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Authors
Fuller, TL
Ducatez, MF
Njabo, KY
et al.

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Avian influenza surveillance in Central and West Africa, 2010–2014

T. L. FULLER[1]*, M. F. DUCATEZ[2,3], K. Y. NJABO[1], E. COUACY-HYMANN[4], A. CHASAR[1], G. L. APLOGAN[5], S. LAO[1], F. AWOUME[6], A. TÉHOU[7], Q. LANGEOIS[2,3], S. KRAUSS[8] AND T. B. SMITH[1,9]

[1] *Center for Tropical Research, Institute of the Environment and Sustainability, University of California, Los Angeles, CA, USA*
[2] *Institut National de la Recherche Agronomique, Unité Mixte de Recherche 1225, Interaction Hôtes Agents Pathogènes, Toulouse, France*
[3] *Université de Toulouse, Institut National Polytechnique, École Nationale Vétérinaire de Toulouse, Toulouse, France*
[4] *Central Laboratory for Animal Diseases (LANADA), Bingerville, Côte d'Ivoire*
[5] *Laboratoire de Diagnostic Vétérinaire et de Sérosurveillance (LADISERO), Parakou, Benin*
[6] *Laboratoire Vétérinaire de Lomé, Lomé, Togo*
[7] *Centre National de Gestion de Réserve de Faune (CENAGREF), Cotonou, Benin*
[8] *Department of Infectious Diseases, St Jude Children's Research Hospital, Memphis, TN, USA*
[9] *Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, USA*

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SUMMARY

Avian influenza virus (AIV) is an important zoonotic pathogen, resulting in global human morbidity and mortality and substantial economic losses to the poultry industry. Poultry and wild birds have transmitted AIV to humans, most frequently subtypes H5 and H7, but also different strains and subtypes of H6, H9, and H10. Determining which birds are AIV reservoirs can help identify human populations that have a high risk of infection with these viruses due to occupational or recreational exposure to the reservoir species. To assess the prevalence of AIV in tropical birds, from 2010 to 2014, we sampled 40 099 birds at 32 sites in Central Africa (Cameroon, Central African Republic, Congo-Brazzaville, Gabon) and West Africa (Benin, Côte d'Ivoire, Togo). In Central Africa, detection rates by real-time RT–PCR were 16·6% in songbirds (eight passerine families, $n = 1257$), 16·4% in kingfishers (family Alcedinidae, $n = 73$), 8·2% in ducks (family Anatidae, $n = 564$), and 3·65% in chickens (family Phasianidae, $n = 1042$). Public health authorities should educate human cohorts that have high exposure to these bird populations about AIV and assess their adherence to biosecurity practices, including Cameroonian farmers who raise small backyard flocks.

Key words: Avian influenza, influenza A, surveillance.

INTRODUCTION

Avian influenza virus (AIV) is an important zoonotic pathogen, resulting in global human morbidity and mortality. Poultry and wild birds have transmitted AIV to humans, most frequently subtypes H5 and

* Author for correspondence: Dr T. L. Fuller, 619 Charles E. Young Drive East, La Kretz Hall Suite 300, Institute of the Environment and Sustainability, University of California, Los Angeles 90049, USA.
(Email: fullertl@ucla.edu)

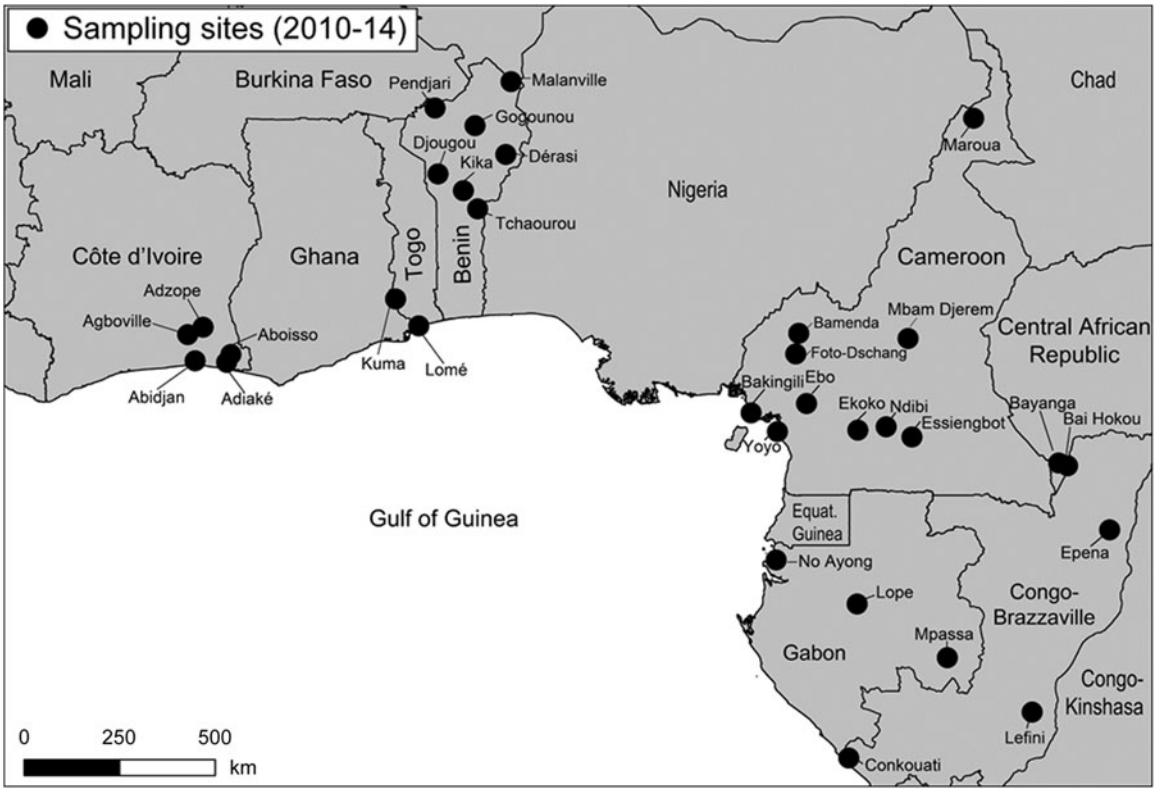

Fig. 1. Location of avian influenza surveillance sites in Central and West Africa.

H7, but also different strains and subtypes of H6, H9, and H10 (reviewed in [1]). Determining which birds are AIV reservoirs can help identify human populations that have a high risk of infection with these viruses due to occupational or recreational exposure to the reservoir species. Public health authorities can prioritize high exposure cohorts for interventions such as educational campaigns.

Such detection efforts are especially important in tropical countries where AIV subtype H5N1 has been isolated from birds but the capacity for sampling and screening is typically limited. A region in which the need for surveillance is particularly great is tropical Africa, where H5N1 has been confirmed in 11 countries [2, 3]. To date, surveillance in the region has focused on poultry, ducks, and shore birds [4–7]; however, the prevalence of AIVs in other birds merits investigation.

For example, a variety of AIV subtypes have been isolated from songbirds in Asia, Europe, and North America, including H1N1, H4N6, H5N1, H5N2, H7N9, and H9N2 [8]. In Africa, AIVs have been detected in passerines but surveillance has been limited to fewer than 300 individuals [4, 7, 9], hence more extensive sampling is needed. The objective of this study was to detect influenza and isolate the virus in tropical birds, including passerines, in Central and West Africa.

METHODS

Domestic and wild birds were sampled at 32 sites in Central and West Africa (Fig. 1, Tables 1 and 2).

Central Africa

A single cloacal sampled was collected from each bird in viral transport media (VTM). All samples were placed immediately on ice after collection, then stored in liquid nitrogen at −196 °C or at −70 °C until processed. Swabs were screened at St Jude Children's Research Hospital, Memphis, TN, USA. Total RNA was extracted from individual swabs using the RNeasy Mini kit or with the QIAmp Viral RNA Mini kit (Qiagen, USA) following the manufacturer's guidelines.

Real-time reverse transcription PCR (qRT–PCR) was used to detect the presence of influenza virus genetic material. Viral RNA was amplified using 4x TaqMan Fast Virus 1-Step Master Mix (Life

Table 1. *Avian influenza surveillance sites in Central and Western Africa (2010–2014) showing the number of cloacal and oropharyngeal samples per site and the timing of data collection*

Region/country	Site	Samples	Sampling date (month/year)	Wild (W) and/or domestic (D) birds
Central Africa				
Cameroon	Bakingili	132	11/2011	W,D
	Bamenda	475	10/2013–12/2013	D
	Ebo	53	6/2013	W
	Ekoko	124	11/2011	W, D
	Essiengbot	190	10/2010–11/2010	W, D
	Foto-Dschang	80	11/2011	W, D
	Maroua	917	1/2013	D
	Mbam Djerem	52	7/2013	W
	Ndibi	204	10/2010	W, D
	Yoyo	85	9/2011	W, D
Central African	Bai Hokou	42	12/2011	W
Republic	Bayanga	151	11/2011–12/2011	W, D
Gabon	Lope	126	12/2012	W
	Mpassa	58	12/2012	W
	No Ayong	92	11/2012–12/2012	W
Congo Brazzaville	Conkouati	111	10/2010–11/2010	W
	Epena	160	11/2010	W, D
	Lefini	134	11/2010	W, D
West Africa				
Côte d'Ivoire	Agboville	4292	10/2011–2/2014	D
	Adzope	3026	10/2011–2/2014	D
	Aboisso	1773	10/2012–4/2013	D
	Adiaké	5836	10/2011–2/2014	D
	Abidjan	2546	11/2011–2/2014	D
Benin	Malanville	300	01/2011–2/2011	D
	Gogounou	2900	01/2011–2/2014	D
	Dérasi	2900	01/2011–1/2014	D
	Djougou	2600	08/2011–2/2014	D
	Kika	900	08/2011–4/2012	D
	Tchaourou	1700	09/2012–2/2014	D
	Pendjari	830	9/2011, 12/2011, 10/2012, 11/2013 1/2014, 4/2014	W
Togo	Kuma	175	10/2011–4/2012	D
	Lomé	7135	8/2011–1/2014	D
Total		40099		

Technologies, USA), including a universal influenza A forward primer (5'-GACCRATCCTGTCACCTCT GAC-3'), reverse primer (5'-AGGGCATTYTGGAC AAAKCGTCTA-3'), and probe (5'-FAM-TGCAG TCCTCGCTCACTGGGCACG-TAMRA-3') under the following cycling conditions: one cycle at 50 °C for 5 min; one cycle at 95 °C for 20 s; 40 cycles at 95 °C for 20 s and 60 °C for 30 s. Samples showing a cycle threshold (C_t) <40 were considered positive by qRT–PCR and were selected for egg culture. Influenza virus A/California/04/2009(H1N1) was used as a positive control throughout all stages of sample processing.

To further assess the evidence for AIV we also performed a separate round of qRT–PCR screening using a different set of primers. In this round, we re-screened the samples collected in Central Africa in 2010 ($n = 817$) with an additional influenza assay using primers known to amplify a conserved segment of the Matrix I gene of AIV strains that circulate in

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Table 2. *Sampling strategy and screening assays utilized in West and Central Africa*

	West Africa	Central Africa
Birds sampled		
Domestic birds in LBMs	×	×
Domestic birds in backyard flocks	×	×
Wild birds near villages		×
Wild birds around ponds	×	
Screening		
RT–PCR	×	×
Egg culture		×

LBMs, Live bird markets.

passerines [10]: 5'-GARATCGCGCAGARACTTGA-3' and 5'-CACTGGGCACGGTGAGC-3' were the forward and reverse primers, respectively. In addition, we attempted to amplify and sequence the second subunit of the AIV HA gene using primers HA-1144 [11]: 5'-GGAATGATAGATGGNTGGTAYGG-3' and Bm-NS-890R [12]: 5'-ATATCGTCTCGTATTAGTAGAAACAAGGGTGTTTT-3'.

Samples positive by qRT–PCR were subsequently grown in the allantoic cavities of 10-day-old embryonated chicken eggs in attempt to isolate influenza A, following established protocols [12].

West Africa

In Côte d'Ivoire and Togo, cloacal and oropharyngeal swabs were collected in VTM from domestic birds. In Benin, cloacal and oropharyngeal swabs were collected in VTM from wild birds. Avian faecal (environmental) samples were also collected in VTM at the Beninese sites. Upon sampling, swabs were stored in liquid nitrogen or on ice and transported to the laboratory within 1 day. In Côte d'Ivoire and Benin, swabs were stored at –80 °C before processing and in Togo they were stored in liquid nitrogen.

Côte d'Ivoire samples were screened at the Central Laboratory for Animal Diseases (LANADA) in Bingerville (Côte d'Ivoire), Benin and Togo samples were either screened on site [at the Veterinary Diagnosis and Serosurveillance Laboratory (LADISERO) in Parakou, Benin or at the Laboratoire vétérinaire central de Lomé, Lomé, Togo] or at UMR 1225 in Toulouse (France). Viral RNA was extracted from individual swabs with the RNeasy Mini kit or with the QIAamp Viral RNA Mini kit. Swabs samples were tested by RT–PCR as described previously [5].

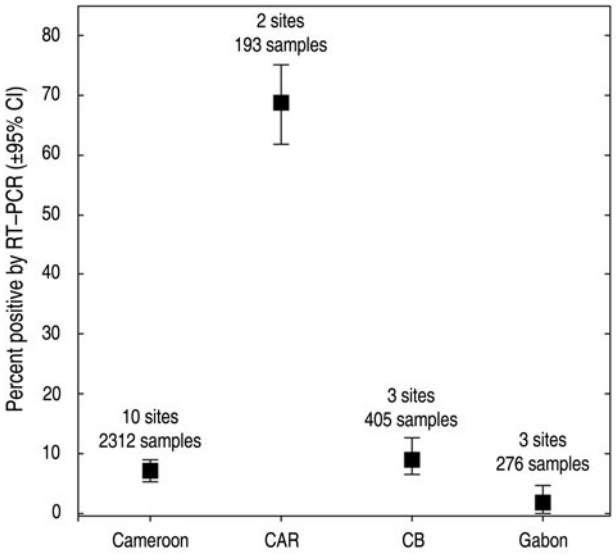

Fig. 2. AIV-positive rates by country in Central Africa based on RT–PCR. Confidence intervals were obtained by logistic regression. CAR, Central African Republic; CB, Congo-Brazzaville; CI, confidence interval.

RESULTS

Regional comparison

The species composition and abundance of bird communities in Central and West Africa were significantly different (Mantel test: $r = 0{\cdot}513$, $P = 1{\cdot}99 \times 10^{-4}$, for description of the Mantel test, see [13]). For example, 55% of the wild birds sampled in West Africa were not collected in Central Africa. In light of this, we analysed the two regions separately.

West Africa

The positive rate by RT–PCR was 0% in Benin, Côte d'Ivoire, and Togo.

Central Africa

The prevalence of AIV differed significantly in the countries sampled (Wald $\chi^2 = 325$, D.F. = 3, $P < 0{\cdot}0001$). Prevalence was highest in the Central African Republic at 65% (Fig. 2); however, this must be interpreted with caution as we only sampled two sites with a total of 193 birds. Elsewhere in Central Africa our sampling was more extensive and the positive rate was 2–9%; for example, prevalence in Cameroon was 7·4% based on 10 sites and 2312 birds.

When we pooled the data across Central African countries, there were significant differences in

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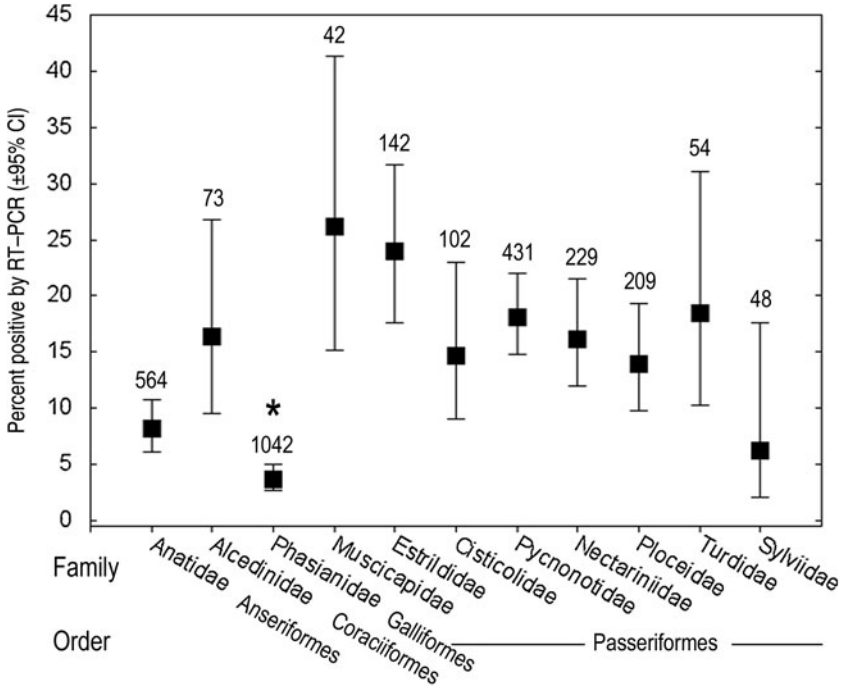

Fig. 3. Passerine birds have AIV positive rates similar to anatid ducks in Central Africa. Only Phasianidae (chickens) had a lower positive rate than anatids by a *t* test with a Holm adjustment for multiple comparisons (* indicates $P = 0{\cdot}0043$). The sample size for each family is listed above the confidence intervals. The plot includes families with $\geqslant 40$ individuals sampled, which comprised 92% of the Central African samples. For families with <40 individuals, the number of samples was insufficient to accurately calculate confidence intervals (CIs).

prevalence in avian families (Wald $\chi^2 = 117$, D.F. = 10, $P < 0{\cdot}0001$). Comparing AIV-positive rates in families reveals a number of interesting patterns (Fig. 3). Based on qRT–PCR using the first set of primers listed in the Methods section, the positive rate was 16·6% in songbirds (eight avian families, $n = 1257$), 16·4% in kingfishers (family Alcedinidae, $n = 73$), 8·2% in ducks (family Anatidae, $n = 564$), and 3·65% in chickens (family Phasianidae, $n = 1042$). The positive rate for chickens was significantly lower than that of songbirds or ducks; however, there was no statistical difference in the positive rate between songbirds and ducks. Within songbirds, the highest positive rate was in flycatchers (family Muscicapidae), which are small insectivorous birds and lowest in warblers (family Sylviidae). These two families had low sample sizes of 40–50 individuals so the confidence intervals for the positive rates are large. Songbird families for which we sampled >200 individuals – including bulbuls (family Pycnonotidae), weavers (family Ploceidae), and sunbirds (family Nectariniidae) – had AIV rates of 14–18%. With respect to the ecological and land use characteristics of sites where we detected AIV, two of the sites were live bird markets (LBMs), Bamenda and Maroua, both of which were in Cameroon. At these LBMs, the positive rates were 0·8% and 1·2%, respectively.

None of the attempts to isolate virus in chicken eggs were successful. Because the amount of RNA extracted from cloacal swabs is generally low, without the ability to grow AIV, it was not possible to further characterize viral subtype via molecular analyses. In order to address the potential of false-positive results from an overly sensitive assay, we compared the C_t values of our positive samples to C_t cut-off values typically used in the influenza literature and found that our results were significantly lower (one-sample *t* test: $t = -3{\cdot}5307$, D.F. = 339, $P = 2{\cdot}36 \times 10^{-4}$; Supplementary Table S1). While the low C_t values of the positive samples provide relative confidence in the presence of AIV in the original samples, the use of the first set of primers listed in the Methods section detected the same rate of AIV positives as the second set (13·3% *vs.* 10·1%, $\chi^2 = 0{\cdot}1261$, D.F. = 1, $P = 0{\cdot}723$). The consistency of the results based on two sets of primers provides additional support that AIV was present in the Central African samples. However, elucidating why AIVs circulating in passerines are refractory to growth in egg culture remains an important area for future research.

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DISCUSSION

Consistent with previous surveillance reports of West Africa [5], our sampling did not detect AIV in the region's poultry or wild birds. For the remainder of the Discussion, we focus on Central African countries. As domestic birds are not vaccinated against AIV in Central or West Africa [14, 15], the positives would be due to natural infections rather than vaccinations. Although AIV was not isolated by culture in chicken eggs, the qRT–PCR assay utilized here is highly sensitive and replicable [16, 17]. Indeed, our estimates of AIV positivity by qRT–PCR are consistent with results of previous surveillance efforts in sub-Saharan Africa [9]. Furthermore, our 8·2% positive rate for anatids is comparable with reported rates for African ducks ranging from 5% to 20% [6, 7, 18].

In Asia, a variety of different poultry species, such as chickens and ducks, are sold at LBMs, and all birds are penned in close proximity at high density, facilitating interspecies transmission of AIV [19, 20]. In comparison with Asian LBMs, those in Africa appear to be less important for the emergence and spread of AIVs because they contain fewer ducks and lower poultry density [5]. Hence, LBM surveillance in sub-Saharan Africa has reported no AIV or a low prevalence of ∼1%. For example, screening of 19 000 domestic birds sampled at LBMs in Benin, Côte d'Ivoire, and Togo in 2006–2008, detected 0% AIV prevalence [5]. In Nigeria, surveillance of 13 597 LBM samples from 25 states in 2006–2007 detected 12 H5N1 positives (∼0·09% prevalence) [21]. LBM surveillance in the state of Sokoto, Nigeria in 2008–2009, detected AIV in ∼1% of chickens ($n = 182$) and pigeons ($n = 19$) [22]. In Kenya, AIV was detected in 0·8% of 5221 LBM samples collected from 2009–2011 [23]. Our results are consistent with these reports in that we found no AIV in West Africa and low prevalence (0·8–1·2%) in LBMs in Central Africa. A factor that could explain the higher rate of AIV positives in Central Africa is that these sites contained more ducks; for example, ducks comprised 53% of the samples at the LBMs in Maroua, Cameroon, whereas the West Africa samples were primarily chickens and guinea fowl.

In addition to domestic birds sampled in LBMs, we found evidence of AIV in backyard poultry and wild birds in Central Africa. Public health authorities should therefore educate human cohorts that have high exposure to these bird populations about AIV and assess their adherence to biosecurity practices. In the countries surveyed here, knowledge of AIV in these cohorts is virtually unknown. However, previous research that measured AIV awareness, biosecurity practices, and infection rates in cohorts exposed to domestic birds in Ghana and Nigeria could provide insights that can inform public health policies in the countries we surveyed.

In Nigeria, workers with occupational exposure to poultry at LBMs and small backyard farms showed evidence of past infection with AIV at a low prevalence of 1·2% [15]. Although experienced workers were well informed about risk factors for AIV transmission and wore personal protective equipment, knowledge of AIV in new workers was poor [14]. In Ghana, adherence to AIV biosecurity practices such as hand washing after handling poultry was low in troops and their families who raised small backyard flocks, and less than half of this cohort knew the symptoms of AIV [24]. This limited awareness led to the question of how best to increase knowledge of AIV via educational campaigns. In Nigeria, poultry workers reported that they were most likely to consult TV, newspapers, and the radio for information about AIV [14], hence these forms of mass media would be the most effective avenues for awareness campaigns.

After Nigeria, Cameroon has the second largest poultry sector in Central and West Africa [25]. In light of this, of the countries we surveyed, rates of occupational exposure to domestic birds are likely to be highest in Cameroon. Although exposure rates of LBM workers and individuals who work with wild birds are unknown, exposure to domestic ducks is high in Cameroonian farmers who raise small backyard flocks [26]. Health authorities should measure levels of AIV awareness and adherence to biosecurity practices at small farm households in Cameroon. If these cohorts have limited knowledge of AIV, they could be targeted for awareness campaigns based on TV, radio, and print media.

The present analysis contributes to the understanding of AIV circulation in wild birds by confirming surveillance reports which have found no AIV in West Africa [5] and sampling Central African Republic, Congo-Brazzaville, and Gabon in Central Africa, which are countries that have not previously been surveyed. The positive rate that we detected in Central Africa's wild birds suggests the possibility that AIVs circulate widely in passerines, a finding that should stimulate further surveillance to isolate these viruses by egg culture. Expanding AIV screening programmes in Central and West Africa can confirm the

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qRT–PCR positives reported here and possibly obtain isolates for molecular characterization and pathogenicity studies. If the AIV subtypes circulating in birds in tropical Africa were low pathogenic with a putative low pandemic risk (as could be assessed using risk assessment tools as described in [27]), controlling the spread of AIVs may not be urgent. However, if highly pathogenic subtypes H5 or H7 occur in the region, then shifts in poultry rearing practices would be warranted to limit spillover from wild birds to domestic animals and humans. Since subtype H5N1 has already caused losses of $20 billion to the global poultry industry [20], it could have a substantial impact on food production in Central and West Africa. Furthermore, subtype H7N9, which has high virulence in humans and circulates in songbirds in China, could result in treatment costs of $5.3 billion if it spreads to a major city [28]. Improving our understanding of the occurrence of AIV in African birds can provide insights useful for the formulation agricultural and biosecurity policies.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268814003586.

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DECLARATION OF INTEREST

None.

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