 months after exposure, MNA titers >40 were considered likely to be positive. In general, MN titers were higher than HAI titers for all cases that could be confirmed by both assays, with the majority of positive reactions having MN titers >80 (Table 1).

Seroprevalence and Participant Demographics

Survey 1: Influenza A Antibodies in Animal Handlers Involved in the (HPAI)H5N2 Outbreak in 2011—Seroprevalence to (AI)H5N2, H7N1 as well as influenza A(H1N1)pdm09, was determined in 207 persons involved in the H5N2 outbreak in 2011 (Table 2). Enrolled participants were 17–74 years of age (mean, 35 years). MNA confirmed 2 individuals (1%) with antibody titers of 80 and 320 to H5N2 (Table 1). Demographic data were available only for the latter participant, a male farmworker aged 41 years, included in Table 2.

MNA identified 4 (2%) positive donors workers in survey 1 with H7N1 antibodies titers ranging from 40 to 80 (median, 80) (Table 1), suggesting a seroprevalence of 7.1% (4 of 56 in abattoir workers relative to 0 of 207 in all other occupations; $P = .06$) (Table 2). Three were male, and 3 were aged 17–30 years.

One hundred of 207 participants (48%) had neutralizing antibodies to A(H1N1)pdm09 at titers of ≥40. Control antisera to H7 and H5 did not cross-react with the A(H1N1)pdm09 antigen. Most seropositive individuals (55 of 89; 61.8%) were women (aged 17–30 years). Most influenza A(H1N1)pdm09 positive participants were abattoir workers, followed by farmworkers. In total 52 of 207 participants (23%) were vaccinated to seasonal influenza in 2011, including 60% of abattoir workers.

Survey 2: Influenza A Seroprevalence in Individuals Involved in the H7N1 2012 Outbreak—Overall, 8 of 66 participants (13%) had neutralizing antibodies to H7 and 1 of 66 (1.5%) to H5 at titers >40 (Table 1). Individuals ranged from 17 to 60 years of age, with no significant difference in positivity between the age groups (Table 3). Most H7-seropositive individuals were male (male 7/50 [14.0; 5.8–26.7], female 1/16 [6.2; 0.1–30.2]; $P = .6$). Most positive participants were abattoir workers (6 of 41; 15%). A single laboratory worker (1 of 4; 25%) responsible for diagnosing AI cases in birds also positive for H7, as well as a veterinary technician who culled ostriches (1 of 8; 12%) ($P = .60$).

The H5-positive participant was a researcher involved in a *Mycoplasma* vaccine trial in 2011 in ostriches, who was later reported to be positive for (AI)H5N2. In a pooled analysis of the data from the 2011 and 2012 surveys, abattoir workers (10 of 97; 10.3%) were significantly more at risk of influenza A(H7N1) infection than any other profession (2 of 171; 1.2%) ($P = .001$).

Seroprevalence in this group was high for both seasonal influenza A(H1N1)pdm09 (67%) and H3N2 (70%), with abattoir workers having the highest seropositivity rates for seasonal influenza strains, at 84% and 94%, respectively, for A(H1N1) pdm09 and H3N2 ($P = .02$ and $P < .001$, respectively). Data on vaccination were not available.

Survey 3: AI Seroprevalence in State Veterinarians Across the Country—Of 37 state veterinarians from across South Africa, 4 (10.8%) had neutralizing antibody titers >40 to H7 and 2 (5%) to H5 (Table 4) . Positive individuals were aged 30–60 years, and 50% were male. H7-positive veterinarians were from Gauteng, North West, and KwaZulu-Natal provinces. Follow-up interviews to determine possible sources of exposure indicated that of 4 veterinarians positive for H7, 1 was involved in investigation of wild bird die-offs in Gauteng, where H7N1 were identified; 2 had inspected quarantine stations where an outbreak of AI H7 had been reported in exotic birds in Gauteng-, and 1 had inspected poultry farms where outbreaks of H6 had been reported in KwaZulu-Natal province. The H5-positive individual was from the WCP and involved in control of AI in ostriches.

Clinical Signs

For survey 1, questionnaires were completed by recollection of time of onset and symptoms during the H5 outbreak. We compared reported signs in H5- and H7-seropositive individuals with A(H1N1)pdm09 seropositivity. Most participants who reported influenzalike illness during the months of May and June were also seropositive for influenza A(H1N1)pdm09, but most were vaccinated. One veterinarian reported sore and red eyes consistent with conjunctivitis during January, which is outside the human influenza season in South Africa and coincided with the time when the H7N1 AI outbreak started.

Protective Gear

Data were collected on use of personal protective equipment (PPE) in survey 1. Of individuals who tested positive to H7N1, 1 of 4 reported wearing gloves ($P = .37$), 2 of 4 had worn masks (N95) ($P = .88$), and 0 of 4 had worn goggles ($P = .23$) when dealing with ostriches in the abattoir.

Discussion

We investigated individuals with a high risk of exposure to AI viruses in South Africa to define the infection potential in animal handlers. All subjects were initially screened with HAI assays against autologous antigen obtained after the 2011 and 2012 outbreaks of (HPAI)H5N2 and (LPAI)H7N1 and confirmed by microneutralization assays, and all donors were screened by MNA in 2012. Human seasonal influenza strains were included to evaluate cross-reaction. Survey 1 included individuals who were directly involved in the H5N2 outbreak, but because the H7N1 outbreak started toward the end of the H5N2 outbreak, they were also screened for H7 antibodies. Only 2 individuals could be confirmed as H5N2 seropositive despite the seemingly high exposure risk during culling of 42 000 ostriches in 2011. In the 2012 group, H5N2 antibodies were detected in a researcher unknowingly exposed to infected ostriches in 2011. This low level of infection with H5N2 is consistent with findings during the 2004 outbreak, in which <2% of persons seroconverted, despite culling of 26 000 ostriches (unpublished data).

Surprisingly, 4 participants from 2011 had antibodies of >80 to H7, all abattoir workers. Serum specimens were collected from this group in August 2011, before the 2012 H7N1 outbreak was detected by serology. Although it cannot be said with certainty when their

exposure occurred, the findings suggest that the virus may have gone undetected for several months before identified serologically, given the low pathogenicity of this strain for ostriches. According to the interviews at the time, the use of PPE was inconsistent in positive individuals.

In the second serosurvey, individuals involved in the 2012 (LPAI)H7N1 outbreak were assessed, some who were also involved in the 2011 H5N2 outbreak. In this group, 7% had antibodies to H7, with the highest seroprevalence among laboratory workers (25%; 1 of 4), followed by abattoir workers (14%; 6 of 41) and veterinary staff (12%; 1 of 8). Statistically significant associations with occupation could not be achieved owing to small sample size and low detection rate; however, combined findings from 2011 and 2012 suggest that abattoir workers (10 of 97; 10.3%) had a significantly higher risk of being infected with H7 strains relative to all other occupations (2 of 171; 1.2%) ($P = .001$). A single case of conjunctivitis and mild influenza-like illness was recorded in a veterinarian during the H7N1 outbreak, which occurred outside the normal influenza season and may have been related to this outbreak. H7 strains are associated with ocular infection and conjunctivitis, which may spread to the respiratory tract [5, 29, 30]

Ostriches had not been culled during the H7N1 outbreak but had been placed under quarantine and tested with RT-PCR until negative tracheal and cloacal swabs samples before they sent for slaughter. According to current recommendations, a sample of birds are assessed serologically on 6 monthly routine surveys, and seropositive birds are slaughtered only when no more RT-PCR–positive cases are detected [21]. Potential exposures of veterinarians and laboratory staff may thus reflect exposure at the time of collection or handling of infected respiratory specimens, whereas exposure may have occurred before the outbreak was detected in abattoir workers. Retrospective interviews with abattoir owners suggested that positive individuals worked on the line where the intestines were removed.

Although studies with (LPAI)H5N1 show that LPAI strains are mostly localized in the lungs in chickens, a recent study showed that systemic distribution of LPAI H7N1, H7N7, H5N2, and H9N2 strains does occur in chickens, with virus detected and isolated in multiple organs, including lung, brain, intestine, peripheral blood mononuclear cells, heart, liver, kidney, and spleen [31, 32]. During 2012, wild birds (sacred Ibis and Egyptian geese) sampled in bird die-offs in Gauteng province tested positive for (LPAI)H7N1 in heart, spleen, and brain samples (Zoonoses Research Unit, University of Pretoria and National Institute for Communicable Diseases, and confirmed by the Onderstepoort Veterinary Institute) (unpublished data). Although (HPAI)H7N1 and (HPAI)H5N2 are known to occur in multiple organs in ostriches [16], tissue tropism and systemic infection of LP strains are lacking and should be addressed.

Finally, 4 of 37 state veterinarians (10.8%) not directly involved in disease management of ostriches also tested positive for H7. One had been responsible for investigating the Sacred Ibis and Egyptian geese die-offs mentioned above in 2012 in Gauteng, and 2 had investigated fatalities in a quarantine station in Gauteng. In 2012, exotic birds due for exports tested positive for a H7 in an animal quarantine station in Gauteng province and were culled out by the veterinary services, which incident was possibly related to the

veterinarians testing positive in the current group [15]. The fourth veterinarian positive for H7 was responsible for inspecting poultry farms in KwaZulu-Natal, including a H6 AI outbreak at the time. Although H7 had not been reported in poultry, they are a possible source of infection. These findings emphasize that all bird die-offs in the country should be treated with caution, irrespective of where they occur.

Several outbreaks of LPAI and (HPAI)H7N1 and (LPAI) H7N3 viruses occurred in poultry in Italy between 1999 and 2003 [33–35]. A serological survey of poultry workers found evidence of anti-H7 antibodies in 3.8% in 2003 when (LPAI) H7N3 virus circulated, and 1 of 185 reported signs of conjunctivitis during the outbreak. A serosurvey conducted after the epizootic outbreak caused by (HPAI)H7N7 in the Netherlands in 2003 identified antibodies in approximately half of exposed individuals and household contacts of infected persons [5, 36]. H5N2 transmission has been reported from poultry and wild birds to humans in Japan and the United States, respectively, although no symptoms were recorded [37, 38].

The current study has several limitations that warrant discussion. Because H5 and H7 do not circulate in the general population and the survey was done several months after exposure, HAI assays were used for screening serum specimens, and only MN titers >40 were considered positive, which may result in either underestimation or overestimation of infections. The majority of positive cases, however, did have titers >80 and were probably true-positives. Active surveillance in humans to detect acute cases had not been performed during the outbreaks. Only basic questionnaires were completed for surveys 2 and 3, limiting information on PPE and symptoms at the time of exposure. Retrospective interviews of state veterinarians may reflect their perceptions of where they may have been infected and did not measure their risk relative to uninfected individuals.

Despite the above shortcomings, our study findings may be used to make specific recommendations. First, biosafety procedures should be enhanced during the handling of AI specimens in veterinary laboratories, during collection of specimens and slaughtering of ostriches, and when investigating any bird die-offs in Southern Africa, irrespective of province. Compliance to biosafety requirements and use of PPE should be monitored during outbreaks and slaughtering of ostriches. Noncompliance has been associated with an increased risk of infection with H7 during culling in other outbreaks [39]. Heat treatment of ostrich meat and products reduces consumer risk, but tissue tropism and the length of time H7 strains remain in ostriches should be investigated to identify exposure risk during slaughter. The high seasonal influenza seroprevalence, particularly in abattoir workers, probably relates to vaccination rates, with 24% overall vaccine uptake versus 60% in abattoir workers suggesting that abattoirs already encourage vaccination among their workers. Active surveillance and vaccination of all high-risk groups for seasonal influenza may help identify AI cases and reduce the opportunity for reassortment events.

In conclusion, humans involved in the control of AI outbreaks in South Africa are at low risk of being infected with influenza A H5N2, but cases do occur. H7 strains seem to pose a greater risk of infection to abattoir workers, veterinarians, and laboratory workers. Clinical signs seem to be limited to conjunctivitis and influenzalike illness, but more active surveillance is needed to describe clinical cases.

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Table 1

HAI and MN Assay Titers for Participants in Survey 1, 2, and 3[a]

		H5N2		H7N1	
Study Identifier	**Year**	**HAI Titer**	**MN Titer**	**HAI Titer**	**MN Titer**
Survey 1	N = 207	n = 3	n = 2	n = 4	n = 4
1695	2011	20	10	10	ND
1846	2011	40	< 10	10	ND
1848	2011	40	< 10	< 10	ND
1874	2011	**20**	**320**	< 10	ND
1875	2011	**20**	**80**	< 10	ND
1877	2011	160	< 10	< 10	ND
1822	2011	< 10	ND	**40**	**80**
1837	2011	< 10	ND	**40**	**80**
1844	2011	< 10	ND	**40**	**80**
1865	2011	< 10	ND	**40**	**40**
Total positive		6 > 20; 3 > 40	2 > 40	4 > 40	4 > 40
Survey 2	N = 66	n = 1	n = 1	n = 1	n = 8
6670	2012	< 10	ND	**10**	**40**
6671	2012	**20**	**160**	< 10	ND
6673	2012	< 10	ND	**10**	**40**
6690	2012	< 10	ND	**10**	**40**
6692	2012	< 10	ND	**10**	**40**
6696	2012	< 10	ND	**10**	**40**
6706	2012	< 10	ND	**10**	**40**
6709	2012	< 10	ND	**10**	**80**
6723	2012	< 10	ND	**40**	**40**
Total positive		1 > 20; 0 > 40	1 > 40	1 > 40	8 > 40
Survey 3	N = 37	n = 1	n = 1	n = 4	n = 4
SAH4	2012	**20**	**160**	ND	ND
SAH14	2012	< 10	< 10	**20**	**160**

		H5N2		H7N1	
Study Identifier	**Year**	**HAI Titer**	**MN Titer**	**HAI Titer**	**MN Titer**
SAH21	2012	< 10	ND	**20**	**160**
SAH27	2012	< 10	ND	**20**	**80**
SAH35	2012	< 10	ND	**20**	**80**
Total positive		1 > 20; 0 > 40	1 > 40	4 > 20; 0 > 40	4 > 40

Abbreviations: HAI, hemagglutination inhibition; MN, microneutralization; ND, not determined; N, denominator, total serum per survey; n, numerator, total positives.

[a]Only participants with HAI titers >20 and MN titers >40 for either H5 or H7 are shown. Those confirmed by both are indicated in bold. For survey 1 (H5N2 outbreak), all individuals were screened by HAI assays for H5 and H7, and HAI titers >20 were confirmed by MN assays. For survey 2 (H7N1 outbreak), all individuals were screened by HAI for H5 and H7, and HAI titers >10 were confirmed by MN assays. For survey 3, all individuals were screened by HAI assays for both H5 and H7, and HAI titers >20 were confirmed by MN assays.

HAI assays were performed with both turkey and horse red blood cells (RBCs) for H5 and H7. Owing to low HAI titers with H5N2 control antigens when horse RBCs were used, compared with turkey RBCs, we elected to use turkey RBCs as the optimum detector cells and HAI screening for this report. All MN assays were performed in duplicate.

Table 2

Influenza-Seropositive Animal Handlers Involved in the Control of the 2011 H5N2 Outbreak in Western Cape Province, South Africa

	Participants With Influenza Positivity, No./Total No. (%; 95% CI)		
Characteristic	**A(H5N2)[a]**	**A(H7N1)**	**A(H1N1)pdm09**
Total	1/207 (0.9; .0012–.04)	4/207 (1.9; .005–.05)	100/207 (48.3; 41.3–55.3)
Age, y	*P* = .56	*P* = .72	*P* = .003
17–30	0/89 (3.4; .0–4.0)	3/89 (3.4; .7–9.5)	55/89 (61.8; 50.9–71.9)
31–45	1/70 (1.4; .03–7.7)	1/70 (1.4; .03–7.7)	30/70 (42.9; 31.1–55.2)
46–60	0/33 (0.0; .0–10.6)	0/33 (0.0; .0–10.6)	10/33 (30.3; 15.6–48.7)
≥61	0/9 (0.0; .0–33.6)	0/9 (0.0; .0–33.6)	2/9 (22.2; 2.8–60.0)
Sex	*P* = .74	*P* = .73	*P* = .02
Male	1/150 (0.7; .01–3.6)	3/150 (2.0; .04–5.7)	65/150 (43.3; 35.3–51.6)
Female	0/52 (0.0; .0–6.8)	1/52 (1.9; .01–10.2)	32/52 (61.5; 47.0–74.7)
Occupation	*P* = .99	*P* = .08	*P* < .001
Abattoir worker	0/56 (0.0; .0–6.4)	4/56 (7.1; 2.0–17.3)	52/56 (92.9; 82.7–98.0)
Farmworker	1/121 (0.8; .01–4.5)	0/121 (0.0; .0–3.0)	39/121 (32.2; 24.0–41.3)
Farmer	0/9 (0.0; .0–33.6)	0/9 (0.0; .0–33.6)	1/9 (11.1; .3–48.2)
Field worker	0/5 (0.0; .0–52.2)	0/5 (0.0; .0–52.2)	2/5 (40.0; 5.3–85.3)
Veterinarian	0/7 (0.0; .0–40.9)	0/7 (0.0; .0–40.9)	3/7 (42.8; 9.9–81.6)
Other	0/4 (0.0; .0–60.2)	0/4 (0.0; .0–60.2)	0/4 (0.0; .0–60.2)

Abbreviation: CI, confidence interval.

[a] Two individuals tested positive for A(H5N2), but no demographic data was available for 1 of them, who was therefore omitted from this analysis although included in the text.

Table 3

Influenza-Seropositive Individuals Involved in the Control of the 2012 H7N1 Outbreak in the Western Cape Province, South Africa

	Participants With Influenza Positivity, No./Total No. (%; 95% CI)			
Characteristic	**A(H7N1)**	**A(H5N2)**	**A(H1N1)pdm09**	**A(H3N2)**
Total	8/66 (12.1; 5.3–22.5)	1/66 (1.5; .1–8.1)	37/55 (67.3; 53.3–79.3)	49/62 (79.0; 66.8–88.3)
Age, y	$P > .99$	$P = .35$	$P = .90$	$P > .99$
20–30	1/7 (14.3; .0–57.9)	0/7 (0.0; .0–40.9)	4/6 (66.7; 22.3–95.7)	6/7 (85.7; 42.1–99.6)
31–45	5/41 (12.2; 4.1–26.2)	0/41 (0.0; .0–8.6)	24/34 (70.6; 52.5–84.9)	32/40 (80.0; 64.3–90.9)
46–60	2/15 (13.3; 1.6–40.4)	1/15 (6.7; .2–31.9)	8/13 (61.5; 31.6–86.1)	10/13 (76.9; 46.2–94.9)
Sex	$P = .67$	$P = .98$	$P = .99$	$P = .16$
Male	7/50 (14.0; 5.8–26.7)	1/50 (2.0; .1–10.6)	28/41 (68.3; 51.9–81.9)	35/47 (74.4; 68.1–99.8)
Female	1/16 (6.2; .1–30.2)	0/16 (0.0; .0–20.6)	9/14 (64.3; 35.1–87.2)	14/15 (93.3; 59.6–86.1)
Occupation	$P = .65$	$P = .04$	$P = .003$	$P < .001$
Abattoir worker	6/41 (14.6; 5.5–29.2)	0/41 (0.00; .0–8.6)	28/33 (84.8; 68.1–94.9)	37/39 (94.9; 82.7–99.4)
Farmworker	0/10 (0.0; .0–30.8)	0/10 (0.0; .0–30.8)	4/9 (44.4; 13.7–78.8)	3/9 (33.3; 7.5–70.1)
Farmer	0/1 (0.0; .0–95.0)	0/1 (0.0; .0–95.0)	0/1 (0.0; .0–95.0)	0/1 (0.0; .0–95.0)
Laboratory worker	1/4 (25.0; .6–80.6)	0/4 (00.0; .0–60.2)	2/3 (66.7; 9.4–99.1)	4/4 (100.0; 39.8–100.0)
Researcher	0/2 (0.0; .0–84.2)	1/2 (50.0; 1.2–98.7)	0/1 (0.0; .0–95.0)	1/1 (100.0; 2.5–100.0)
Veterinarian or veterinary technician	1/8 (12.5; .3–52.6)	0/8 (0.0; .0–36.9)	3/8 (37.5; 8.5–75.5)	4/8 (50.0; 15.7–84.3)

Abbreviation: CI, confidence interval.

Table 4

Avian Influenza Antibodies in State Veterinarians From Across South Africa in 2012

	Participants With Influenza Positivity, No./Total No. (%; 95% CI)	
Characteristic	**A(H7N1)**	**A(H5N2)**
Total	4/37 (10.8; 3.0–25.4)	1/37 (2.6; .1–14.2)
Age, y	*P* = .98	*P* = .99
26–30	0/6 (0.0; .0–45.9)	0/6 (0.0; .0–45.9)
31–45	1/6 (16.7; .4–64.1)	0/6 (0.0; .0–45.9)
46–60	3/21 (14.3; 3.0–36.3)	1/21 (4.8; .1–23.8)
≥61	0/3 (0.0; .0–70.7)	0/3 (0.0; .0–70.7)
Sex	*P* = .58	*P* > .99
Male	2/25 (8.0; 1.0–26.0)	1/25 (2.7; .1–20.3)
Female	2/12 (16.7; 2.0–48.4)	0/12 (0.0; .0–26.5)
Province or country	*P* = .35	*P* = .62
Eastern Cape	0/3 (0.0; .0–70.7)	0/3 (0.0; .0–70.7)
Free State	0/1 (0.0; .0–95.0)	0/1 (0.0; .0–95.0)
Gauteng	2/14 (14.3; 1.8–42.8)	0/14 (0.0; .0–23.1)
KwaZulu Natal	1/5 (20.0; .5–71.6)	0/5 (0.0; .0–52.2)
Limpopo	0/2 (0.0; .0–84.2)	0/2 (0.0; .0–84.2)
North West	1/1 (100.0; 5.0–100.0)	0/1 (0.0; .0–95.0)
Northern Cape	0/1 (0.0; .0–95.0)	0/1 (0.0; .0–95.0)
Western Cape	0/9 (0.0; .0–33.6)	1/9 (11.1; .3–48.2)
Zimbabwe	0/1 (0.0; .0–95.0)	0/1 (0.0; .0–95.0)

Abbreviation: CI, confidence interval.