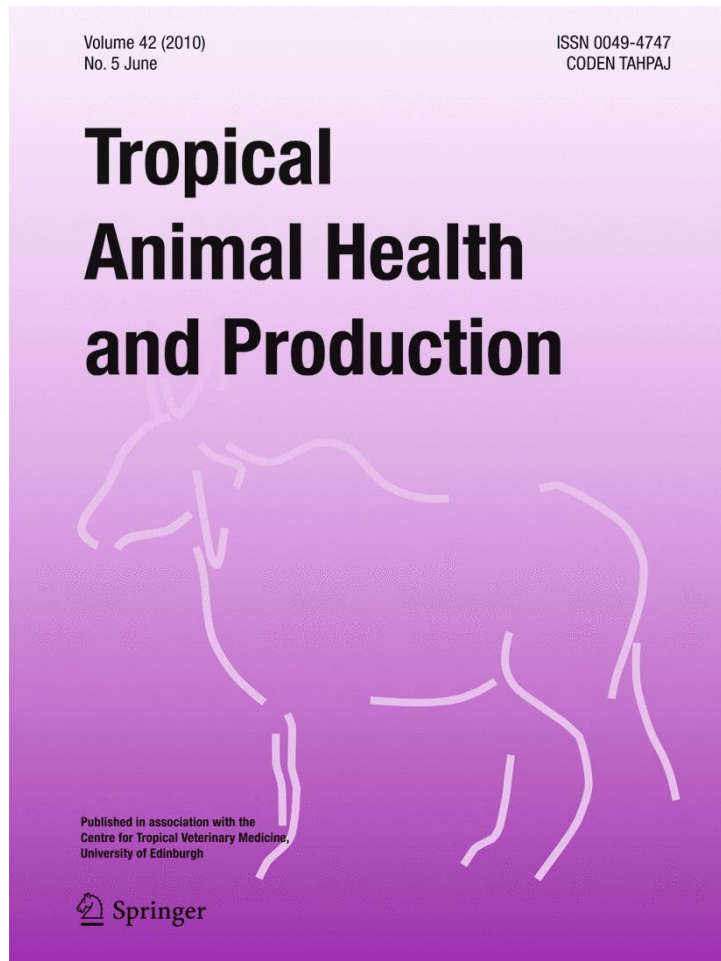


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Avian influenza in backyard poultry of the Mopti region, Mali

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Abstract This study reports the first evidence of circulation of avian influenza viruses (AIV) in domestic poultry in Mali. In the Mopti region, where AIV have already been isolated in migratory water birds, we sampled 223 backyard domestic birds potentially in contact with wild birds and found that 3.6% had tracheal or cloacal swabs positive by real-time reverse transcription PCR (rRT-PCR) for type A influenza viruses (IVA) and that 13.7% had sera positive by commercial ELISA test detecting antibodies against

IVA. None of the birds positive by rRT-PCR for IVA was positive by rRT-PCR for H5 and H7 subtypes, and none showed any clinical signs therefore indicating the circulation of low pathogenic avian influenza. Unfortunately, no virus isolation was possible. Further studies are needed to assess the temporal evolution of AIV circulation in the Mopti region and its possible correlation with the presence of wild birds.

Keywords Avian influenza · Poultry · Backyard · Mali · Africa

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The Mopti region of Mali encompasses most of the inner delta of the Niger River, the second largest wetland in Africa and the winter habitat for millions of migratory water birds. A 2006 survey found that aquatic wild birds sampled in this region were infected with avian influenza viruses (AIV), including subtypes H5N3, H11N9, and H12N5 (Gaidet et al. 2007). This evidence for circulation of AIV in wild birds of the Mopti region brought concern over the possible transmission to domestic poultry. Contact between wild and backyard domestic birds is indeed common in this area where free-ranging poultry is kept in most households. We therefore investigated the circulation of AIV in backyard domestic poultry possibly in contact with wild birds in the Mopti region.

In February 2007, which corresponds to the winter period when large numbers of palearctic migratory birds are present in the inner delta of the Niger River, six villages of the Mopti region were conveniently

selected because they were located near the Niger River ($n=5$) or a pond ($n=1$) and were therefore exposed to aquatic wild birds. In each village, tracheal swabs, cloacal swabs, and blood were collected from a number of birds sufficient to detect AIV with 95% confidence if the prevalence was 10% or more. Sampled birds were conveniently chosen, but whenever possible, a larger number of ducks was sampled because we assumed they were more likely to be infected with AIV. In addition, 28 birds were sampled at the poultry market in Mopti, which attracts sellers and middlemen from the whole region. A total number of 223 birds were sampled. Swab samples were tested by real-time reverse transcription-polymerase chain reaction (rRT-PCR) for type A influenza viruses (IVA; Spackman et al. 2002), and positive samples were further tested by rRT-PCR specific for H5 and H7 subtypes and virus isolation on embryonated chicken eggs. Serum samples were tested for the presence of antibodies against IVA with a commercial ELISA kit (FluA, IdVET, Montpellier, France) according to manufacturer's instructions.

Diagnostic tests results are presented in Table 1. Eight (3.6%) of the 223 birds had at least one swab positive by rRT-PCR for IVA, either tracheal or cloacal but never both. Three (1.3%) of the 223 cloacal swabs and five (2.2%) of the 223 tracheal swabs were positive, of which, respectively, two and three were borderline positive. None of these positive samples was positive by rRT-PCR for H5 and H7 subtypes and no virus isolation was possible. Among the 190 non-hemolyzed serum samples tested by ELISA, 26 (13.7%) were positive. Neither the proportion of birds with a positive result by rRT-PCR for IVA nor the proportion of birds seropositive

by ELISA differed significantly depending on species, sex, or age of the bird. The odds of having a positive versus negative rRT-PCR by IVA were significantly greater ($OR=6.8$; $p=0.037$) for birds seropositive by ELISA (3/23) than for birds seronegative by ELISA (3/156).

This study provides the first evidence of circulation of AIV in domestic poultry in Mali. No virus isolate could be obtained from the eight birds with a positive rRT-PCR result for IVA, thus preventing the comparison with AIV strains previously isolated from wild birds in the Mopti region (Gaidet et al. 2007). Because all of these eight birds had negative rRT-PCR results for H5 and H7 subtypes and because they showed no clinical signs, we can nevertheless assume that they were infected with low pathogenic strains. Based on the lack of concurrent positivity of tracheal and cloacal swabs from the same bird, we recommend combining cloacal with oropharyngeal swabbing when surveying AIV in domestic poultry, as already recommended for wild mallards (Ellstrom et al. 2008). Additionally, the proportion of seropositive birds (13.7%) we found provides a first although clearly biased estimate of individual seroprevalence of AIV in African backyard poultry. This estimate should be treated very cautiously because it is based on a non-random sample of villages and birds and because no confirmatory serological tests were run. Indeed, a 7% AIV seroprevalence found by indirect ELISA in commercial farm chickens in Nigeria decreased to 0% when assessed with more specific tests (Owoade et al. 2006). Other available AIV individual seroprevalence data relate mostly to birds reared in commercial farms and vary greatly: 0% overall in Nigeria (Joannis et al. 2008), 0.9% overall

Table 1 Results of diagnostic tests for type A influenza viruses for 223 birds sampled in the Mopti region, Mali, in February 2007, stratified by species, sex, and age

rRT-PCR real-time reverse transcription PCR, IVA type A influenza viruses

	rRT-PCR for IVA		ELISA detecting antibodies against IVA		
	Negative	Positive	Negative	Doubtful	Positive
Duck	127	7 (5.2%)	85	4	19 (18.3%)
Chicken	85	1 (1.2%)	72	1	7 (8.9%)
Guinea fowl	3	0 (0.0%)	2	0	0 (0.0%)
Female	148	5 (3.2%)	104	3	18 (14.9%)
Male	67	3 (4.3%)	55	2	8 (12.7%)
Young (<6 months)	31	0 (0.0%)	26	0	2 (7.1%)
Adult	184	8 (4.2%)	133	5	24 (15.3%)
All birds	215	8 (3.6%)	159	5	26 (13.7%)

in the Netherlands (de Wit et al. 2004), 7.3% overall in Korea (Woo and Park 2008), 6.7% to 100% within-flock in Jordan (Al-Natour and Abo-Shehada 2005), and 88% to 100% within-flock in Pakistan (Naeem et al. 2003). Further serological and virological studies are needed to assess the temporal evolution of AIV circulation in the Mopti region and its possible correlation with the presence of wild birds.

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