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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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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 N22,000 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.

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 (Maminaina et al., 2010; Couacy-Hymann et al., 2012a). The infectious agent of ND, Newcastle disease virus (NDV), is a single stranded, non-segmented, negative-sense 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: APMV10 (Miller et al., 2010), APMV11 (Briand et al., 2012) and APVM12 (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, positive-strand 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%.

http://dx.doi.org/10.1016/j.rvsc.2015.07.015
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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, live-poultry 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
examined animals and processed to yield serum. Individual swabs were used in this survey. Tracheal and cloacal swabs were also collected from the same birds and placed in viral transport medium (VTM) (50% sterile glycerin; 45% sterile PBS 1 M, pH 7.2–7.4; 2% antibiotic solution with Penicillin and Streptomycin; 0.5% Gentamicin; 1% Nystatin; 1.5% Polymyxin B) with the final antibiotic concentration of Penicillin 1000 units/mL, Streptomycin 200 μg/mL, Nystatin 50 units/mL, Gentamicyn 250 μg/mL, and Polymyxin B 100 units/mL. Each tracheal and cloacal swab was stored in a sterile individual tube containing the VTM. In the field, collected swab samples were kept in liquid nitrogen to prevent any degradation of biological materials. At the laboratory, serum samples were stored at −20 °C and swabs were transferred to a −80 °C freezer until used for analysis (Tables 1 & 2).

2.3. Serological tests

2.3.1. Detection of anti-NDV antibodies

Serum samples (n = 1943) were screened for anti-NDV antibodies using the haemagglutination/haemagglutination inhibition test (HA/HAI), the gold standard test, following the reference method (OIE, 2012) with reference NDV antigens (batch no. 1/08 Ulster 2C) and corresponding reference positive serum as positive control. The reference reagents were provided free of charge by the World Organization for Animal Health (OIE)/Food and Agriculture Organization of the United Nations (FAO) Reference Laboratory in Padova (Italy) and by St Jude Children’s Research Hospital, Memphis (TN, USA).

2.3.2. Detection of anti-IBV antibodies

Serum samples were also screened for specific anti-IBV antibodies by ELISA using the IDEXX IBV kit (IDEXX, The Netherlands with specificity = 100% and sensitivity > 90%) according to the protocol recommended by the manufacturer. Only 1938 serum samples were used for this analysis, as five (5) serum samples from guinea fowl were not available anymore to perform this test.

2.4. Molecular detection of avian viral genomes

Tracheal and cloacal swabs were processed as described (Kho et al., 2000; Snoeck et al., 2009). In the laboratory, each individual swab in an individual tube with VTM was processed and the suspension was kept individually. Then 5 individual swab-suspensions were pooled from the same species, farm or vendor in the live market. Finally, the samples were screened in pools of 5 swabs (Couacy-Hymann et al., 2012a). However some pools could contain less than 5 individual samples depending upon the number of available samples. The procedure for RNA isolation was as recommended by the manufacturer, using the RNeasy Mini Kit (Qiagen, Germany). The RNA was eluted in 50 μL of nuclease-free water. The RT step was performed by using random hexamer primers (Introgen, Carlsbad, CA, USA) with 10 μL of extracted RNA and the First-strand cDNA Synthesis Kit (GE Healthcare Europe GmBH, Orsay, France) as recommended by the manufacturer’s protocol. Then, 5 μL of the cDNA obtained was used as the template for the PCR step with each outer set of primers specific for NDV F (Kho et al., 2000) or IBV S1 (Akin et al., 2001). Conventional PCR was carried out with the GeneAmp PCR System 2400 (Perkin-Elmer, Applied-Biosystems, Paris, France) using a

Table 1

| Year | Region | Locality | Species/prod syst | Collected serum | NDV positive | IBV positive | NDV prevalence (× 100) | IBV Prevalence (× 100) |
|------|--------|----------|-------------------|-----------------|--------------|--------------|-----------------------|-----------------------|
| 2010 | Agneby | Agboville/Adzope | BYC | 382 | 91 | 244 | 23.8 | 63.9 |
|      |        |           | CMF | 7 | 0 | 7 | – | – |
|      |        |           | Ducks | 5 | 3 | 2 | 60 | – |
|      | District of Abidjan | Bingerville/Abidjan Market | BYC | 17 | 7 | 11 | 41.2 | 64.7 |
|      |        |           | CMF | – | – | – | – | – |
|      |        |           | Ducks | 25 | – | 4 | 16 | – |
|      |        |           | Guinea fowl | – | – | – | – | – |
|      | South Comoe | Aboisso | BYC | 292 | 110 | 217 | 37.6 | 74.3 |
|      |        |           | CMF | – | – | – | – | – |
|      |        |           | Ducks | 23 | 9 | 1 | 39.1 | 4.3 |
|      |        |           | Guinea fowl | – | – | – | – | – |
|      | Subtotal |         |        | 751 | 220 | 477 | 29.3 | 63.9 |
| 2011 | Agneby | Agboville/Adzope | BYC | 91 | 8 | 77 | 8.8 | 84.6 |
|      |        |           | CMF | – | – | – | – | – |
|      |        |           | Ducks | 31 | 0 | – | – | – |
|      | District of Abidjan | Bingerville/Abidjan Market | BYC | – | – | – | – | – |
|      |        |           | CMF | – | – | – | – | – |
|      |        |           | Ducks | – | – | – | – | – |
|      |        |           | Guinea fowl | – | – | – | – | – |
|      | South Comoe | Aboisso | BYC | 351 | 62 | 293 | 17.7 | 83.5 |
|      |        |           | CMF | – | – | – | – | – |
|      |        |           | Ducks | 9 | 1 | – | 11.1 | – |
|      | Subtotal |         |        | 482 | 71 | 370 | 14.7 | 76.8 |
| 2012 | Agneby | Agboville/Adzope | BYC | 310 | 33 | 284 | 10.6 | 91.6 |
|      |        |           | CMF | – | – | – | – | – |
|      |        |           | Ducks | – | – | – | – | – |
|      | District of Abidjan | Bingerville/Abidjan Market | BYC | 8 | 3 | 8 | 37.5 | 100 |
|      |        |           | CMF | – | – | – | – | – |
|      |        |           | Ducks | 6 | 2 | – | 33.3 | – |
|      | South Comoe | Aboisso | BYC | 386 | 93 | 260 | 24.1 | 67.4 |
|      |        |           | CMF | – | – | – | – | – |
|      |        |           | Ducks | – | – | – | – | – |
|      | Subtotal |         |        | 710 | 129 | 554 | 18.2 | 78 |
|      | Total |         |        | 1943 | 420 | 1401 | 21.6 | 72.3 |

BYC: backyard chicken. CMF: commercial poultry farm. Prod syst: production system. * Minus 5 guinea fowl for IBV total serum.
and nervous signs, inappetence, diarrhoea), a total of 1254 chickens (11%) of the total 11,403 birds sampled presented apparent signs of disease (340 in 2010, 503 in 2011 and 411 in 2012). The total number of dead and sick chickens was 1488 from which we collected both cloacal and tracheal swabs giving a total of 2976 swab samples.

A total of 22,806 samples, consisting of 11,403 cloacal and 11,403 tracheal swabs, were collected during the period 2010–2012 during the monthly surveys, including the samples from dead and sick chickens. These collected materials were pooled using maximum of 5 individual samples per pool, giving 4562 pools of samples (2281 pools of each type of swab) including 595 pools from sick and dead birds. During the same period, 1943 serum samples were collected (serum sampling only every 3 months) with 186 sera from apparently sick chickens (9.6%). These samples were obtained from backyard poultry farms, commercial farms and at live-bird markets within the three selected regions and involved samples from chickens, ducks and guinea fowl, with chickens representing 95.6% (23,667/24,749), duck, 3.04% (753/24,749) and guinea fowl, 1.3% (329/24,749) of the total collected samples, including serum samples (Tables 1 & 2). An average of 687.5 samples (24,749/36) was collected each month during the survey period. Samples collected from live bird markets represented 19% (4704/24,749) of the total.

3.2. NDV- and IBV-specific antibodies

Of a total of 1943 serum samples screened using the HA/HI test, 420 sera were positive, with an overall NDV prevalence of 21.6% (95% CI
4. Discussion

The avian influenza crisis, starting in Asia, reached Africa and in particular Ivory-Coast in 2006, causing huge economic losses. This situation greatly affected local poultry industries along with the loss of an important source of proteins for middle income and poor populations. Interestingly, the avian crisis highlighted the importance of ND (of which the main concern is the velogenic form) alongside other respiratory diseases such as IB. We took advantage of the ongoing surveillance for avian influenza virus within Ivory-Coast which followed the detection of 12 outbreaks of H5N1 (Couay-Hymann et al., 2009). Birds that were sampled were clinically examined for any signs of disease. Animals showing clinical signs were included in the survey along with dead animals found on the site of sampling. The collected samples were screened for avian influenza virus type A RNA and for specific subtype H5, H7 and H9 antibodies and the overall result remained negative (Couay-Hymann et al., 2012b).

These same samples have been screened in the present study for the presence of NDV and IBV, using assays for both genetic material and antibodies, for the period 2010–2012. The study has demonstrated the importance of ND in these mainly rural areas with poor populations, whose backyard poultry farms contribute significantly to household income and so contribute to poverty alleviation. Particularly, essentially all chickens found dead or sick were positive for NDV genome, with 96.3% prevalence. Partial sequencing of the F gene from samples collected on dead chickens showed the presence of polybasic sequence at the F protein cleavage site, corresponding with that expected for a velogenic strain of NDV. NDV-specific antibody prevalence ranged from 18.2% to 29.3% over the period of the study, with an overall average value of 21.6%, while the NDV F gene detection gave an overall prevalence of 14.7%, showing widespread distribution of the virus even among apparently healthy animals. These results confirm a previous study undertaken in Ivory-Coast on the burden of NDV in backyard poultry units, when compared to commercial farms where vaccinations are implemented in a correctly and thoroughly applied programme (Couay-Hymann et al., 2012a). In rural regions, no vaccination against NDV is implemented on free range poultry. Among the three avian species studied, chickens, with 22.1% seroprevalence, are of main concern. The widespread nature of NDV in these populations contributes to the maintenance of the endemic pattern of the disease, causing mass seasonal death and impacting negatively on food security and poverty alleviation in those rural populations.

If ND is well known and studied in Africa, this is not the case with IB, which remains less investigated, with few data available presently (Ducatez et al., 2009). Cases of IB are reported mainly from commercial layer farms based on clinical signs such as respiratory distress, decline of the egg production, and damage of the shape of the eggs. Vaccination against the disease is strongly recommended in commercial poultry farms. Although field veterinary personnel and rural farmers report from time to time cases of low egg size or change of the shape of the eggs, any respiratory signs in the field are associated with, and reported as, ND. Little is also known on IB in backyard poultry units, since ND is still reported as the most important disease in that type of poultry farming. The study undertaken in Ivory-Coast on the burden of NDV in backyard poultry farms. Among the three avian species studied, chickens, with 22.1% seroprevalence, are of main concern. The widespread nature of NDV in these populations contributes to the maintenance of the endemic pattern of the disease, causing mass seasonal death and impacting negatively on food security and poverty alleviation in those rural populations.

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While we observed a much higher seroprevalence for IBV than for NDV (72% versus 22%), the virus prevalences were similar for both viruses. The sequenced NDV F cleavage sites highlighted the circulation of velogenic strains of the virus in the country. Taken together these results suggest that while healthy birds have been detected positive for NDV velogenic strains, the pathogen likely causes severe mortality in the field that may explain a lower seroprevalence for NDV than for IBV. A recent study in domestic poultry reported 8.7% NDV prevalence by virus isolation in Uganda with circulation of mainly velogenic viruses as well. In the Ugandan study, 28.6% (6/21) and 9.0% (108/1229) of the chickens from which NDV could be isolated were sick and healthy, respectively, confirming both the morbidity caused by velogenic NDV in the field and the detection of these strains in asymptomatic birds (Byarugaba et al., 2014).

Forty nine (49) of the pooled samples were positive for both NDV and IBV. Since each pool contained material from 5 birds, this result could be that the two viruses came from different birds or was a dual infection of the same individual bird. To clarify this situation, further work clearly needs to be done on individual samples from each positive pool. This study on IBV in free range poultry farms is the first investigation on this disease undertaken in the country. Commercial poultry farms in the country used to vaccinate their flocks with vaccine having the Massachusetts 41 (M41) strain of IBV while several serotypes circulate worldwide and there is not always cross-protection from one serotype to another (reviewed in Cavanagh, 2003). There needs to be fuller investigation to determine the genotype(s) and serotype(s) of the strains which are present in a concerned area prior to any vaccination. Re-use of samples collected for AIV surveillance may provide the opportunity to characterise the IBV strains currently circulating in Ivory-Coast.

Acknowledgements

We are grateful to Pr. C.P. Muller and Dr C. Snoeck, Laboratoire National de Santé, Centre de Recherche Public Santé, Luxembourg, who provided the ELISA kit for the IBV antibody detection.

We would like to thank the field veterinary services and personnel for their collaboration as well as all the poultry owners, vendors and other stakeholders for their cooperation during this study.

We specially thank Dr M. Baron, The Pirbright Institute, Ash Road, Pirbright GU24 ONF, UK, for his comments and the editing of this manuscript.

This study was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services under contract no. HHSN266200700005C, by the American Lebanese Syrian Associated Charities (ALSAC).

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