Emerging Problems in Infectious Diseases

Prevalence of infectious bronchitis and Newcastle disease virus among domestic and wild birds in H5N1 outbreaks areas

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Abstract

Introduction: The first H5N1 outbreak in Burkina Faso was reported to the World Organization for Animal Health on 3 April 2006. This study aimed to determine the prevalence of avian influenza virus, infectious bronchitis virus, and Newcastle disease virus among domestic and wild birds in highly pathogenic avian influenza (HPAI) H5N1 outbreaks areas.

Methodology: We collected paired tracheal and cloacal swabs from 283 birds including 278 domestic and five wild birds (three vultures, one sparrowhawk and one Western Grey Plantain-eater) in the Central Region (Ouagadougou) and the Western Region (Bobo-Dioulasso and Sokoroni) of Burkina Faso. Total RNA extracted from samples were subjected to reverse transcription and resulting cDNA amplified by PCR using specific primers for detection of Avian Influenza Virus (AIV mainly highly pathogenic H5N1), Infectious Bronchitis Virus (IBV), and Newcastle Disease Virus (NDV) for the first time in Burkina Faso.

Results and conclusions: Our results show that 13.8% (39/283) samples were reactive for NDV, and the prevalence of IBV was 3.9% (11/283). None of the 283 birds were co-infected by AIV, IBV and/or NDV in our study areas. The prevalence of influenza A virus was 3.2% (95% CI: 0-6.6) with a 1.7% (95% CI: 0-3.2) prevalence of H5N1 being detected. Positive cases of H5N1 virus were found in two out of three vultures in Ouagadougou, and in three out of 203 local chickens in the Western Region. These results confirm the presence of influenza A H5N1 virus, IBV and NDV in domestic and wild birds in Burkina Faso.

Key words: avian influenza; H5N1; infectious bronchitis virus; Newcastle disease virus

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Introduction

Infectious bronchitis (IB) and Newcastle disease (ND) are two major causes of economic losses in the poultry industry [1,2]. Clinical signs occurring in these avian respiratory diseases are often non-specific. IB and ND are characterized by respiratory signs including gasping, coughing, sneezing, tracheal rales, and nasal discharge and they are believed to be involved in poor egg production in layers and acute highly contagious respiratory diseases in infected chickens [3,4].

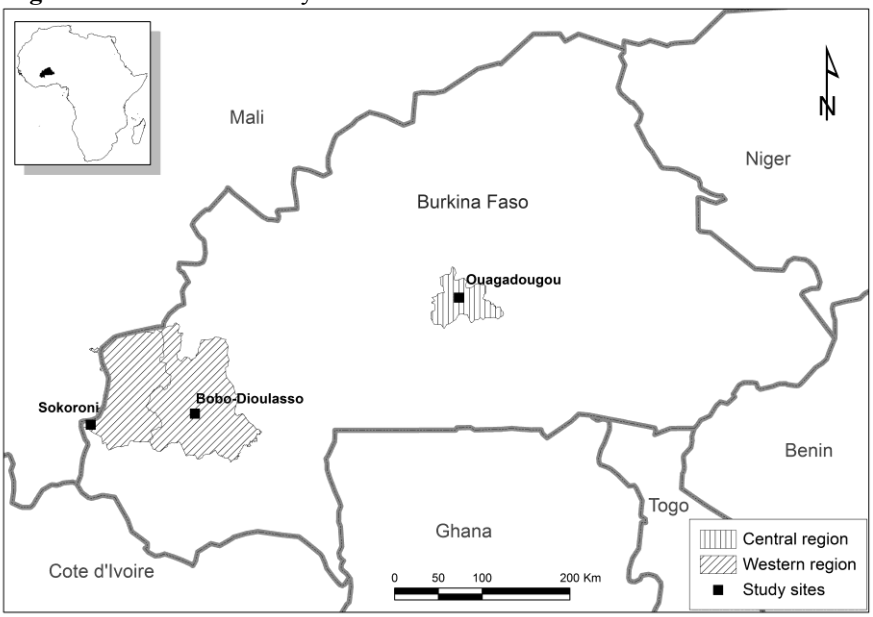
In young chickens severe respiratory distresses may occur, while in layers only mild respiratory distress is usually observed [1,5,6]. Intensive research and survey on these diseases has been conducted in developed countries because of their impact on the economy and possible transmission to humans. The poultry business is traditionally managed and is of wide interest to many farmers but few studies have been done in African countries to survey these threatening infectious diseases, which has led to high losses of poultry birds [1,6,7-12,18,19]. Unfortunately, these two diseases have not been previously studied in Burkina Faso despite the importance of the poultry industry in this country; approximately 32 million chickens per year are slaughtered. After the first reported outbreak of highly pathogenic avian influenza (HPAI) H5N1 in Burkina Faso [13,14], it became imperative to investigate the prevalence of avian influenza virus (AIV) and other avian respiratory diseases viruses, including infectious bronchitis virus (IBV) and Newcastle disease virus (NDV). The aim of our study was to determine the prevalence of IBV, NDV and AIV in familial flocks in two regions of the country.

Methodology

Sample collection

From March to June 2006 (at the beginning of a suspected AIV outbreak in the country), flocks with atypical morbidity and mortality were sampled in the Central Region (Ouagadougou) and the Western

Figure 1. Locations of study sites



Region (Bobo-Dioulasso and Sokoroni) of Burkina Faso (Figure 1). A questionnaire regarding the vaccination status of chickens was administered to domestic poultry owners during sampling. Paired tracheal and cloacal swabs were collected from 283 birds (273 domestic chickens, three turkeys, two ducks, one Western Grey Plantain-eater (grey turaco), one sparrowhawk and three hooded vultures). The five wild birds (one Western Grey Plantain-eater, one sparrowhawk and three hooded vultures) were easily captured because they were affected by locomotion ataxia. All swabs were soaked in virus transport medium (VTM: RPMI + bovine serum albumin + penicillin + streptomycin + amphoteracin B, Invitrogen, Merelbeke, Belgium). The samples were stored at -80°C until further processing.

Viral RNA extraction and RT-PCR

Viral RNA was extracted with the QiAamp viral RNA Mini Kit (Qiagen, Leusden, the Netherlands) following the manufacturer's instructions.

Two step RT-PCR was performed to amplify parts of the AIV, IBV, and NDV genomes. Briefly, the RNA (5 µl) was first transcribed into cDNA by reverse transcriptase using random primers (Invitrogen, Merelbeke, Belgium). A volume of 0.5 µl of the reverse transcriptase product was used as the template for each PCR reaction.

For AIV, a highly conservative region of the matrix gene was targeted for influenza A detection [15] and the positive samples were further tested with H5 and N1 specific PCR respectively.

Infectious bronchitis virus genome amplification was performed as described by Akin *et al*. [16] in a highly sensitive nested PCR format. Newcastle disease virus was detected as described by Kho *et al*., using primers FOP1 (50-TACACCTCATCCCAGACAGGGTC-30) and FOP2 (50-AGGCAGGGGAAGTGATTTGTGGC-30) at a concentration of 0.1 lM for the first round and FIP1 (50-TACTTTGCTCACCCCCCTT-30) and FIP2 (50-CATCTTCCCAACTGCCACT-30) at a concentration of 0.5 lM for the nested PCR [17]. All programmed cycling was performed in a thermocycler (Mastercycler Gradient, Eppendorf, Hambourg, Germany). PCR amplicons were analyzed in a 1.5% agarose gel using a 1xTBE as electrophoresis running buffer and ethidium bromide stain.

Inhibition control for AIV analysis

To control eventual inhibitors, we also constituted pools of collected samples. RNA was extracted as described above, and each pool was composed of 20 µl of measles virus (MV) suspension and three different samples (20 µl of tracheal specimen + 20 µl of cloacal specimen for each sample). All constituted pools were analyzed as individual samples. Positive and negative controls were also included to validate AIV PCR results when all pools were positive for MV in the analysis.

Table 1. Main observed symptoms among investigated birds

Symptoms	**Observed Cases**	
	Number of cases (n)	**Percentage** (%)
Apathy	04	1.4
Diarrhoea	06	2.1
Asthenia	13	4.6
Decrease in egg production	19	6.7
Locomotion ataxia	09	3.1
General paralysis	20	7.1
Asymptomatic cases	212	75
Total	283	100

Table 2. Prevalence of AIV, H5N1, IBV and NDV in Central Region, and Western Region, Burkina Faso

	Central Region			**Western Region**			**General Total**
	Domestic poultry	**Wild birds**	**Total**	**Domestic poultry**	**Wild birds**	**Total**	
Number tested	76	4	80	202	1	203	283
AIV Positive % 95% CI	0 0%	2 50% (21.7-78.2)	2 2.5% (0-5.9)	7 3.4% (0.9-5.9)	0 0%	7 3.4% (0.9-5.9)	9 3.2% (0-6.6)
H5N1 Positive % 95% CI	0 0%	2 50% (21.7-78.2)	2 2.5% (0-5.9)	3 1.5%	0 0%	3 1.4%	5 1.7%
IBV Positive % 95% CI	4 5.2% (0.2-10.2)	0 0%	4 5% (0.2-9.7)	7 3.4% (0.9-5.9)	0 0%	7 3.4% (0.9-5.9)	11 3.9% (1.6-6.1)
NDV Positive % 95% CI	20 26.3% (16.1-35.8)	2 50% (21.7-78.2)	22 27.5% (17.7-37.2)	17 8.4% (4.5-12.2)	0 0%	17 8.3% (4.5-12.1)	39 13.8% (9.7-17.8)

Results

Study population

Based on interview data from the farmers, the study population was composed as follows: 278/283 (98.2%) were domestic birds; 216/283 (76.3%) were local race; 55/283 were (19.4%) industrial poultry (leghorn 30/283, 10.6%; Isa Brown 25/283, 8.8%). The population also included two ducks and five turkeys. Only five wild birds (1.7%) were investigated: three hooded vultures (*Necrosyrtes monachus*), one African sparrowhawk (*Accipiter nisus*) and one Western Grey Plantain-eater (*Crinifer piscator*).

According to the collected information, few birds were vaccinated against Newcastle disease virus (NDV), infectious bronchitis virus (IBV) or infectious bursal disease virus (IBDV). Only 50 industrial chickens and 17 local chickens were vaccinated. The others species were not vaccinated. There was no report of anti-AIV vaccination in our study areas.

Primary observed symptoms

The primary symptom observed in live birds was dyspnoea which was frequently associated with other symptoms, including apathy, diarrhoea, asthenia, locomotion ataxia, or general paralysis. Most birds (75%), however, were asymptomatic, even in culling areas where HPAI H5N1 was initially detected (Table 1).

Diagnosis and prevalence of AIV, IBV and NDV

In this study, we observed that ND was the most prevalent respiratory disease among birds (13.8%) followed by IB (3.9%) and AI (3.2%). Of the total number of samples tested by PCR from the Central and Western Regions, 59/283 (20.8%) were positive for AIV, IBV or NDV. Twenty-eight out of 80 samples (35%) from the Central Region (Ouagadougou) were PCR-reactive compared to 31/203 (15.1%) reactive samples from the Western Region (Bobo-Dioulasso and Sokoroni). None of the 283 birds were co-infected by AIV, IBV and/or NDV in our study areas. Two out of 80 (2.5%) birds in the Central Region and 7/203 (3.4%) birds in the Western Region were AIV reactive (Table 2). In the Central Region, the two positive samples for AIV were from wild birds (hooded vultures). These two AIV cases were confirmed to be HPAI (H5N1). In the Western Region (Bobo-Dioulasso and Sokoroni) AIV was present in seven domestic chickens, and three of these samples were confirmed to be HPAI (H5N1). The overall prevalence of AIV in all collected samples was 9/283 (3.2%), with five confirmed cases (1.7%) of HPAI (H5N1).

A total of 39 out of 283 (13.8%) samples were reactive for NDV in PCR; of these, 23/39 (59%) were from chickens vaccinated against NDV while 16/39 (41%) were from chickens not vaccinated against this disease. All 11 IBV positive samples were from chickens that were not immunized against IBV.

Alert signs in the flocks were high morbidity and mortality, decrease in egg production, and respiratory distress.

Individually examined morbid birds had the same clinical signs, including dyspnoea, diarrhoea, asthenia, and neurological signs, regardless of species or locality. Some of the birds that were reactive for one of the three viral diseases were asymptomatic (Table 1).

Of the total number of samples tested for IBV from both sites (n = 283), 11 were positive (four from the Central Region and seven from the Western Region), resulting in an overall prevalence of 3.9% (11/283), (Table 2). All of the samples that were positive for IBV were from domestic chickens.

NDV reactivity in the Central Region (27.5%) was higher than that in the Western Region (8.3%, Table 2). Two of the positive cases from the Central Region were from wild birds (one hooded vulture and one Western Grey Plantain-eater).

Discussion

Burkina Faso is a West African country where very few studies have examined the prevalence of avian viruses. This study was conducted after the first outbreak of highly pathogenic avian influenza (HPAI) H5N1 virus was reported in Ouagadougou in April 2006. We determined for the first time the prevalence of IBV (3.9%), NDV (13.8%) and AIV (3.2%) in the context of the HPAI H5N1 outbreak in Burkina Faso. The overall prevalence for the three investigated viruses (AIV, IBV and NDV) reached 20.8% in our sample of domestic and wild birds. This prevalence is relatively high and it is of concern because these are three of the most dangerous viruses in poultry. While there was significant geographical variation in the prevalence of NDV, no geographic difference was observed for AIV prevalence, indicating that birds in the two regions are equally exposed to AIV infections. This was also observed for HPAI H5N1 infection in the Central Region (2.5%) and in the Western Region (1.4%). The three positive cases of H5N1 within local chickens in the

Western Region (one case in Bobo-Dioulasso and two cases in Sokoroni) and above all the two cases of HPAI H5N1 in vultures in Ouagadougou (Central Region) possibly indicate that this zoonotic disease might be spreading from wild to domestic birds and vice-versa. This is consistent with the results of previous studies [18,19]. Vultures are essentially carrion-eaters, and can fly over dozens kilometers within a few hours. These two characteristics render vultures suitable reservoirs and hosts for avian flu [13].

Concerning IBV, all observed cases in the two study sites occurred in non-vaccinated domestic poultry. This could be a result of the low number of IBV-reactive cases in our study. On the other hand, our results showed that (59%) of detected cases of NDV were within vaccinated poultry and NDV infection was slightly more prevalent in the Central Region (27.5%) than in the Western Region (8.3%). Considering that the majority of industrial farms in the Central Region (Ouagadougou) were vaccinated against NDV, one would have predicted that transmission should be less prevalent in the Central Region (Ouagadougou) compared to the Western Region. In the Western Region, observed chickens were from backyards and were mostly non-immunized against NDV. This was, however, not the case as NDV prevalence in the Central Region (27.5%) was three-fold higher than in the Western Region (8.3%). These cases of NDV are most probably vaccine derived strains because several vaccine strains are used for vaccination in the region [20]. It is also possible that these findings indicate a serious quality problem with the vaccines used against NDV or with the methods of their storage in Burkina Faso. Burkina Faso imports poultry from all over the world, and the lack of adequate biosecurity measures, such as quarantine and prophylaxis, could explain this epizootic situation in the country. Our findings are similar to reports of other countries in West Africa, which show relatively high prevalence of NDV, IBV and outbreaks of HPAI H5N1 [19,10]. There is, therefore, an urgent need for the implementation of routine well-managed vaccination against NDV, IBV and AIV in poultry.

These vaccination strategies should aim to reduce viral loads and disease prevalence, animal and public health consequences, and economic damage.

Conclusion

Respiratory infectious diseases are the leading cause of loss of poultry in industrial and traditional farms. During H5N1 outbreaks in Burkina Faso, we reported a high prevalence of NDV (13.8%); as well as the presence IBV (3.9%), and AIV (3.2%) infection in domestic and wild birds for the first time. There is an urgent need for the implementation of routine laboratory surveillance for poultry disease and for the development of strategies to improve the quality of the vaccines to safeguard the important poultry industry in Burkina Faso.

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