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Prevalence of Newcastle disease virus and infectious bronchitis virus in avian influenza negative birds from live bird markets and backyard and commercial farms in Ivory-Coast



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1. Introduction

Newcastle disease (ND) and infectious bronchitis (IB) are two viral diseases affecting the respiratory tracts of many species of birds and placing a severe economic burden on the poultry industry (Alexander, 1997; Cavanagh and Gelb, 2008; Jackwood et al., 2012).

ND has a worldwide distribution. In Africa, it is the major constraint of chicken development, mainly in rural areas (Maminiaina et al., 2010; Couacy-Hymann et al., 2012a). The infectious agent of ND, Newcastle disease virus (NDV), is a single stranded, non-segmented, negativesense RNA virus belonging to the order *Mononegavirales*, family *Paramyxoviridae*, sub-family *Paramyxovirinae*, and genus *Avulavirus* (Lamb and Parks, 2007; Cattoli et al., 2011). However, only virulent strains of NDV cause ND when they infect birds. This genus contains at least 9 serogroups of avian paramyxoviruses (APMV-1 to -9) previously described and recently 3 more serogroups have been added: APVM10 (Miller et al., 2010), APMV11 (Briand et al., 2012) and APVM12

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ABSTRACT

Newcastle disease (ND) and infectious bronchitis (IB) are two major viral diseases affecting the respiratory tracts of birds and whose impact on African poultry is still poorly known. In the present study we aimed at assessing NDV and IBV prevalences in Ivory-Coast by molecular screening of >22,000 avian swabs by nested PCR and by serology testing of close to 2000 avian sera from 2010 through 2012. The NDV and IBV seroprevalences over the study period reached 22% and 72%, respectively. We found 14.7% pooled swabs positive by PCR for NDV and 14.6% for IBV. Both pathogens are therefore endemic in Ivory-Coast. Economic losses associated with NDV and IBV infections still need to be evaluated.

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(Terregino et al., 2013). According to their virulence in poultry, APMV-1 isolates can be grouped into three pathotypes: lentogenic, mesogenic or velogenic (Alexander, 1997; Cattoli et al., 2009). The velogenic strains may cause 100% mortality in infected chicken flocks (Kho et al., 2000); they are further classified as neurotropic or viscerotropic based on their pathological manifestations (Alexander, 1998; Wise et al., 2004). Mesogenic strains cause primarily respiratory disease while lentogenic isolates are of low virulence and may cause mild respiratory or enteric infections. The virulent NDV isolates (mesogens and velogens) are notifiable agents that require reporting to the OIE (OIE, 2000).

IB, in contrast, remains less known in Africa, and is found mainly in the backyard poultry production system. It is a highly contagious upper-respiratory tract disease of chickens. The causative agent, infectious bronchitis virus (IBV), is a coronavirus, an enveloped, positivestrand RNA virus with a genome of about 27 kb. It belongs to the family *Coronaviridae* and subfamily *Coronavirinae* within the genera of Gammacoronaviridae (Jackwood et al., 2012). Clinical signs of IB disease in chickens are watery eyes, mucus in the nares and trachea, gasping, coughing, and tracheal rales. The disease can also cause a decrease in egg production and egg quality and some strains of the virus can cause an interstitial nephritis (Jackwood et al., 2012). Morbidity is close to 100%, while mortality can be variable, ranging from 14% to 82%,

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depending on the age of the birds, strain of the virus and secondary infections (Cavanagh and Gelb, 2008).

Up to now little is known about the distribution and impact of IBV in sub-Saharan African countries including Ivory-Coast. A recent study undertaken on chickens from commercial farms, live bird markets and backyard farms in Nigeria and Niger revealed the presence of IBV genome. Phylogenetic analysis of the S1 coding sequence revealed a new genotype of IBV. This strain did not cross-react with antisera against known strains such as IT02, M41, D274 or Connecticut in virus neutralisation tests (Ducatez et al., 2009). In Ivory-Coast, poultry technicians report on a regular basis the presence of IB in commercial farms and recommend the use of vaccine, mainly based on the M41 strain, although there is no prior study of the presence of IBV in the country or on the type of strains circulating. These reports, based on clinical signs, were never confirmed by the laboratory.

Both ND and IB affect the respiratory tract, so the differential diagnosis between them and with respect to other respiratory diseases such as *Mycoplasma gallisepticum* (chronic respiratory disease), infectious laryngotracheitis, *Haemophilus paragallinarum* (infectious coryza) and avian influenza virus (AIV) infections, remains a challenge (Ducatez et al., 2009).

The present study took advantages of the surveillance for avian influenza viruses carried out within Ivory-Coast to determine the prevalence of NDV and IBV in poultry farms (both backyard and commercial farms) and at live poultry markets.

2. Materials and methods

2.1. Sampling sites

Outbreaks of avian influenza due to H5N1 strains were detected in Ivory-Coast in 2006. From that date on a continuous surveillance of poultry farms, both backyard and commercial production systems, has been implemented. Every month, the team of the Virology Laboratory was sent to the field to collect tracheal and cloacal swabs and serum samples. These samples were collected in the southern regions (Agneby, District of Abidjan, South Comoe), which are the biggest large-scale poultry production areas in the country. In addition, the south-eastern region (South Comoe) includes lakes and rivers with large populations of various water bird species (Fig. 1). The sampling was carried out following a validated protocol previously described with data from 2007 through 2009 previously reported (Couacy-Hymann et al., 2012a). In each region, a minimum of 5 villages were randomly selected from a known list of villages. In addition, following the same protocol, 5 commercial farms were selected per region. However, any commercial farm, having reported any diseases to the veterinary field technician, was systematically included in the survey (in addition to the 5 commercial farms randomly selected). Within a selected village, any backyard poultry's owner having a poultry flock (flock size varying between 5 and 20 birds per household) was systematically included in the survey. At live bird markets (mainly one big live market per region), 5 vendors were randomly selected (average number of vendors per market = 10). In addition, farmers were interviewed regarding the case mortality that occurred on their farms.

2.2. Sample collection

At the sampling sites (backyard and commercial poultry farms, livepoultry markets), clinical examination of each bird (chicken, guinea fowl or duck) was undertaken for any signs of disease prior to sampling. In each selected village, a minimum of 30 birds were sampled. From a commercial farm, 30 to 50 chickens were selected and at live bird market, 5 birds were selected from each selected vendor in a given market. Any dead or sick animals were systematically included in the survey at any sampling sites and sampled. Blood samples were obtained from



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