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Serological survey of avian influenza virus infection of unvaccinated backyard chickens in Mandlhakazi, Southern Mozambique

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ABSTRACT

Objective: To investigate serologically the presence of avian influenza virus (AIV) in backyard chickens from Mandlhakazi district, Southern Mozambique.
Methods: A total of 439 sera samples from unvaccinated and apparently healthy backyard chickens from 4 villages (Chidenguele, Macuacua, Chizavane, and Nwadjahane) were tested for the presence of AIV antibodies through commercial enzyme-linked immunoabsorbent assay (ELISA) kit used according to manufacturer instructions.
Results: Anti-AIV antibodies were detected in all villages surveyed. The overall seroprevalence obtained was 32.6% (95% *CI* 28.2%–37.0%). The highest prevalence of 51.3% (95% *CI* 42.3%–60.2%) was recorded in Macuacua village, while the lowest prevalence of 13.0% (95% *CI* 6.2%–19.9%) was found in Chizavane village. The results of logistic regression analyses suggested that chicken being located in Chizavane and Macuacua villages were more unlikely for getting the virus exposure ($P < 0.05$).
Conclusions: Our findings suggested that AIV is widespread within backyard chickens in the studied villages. Further research is needed to identify the circulating virus genotypes and determine the potential role of backyard chickens in the zoonotic transmission of AIV in Mozambique.

1. Introduction

Backyard village chickens play a vital role in the improvement of nutritional status and income of many poor rural households and are a global asset for many millions who live below the poverty line [1]. However, the productivity of backyard chickens is hindered by several factors, including a variety of infectious diseases. Furthermore, these birds may act as reservoirs for avian influenza [2].

Avian influenza, also known as fowl plague, is a highly contagious and zoonotic disease of domestic and wild avian species. The causal Orthomyxoviruses are type A influenza viruses. There are 16 known serologically distinct subtypes based on the surface hemagglutinins and 9 based on neuraminidases. Grounded on the severity of the illness caused, avian influenza viruses are divided into two distinct phenotypes: the highly pathogenic avian influenza (HPAI) and the low pathogenic avian influenza virus (LPAI). HPAI viruses induce a highly virulent systemic disease causing high morbidity and mortality, where the most severe lesions are generally characterized as congestive and hemorrhagic. LPAI viruses are asymptomatic or associated to head and face edema, mild to severe respiratory symptoms, decreased egg production and sometimes low mortality [3–5].

Domestic birds that are predisposed to the AIV infections include chickens, turkeys and guinea fowls, while many species of wild birds are carriers of the virus [4]. Since 2014, HPAI virus has cost the loss of millions of birds either by death or culling worldwide [3]. From 2006, about 11 African countries have reported AI outbreaks in poultry, raising fear of a massive spread of the virus within the continent [6–8]. In fact, in the last decades, several outbreaks of many distinct influenza subtypes in chickens and ostriches have been reported in South Africa and Zimbabwe within the Southern Africa region

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[9–11]. Mozambique shares border with those two countries, in a circumstance where the cross-border trade of poultry is very common and is largely unchecked by veterinary services. This reality makes Mozambique at high risk of getting avian influenza outbreaks as well. Taking into account the current epidemiological situation of avian influenza in Southern Africa, this study aimed to investigate serologically the presence of AIV in backyard chickens from Southern Mozambique.

2. Materials and methods

2.1. Study area and sampling

A total of 439 sera samples from apparently healthy backyard chickens were tested for the presence of antibodies against AIV. These chickens were from 4 villages: Chidenguele, Macuacua, Chizavane and Nwadjahane, district of Mandlhakazi, Southern Mozambique. These villages were selected because poultry development programs are being implemented with the support of local Government and Non-Government Organizations. The required minimum sample size was calculated using the formula $n = (Z\alpha^2 * p * q / L^2)$, where n = sample size required; $Z\alpha = 1.96$ is the value required for confidence of 95%; p = a priori estimate of the prevalence; q = 1−p the complementary of prior estimate and L = 5%, the precision of estimate [12]. A priori estimate of the prevalence of 50% was used, once there were no previous studies regarding AIV in Mozambique. We hypothesized that backyard chickens can be a possible AIV source and a risk factor for virus transmission to farm poultry and human beings in that region.

2.2. Serological survey

Approximately 3 mL of blood was collected from wing vein, left horizontally for about 3 h, and sera were collected in 2 mL cryovial tubes and kept at −20 °C until testing. All experimental procedures involving birds were conducted in accordance to ethical protocols in animal research and approved by the Scientific and Ethical Committee of the Veterinary Faculty of Eduardo Mondlane University.

Sera samples were analyzed using standard and commercially available indirect enzyme-linked immunoabsorbent assay (ELISA) (ProFLOK® Avian Influenza Virus Antibody Test Kit, Synbiotics Corp., San Diego, CA, item number 96-6539). This ELISA kit was used according to manufacturer instruction and is designed to detect influenza A group specific virus in chicken sera.

2.3. Data analysis

All data were entered in MS Excel (Microsoft Corporation) spreadsheet and exported to STATA version 12.1® (Stata IC 12.1 for Windows) software for analysis. Descriptive statistics were based on frequencies and percentages for qualitative variables, means and confidence intervals for quantitative variables. The proportion of positive sample data was calculated using either Fisher's exact test or the χ^2-test. *Chi*-square analysis was used to compare the association between dependent (seroconversion status: positive or negative) and independent variable (location). In all *chi*-square tests a probability level of $P < 0.05$ was considered statistically significant.

Only serum samples with Sample to Positive (SP) value greater or equal to 0.5 were considered positive.

3. Results

Table 1 shows the prevalence of anti-AIV antibodies in the tested chicken sera. Results of the serological survey revealed that all 4 villages had unvaccinated free-range indigenous chickens that were positive for anti-AIV antibodies. The overall seroprevalence was 32.6% (95% *CI* 28.2%–37.0%). The highest prevalence of 51.3% (95% *CI* 42.3%–60.2%) occurred in Macuacua village, while the lowest prevalence of 13.0% (95% *CI* 6.2%–19.9%) was encountered in Chizavane village. Results of logistic regression indicated that chicken being located in Chizavane and Macuacua villages was found to be significant ($P < 0.05$) factor for getting the virus exposure with odds ratios of 0.33 and 2.29, respectively.

Table 1
Seroprevalence and proportion of AIV in unvaccinated backyard chickens in Southern Mozambique.

Variable	Number examined (*n*, %)	Number positive (*n*, %)	Univariate analysis		
			OR	*P*-value	95% *CI*, *OR*
Chidenguele	89 (20.3)	28 (31.5)	RF	–	–
Chizavane	92 (20.9)	12 (13.0)	0.33	0.004	0.15–0.69
Macuacua	119 (27.1)	61 (51.3)	2.29	0.005	1.29–4.07
Mwadjahane	139 (31.7)	42 (30.2)	0.94	0.842	0.53–1.68

OR = odd ratio; *CI* = confidence interval of *OR*; RF = reference factor.

4. Discussion

The present study conducted on the prevalence of avian influenza in backyard chickens in Southern Mozambique reveals the presence of AIV antibodies in all villages surveyed. Since there are no routine vaccination programs against avian influenza in commercial or indigenous chicken production system in Mozambique, anti-AIV antibodies detected in these birds indicate that LPAV viruses presently circulate in chicken flocks in Southern Mozambique. Moreover, the antibodies detected could only have derived from seroconversion following natural infection with the viruses. Consequently, the infected birds may be playing a crucial role in the epidemiology of the disease through viruses shedding into the environment. These findings are in agreement with previous reports of AIV infection in poultry elsewhere [13–18].

The overall presence of AIV specific antibodies was found to be 32.6% (Table 1). This finding was in agreement with other ELISA serological survey results that were obtained by Shekaili *et al.* who reported 37.5% [16] and Fallah Mehrabadi *et al.* who found 42% [15]. Lower seroprevalences had been also reported in Mali [19], West Africa [20], Grenada [21] and Uganda [22]. Differences in the management system and

agro-ecological conditions of the study sites may in part explain this disagreement.

In our study, the backyard chickens in the studied villages were allowed to scavenge with ducks, pigs, and goats in the yard and in the crop field, where they were numerous and different species of wild birds. This husbandry practice may have contributed to the natural infection of the backyard chickens and may explain the high prevalence detected [23,24].

This survey shows that AIV is circulating among backyard chickens in studied villages. These birds may be asymptomatic carriers of the AIV. Further research is needed to identify the circulating virus genotypes and determine the potential role of backyard chickens in the zoonotic transmission of AIV in Mozambique.

Conflict of interest statement

We declare that we have no conflict of interest.

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