Surveillance for avian influenza and Newcastle disease in backyard poultry flocks in Côte d'Ivoire, 2007–2009

E. Couacy-Hymann [(1)*], A.V. Kouakou [(1)], C.K. Kouamé [(1)], A.L. Kouassi [(1)], Y.M. Koffi [(1)], P. Godji [(1)], P. Nana [(1)], Z. Tarnagda [(2)] & C. Akoua-Koffi [(3)]

(1) Laboratoire National d'Appui au Développement Agricole (LANADA)/Laboratoire Central de Pathologie Animale (LCPA), B.P. 206, Bingerville, Côte d'Ivoire
(2) Institut de Recherche en Sciences de la Santé (IRSS), 399 Avenue de la liberté, B.P. 545 Bobo-Dioulasso, Burkina Faso
(3) Institut Pasteur, Département de Virologie – Épidémiologie, 01 B.P. 490 Abidjan, Côte d'Ivoire
*Corresponding author: chymann@hotmail.com; e.couacy-hymann@lanada.ci

Summary

Between 2007 and 2009, active surveys were conducted on backyard poultry (chickens, guinea fowls and ducks) in four areas of Côte d'Ivoire, including two areas where avian influenza H5N1 outbreaks occurred in 2006. Each bird underwent clinical examination. In total, 5,578 sera, 4,580 tracheal swabs and 5,120 cloacal swabs were collected, plus tissues from 35 sick chickens.

Using the haemagglutination inhibition (HI) test, 277 and 36 serum samples were positive for H5 and H7, respectively; all were negative for H9. All samples were negative by reverse transcription polymerase chain reaction. These results confirm the circulation of H5 and H7 influenza subtypes in backyard poultry in Côte d'Ivoire. Given that the seropositive birds were healthy, the circulating subtypes may be low pathogenicity avian influenza strains.

Half (2,680) of the sera collected from chickens were tested by HI for Newcastle disease virus (NDV) antibody: 531 were positive. The seroprevalence of 19.8% confirms the endemic status of NDV, but may underestimate its true prevalence in Côte d'Ivoire.

Keywords

Avian influenza – Côte d'Ivoire – H5 subtype – H7 subtype – H9 subtype – Haemagglutination inhibition test – Newcastle disease virus – Reverse transcription polymerase chain reaction.

Introduction

Avian influenza (AI) viruses belong to the *Influenzavirus A* genus, family *Orthomyxoviridae* (8). They are classified into various subtypes on the basis of the antigenic properties of two surface glycoproteins that are associated with virus attachment and cellular release, namely the haemagglutinin (HA), with 16 subtypes (H1–H16), and the neuraminidase (NA), with nine subtypes (N1–N9) (5, 7, 15). The viruses can be divided into two distinct groups, based on their ability to cause clinical disease in susceptible birds. The first group comprises highly pathogenic avian influenza (HPAI) viruses, which cause severe disease in domestic poultry, resulting in millions of deaths in birds throughout the world. Outbreaks of HPAI inflict major economic damage on the poultry industry (2, 3, 6), and also on backyard poultry flocks. These viruses are restricted to subtypes H5 and H7; however, not all viruses of these subtypes cause HPAI (2). The second group comprises low pathogenicity avian influenza (LPAI) viruses, which produce a milder disease with respiratory signs, depression and problems with egg production (2, 9). In 2006, HPAI H5N1 was detected in seven African countries (Nigeria, Egypt, Niger, Cameroon, Burkina Faso, Côte d'Ivoire and Djibouti), and in 2007 it was detected in three more countries (Ghana, Togo and Benin). Most of the reported cases occurred on backyard poultry farms, except in Nigeria and Egypt where layers and broilers from the poultry industry were predominantly affected.

Between April and November 2006 there were 12 cases of H5N1 in Côte d'Ivoire: 11 in free-range poultry and one in a wild sparrow hawk (*Accipiter nisus*) (4). Since that date, a surveillance programme has been implemented to detect cases of AI in the country.

The present study was based on an active surveillance programme, which principally targeted backyard poultry. The main objective was to determine the circulation of H5, H7, and H9 virus subtypes in the local avian populations. In addition, a seroprevalence study of Newcastle disease virus (NDV) was conducted in the same population.

Materials and methods

Animal sampling sites

Since the first outbreak of avian influenza virus (AIV) in Côte d'Ivoire in 2006 (Fig. 1), the surveillance of poultry farms and backyard poultry by livestock farmers has been strengthened all over the country. For this study, samples were taken from 50 randomly selected villages in four regions: 15 in the southern region, which is the biggest large-scale poultry production area in the country; 15 from the eastern region, which has a border with an infected country and is the second largest poultry production area in the country; 15 from the northern region, which has a border with an infected country (Burkina Faso) and 5 from the western region, where an H5N1 positive case was found in 2006. The selected villages were not involved in vaccination programmes against AIV or NDV.

X : sampling sites

+ : H5N1 outbreaks in 2006

● : Main city

Fig. 1

Sampling sites used for the survey of avian influenza virus in Côte d'Ivoire

Clinical examination and sample collection

Each bird (chicken, guinea fowl or duck) was clinically examined for signs of disease before sampling. Blood samples were obtained from a minimum of 30 birds per village and processed to yield serum. Tracheal and cloacal swabs were also collected from the same birds. A total of 5,578 serum samples, 4,580 tracheal swabs and 5,120 cloacal swabs were obtained (Table I). Serum samples were stored at –20°C and swabs at –80°C until used for analysis.

Twenty-six and nine sick birds from the northern and western regions respectively were slaughtered, and tissue samples were collected at post-mortem examination. These tissue samples were stored at –80°C until processing.

Serological testing

Haemagglutination/haemagglutination inhibition test for avian influenza and Newcastle disease

The haemagglutination/haemagglutination inhibition (HA/HAI) tests were performed following the reference method (15), using reference antigens from H5N3 (Batch No. 01/06 – A/duck/It/775/04), H7N1 (Batch No. 06/06 – A/Ck/It/1067/V99), H9N2 (Batch No. 13/06 and No. 16/06 – A/Turkey/Wisconsin/66), and NDV (Batch No. 1/08 – Ulster 2C); their corresponding positive sera were used as positive controls.

Half (2,680) of the serum samples collected from chickens (consisting of 910 sera obtained in 2007, 1,024 from 2008 and 746 from 2009) were randomly selected and tested for antibodies against NDV.

The reference reagents were provided free of charge by the World Organisation for Animal Health (OIE)/Food and Agriculture Organization of the United Nations (FAO) Reference Laboratory in Padua (Italy).

Processing of swabs and tissue samples, and RNA extraction

The tissue samples, and tracheal and cloacal swabs, were processed as described previously (10, 13). 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 and 10 µL was used as the template for the reverse transcription polymerase chain reaction (RT-PCR).

Primers, single-stranded complementary DNA synthesis and conventional polymerase chain reaction technique

Different sets of primers (Table II) were used for the detection of influenza A virus subtypes H5, H7 and H9 (10, 12, 14).

Table I
Description of samples collected from poultry between 2007 and 2009
Samples were collected from 50 randomly selected villages: 15 villages from each region, except the western region, where five villages were selected

Year	Species	Type of sample	No. of samples from each region				Total no. of samples
			South[a]	North[b]	East[c]	West[d]	
2007	Chicken	Tr sw	448	450	470	160	1,528
		Cl sw	452	471	471	158	1,552
		Serum	515	449	696	160	1,820
	Guinea fowl	Tr sw	13	–	6	–	19
		Cl sw	14	–	12	–	26
		Serum	10	2	17	–	29
	Duck	Tr sw	–	–	2	–	2
		Cl sw	4	2	4	–	10
		Serum	–	–	2	–	2
Subtotal of collected samples			**1,456**	**1,374**	**1,680**	**478**	**4,988**
2008	Chicken	Tr sw	484	459	440	155	1,054
		Cl sw	485	458	452	155	1,550
		Serum	831	467	597	152	2,047
	Guinea fowl	Tr sw	31	–	–	–	31
		Cl sw	32	–	–	–	32
		Serum	47	3	2	–	52
	Duck	Tr sw	90	–	5	–	95
		Cl sw	90	–	5	–	95
		Serum	90	–	5	–	95
Subtotal of collected samples			**1,696**	**1,387**	**1,506**	**462**	**5,051**
2009	Chicken	Tr sw	460	457	647	163	1,727
		Cl sw	458	456	657	160	1,731
		Serum	464	445	426	158	1,493
	Guinea fowl	Tr sw	21	–	–	–	21
		Cl sw	21	–	–	–	21
		Serum	–	–	–	–	–
	Duck	Tr sw	66	–	37	–	103
		Cl sw	66	–	37	–	103
		Serum	14	–	26	–	40
Subtotal of collected samples			**1,570**	**1,358**	**1,830**	**481**	**5,239**
Total of collected samples			4,722	4,119	5,016	1,421	15,278

a) Biggest large-scale poultry production area and detection of 10/12 H5N1 positive cases
b) Border with infected country, Burkina Faso, and movements of people and live poultry between the two countries
c) Second biggest large-scale poultry production area and border with Ghana, infected country
d) One H5N1 positive case
Tr sw: tracheal swab
Cl sw: cloacal swab

Table II
Primers used in the conventional reverse transcription polymerase chain reaction for diagnosis of avian influenza virus (AIV) subtypes H5, H7 and H9

Subtype	Primer
AIV subtype H5 (ref. 10)	
H5Kha- (793–819)	5′-CCT CCA GA**R** TAT GC**M** TA**Y** AAA ATT GTC
H5Kha-3 (1081–1103)	5′-TAC CAA CCG TCT ACC AT**K** CC**Y** TG
Size of amplified product: 300–320 bp	
AIV subtype H7 (ref. 11)	
H7HA-1 (1–20)	5′-AGC AAA AGC AGG GG**W** TAC AA
H7HA-2 (616–635)	5′-GA**R** CAG ACC AA**R** **Y**T**M** TAT GG
Size of amplified product : 634 bp	
AIV subtype H9 (ref. 9)	
H9-151 F (151–171)	5′-CT**Y** CAC ACA GA**R** CAC AAT GG
H9-638 R (619–639)	5′-TC ACA CTT GTT GTT GT**R** TC
Size of amplified product: 488 bp	

bp: base pairs
Abbreviations for wobble positions (in bold): R = A/G; Y = C/T; W = A/T; M = C/A; K= G/T

Reverse transcription was performed using 10 µl of extracted RNA in a 200 µl Eppendorf tube with the First Strand cDNA Synthesis kit (Healthcare, Germany). The manufacturer's protocol was followed, using the random primer $pdN_{(6)}$ provided.

Following this, 5 µl of the RT product was used as a template for the PCR in a 200 µl thin-walled tube. The conventional PCR was carried out with the Gene Amp PCR system 2400 (Perkin-Elmer) using a reaction mixture (50 µl) as previously described (4, 10, 14).

Results

Clinical signs in selected poultry

All birds were examined for clinical signs prior to sampling. In 6 out of 15 villages in the northern region a

total of 43 (17 and 26 sick chickens in 2007 and 2009, respectively) of 180 chickens examined showed the following clinical signs: cough, inappetence, depression and diarrhoea, and 26 sick birds were slaughtered for the collection of tissue samples. In the western region, clinical signs were recorded in 13 (4 sick chickens in 2007 and 9 in 2008), of a total of 150 chickens, with inappetence/depression and nasal discharge; 9 sick individuals were slaughtered. The remaining sampled poultry were apparently healthy and no case of recent mass mortality of poultry had been reported within the two months before the investigation (Table III).

Serological testing

Antibodies against H5, H7 and H9

From a total of 5,578 serum samples collected throughout the country and analysed by the H5 HA/HAI technique, 277 sera (5%) from 32 villages were positive, with titres ranging from 1/16 to 1/1,024. With regard to H7, 36 sera (0.6%) from 26 villages were positive, with titres ranging from 1/16 to 1/64. No serum sample was positive for both H5 and H7. All 5,578 serum samples were negative for H9 antibodies. The detailed breakdown of positive cases is shown in Table III.

Detection of antibodies against Newcastle disease virus

Of a total of 2,680 serum samples collected from backyard chickens, 531 (19.8%) were positive, with titres ranging from 1/16 to 1/1,024 (Table III). Positive samples were found in all four regions and throughout the study period.

Reverse transcription polymerase chain reaction

Using the RT-PCR technique with specific sets of primers for H5, H7 and H9, tissue samples from 35 sick chickens, in addition to 4,580 tracheal swabs and 5,120 cloacal swabs, were analysed. All the samples were negative with all three primer sets (Table III).

With regard to the sick chickens, samples from 25 out of the 35 individuals were NDV positive (data not shown).

Discussion

The H5N1 HPAI virus was detected in Côte d'Ivoire in 2006, and from April to November 12 outbreaks were confirmed. They involved mainly backyard poultry flocks (11/12 cases); a twelfth case was found in a wild sparrow hawk (*Accipiter nisus*) (4). Since that date, the Veterinary Services, along with the National Laboratory for the Support of Agricultural Development/Central Laboratory for Animal Diseases (LANADA/LCPA) in Bingerville, have strengthened field surveillance for AIV. Actions have been implemented to increase public awareness and to train field staff in areas such as the recognition of clinical signs and control measures to be taken. For modern farms, biosecurity measures have been upgraded on and around the farms. As yet, no case of influenza has been recorded in poultry in this type of production system in Côte d'Ivoire.

This active investigation, focused on backyard poultry flocks, was based on the authors' previous findings. Subtypes H5 and H7 were found in three of the four

Table III
Results of field investigation of clinical signs in individual birds, with results from serology and reverse transcription polymerase chain reaction analysis for the period 2007–2009

Sampling region	Clinical signs	Serology results: number of positive serum samples in each year												RT-PCR results
		2007				2008				2009				
		H5	H7	H9	NDV	H5	H7	H9	NDV	H5	H7	H9	NDV	H5/H7/H9
South	No clinical signs	8 (16–64)**	Neg.	Neg.	48 (16–512)	Neg.	1 (16)	Neg.	67 (16–256)	Neg.	2 (16)	Neg.	59 (16–128)	Neg.
North	Cough, inappetence, diarrhoea (43/180)*	9 (16–64)	Neg.	Neg.	50 (16–64)	39 (16–512)	13 (16–64)	Neg.	26 (16–256)	Neg.	Neg.	Neg.	33 (16–256)	Neg.
East	No clinical signs	196 (16–1,024)	Neg.	Neg.	85 (16–1,024)	Neg.	3 (16–32)	Neg.	65 (16–64)	25 (16–128)	17 (16–64)	Neg.	42 (16–512)	Neg.
West	Inappetence, nasal discharge (13/150)*	Neg.	Neg.	Neg.	30 (16–1,024)	Neg.	Neg.	Neg.	18 (16–128)	Neg.	Neg.	Neg.	8 (16–128)	Neg.
Total positive		**213/1,851**	**0**	**0**	**213/910**	**39/2,194**	**17/2,194**	**0**	**176/1,024**	**25/1,533**	**19/1,533**	**0**	**142/746**	**0**

* : number of positive cases out of the total number of birds examined for clinical signs
**: titre range of tested serum samples
Neg.: negative
NDV: Newcastle disease virus
RT-PCR: reverse transcription polymerase chain reaction

surveyed regions within the study period. The highest seroprevalence rates were 11.5% in 2007 and 1.2% in 2009. The H9 subtype was not found in any region during the period of the survey. No avian influenza viruses of the H5, H7 or H9 subtypes were detected in the western region.

The tissue samples collected at post-mortem examination from sick birds, and the tracheal and cloacal swabs, were negative by RT-PCR. This confirmed the absence of actively circulating HPAI viruses in the free-ranging poultry in the villages surveyed. All 5,535 chickens, ducks and guinea fowls (5,578 minus 43 sick chickens) from which serum samples were obtained were healthy and showed no clinical signs at the time of sampling. In addition, the owners of the poultry did not report any outbreaks of mass mortality before the present study. The results from the active surveillance in backyard poultry indicate that H5 and H7 LPAI strains are circulating in free-ranging poultry flocks in these regions. These findings are of importance because LPAI strains can mutate to HPAI strains (1, 13). Therefore, the surveillance on backyard poultry farms needs to be reinforced all over the country, with an additional goal of isolating circulating AIV strains in order to confirm the presence of LPAI and allow further studies on the characteristics of the virus strains.

After the detection of H5N1 in Côte d'Ivoire, the Ministry of Agriculture decided to introduce vaccination. However, the vaccination programme did not cover the entire country and the active surveillance in the current study did not involve any vaccinated poultry populations. Therefore, the serological detection of H5 and H7 in healthy poultry indicates the natural circulation of LPAI strains.

This study also provided an opportunity to determine the seroprevalence of NDV, which is known to be endemic in Côte d'Ivoire and is not subject to countrywide control measures. Annual outbreaks are mainly observed during the rainy season (July to August) and during the dry cold season (December to February), which is the harmattan period (Couacy-Hymann, unpublished observations). Newcastle disease remains the major disease affecting free-range poultry production, but this is not the case in modern farms, where vaccination against NDV is adequately implemented (Couacy-Hymann, unpublished observations). The 19.8% seroprevalence rate found in this study clearly shows the importance of this disease in backyard poultry flocks. (This seroprevalence rate is probably lower than the actual rate, but it is not possible to know for sure because there are very few reports from owners of free-range poultry and there is no reporting system at national or regional levels.) In addition, samples were taken from 35 out of the 43 sick birds, and 25 of them were NDV positive using RT-PCR (data not shown). This finding emphasises the need to pay more attention to Newcastle disease, the control of which could improve farmers' livelihoods.

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Surveillance de l'influenza aviaire et de la maladie de Newcastle dans les élevages aviaires familiaux de Côte d'Ivoire de 2007 à 2009

E. Couacy-Hymann, A.V. Kouakou, C.K. Kouamé, A.L. Kouassi, Y.M. Koffi, P. Godji, P. Nana, Z. Tarnagda & C. Akoua-Koffi

Résumé

De 2007 à 2009, une surveillance active a été exercée dans les élevages familiaux de volailles (poulets, pintades et canards) de quatre zones de la Côte d'Ivoire, dont deux zones qui avaient enregistré en 2006 des foyers d'influenza aviaire dus au virus de type H5N1. Chaque volaille a été examinée cliniquement. Au total, 5 578 échantillons sériques, 4 580 écouvillons trachéaux et 5 120 écouvillons cloacaux ont été analysés, en plus des échantillons tissulaires provenant de 35 poulets malades.

L'épreuve d'inhibition de l'hémagglutination (IH) a mis en évidence la présence d'anticorps dirigés contre le sous-type H5 du virus de l'influenza dans 277 sérums, et contre le sous-type H7 dans 36 autres sérums. Les résultats obtenus par amplification en chaîne par polymérase couplée à une transcription inverse ont été négatifs pour tous les prélèvements. Ces résultats confirment la circulation des sous-types H5 et H7 du virus de l'influenza dans les élevages familiaux de volailles en Côte d'Ivoire. Les volailles séropositives étaient en bonne santé, ce qui laisse penser que les sous-types présents sont des souches d'influenza aviaire faiblement pathogènes.

Les moitié des échantillons de sérum de poulet (2 680) ont été soumis à une épreuve d'inhibition de l'hémagglutination afin de détecter la présence d'anticorps dirigés contre le virus de la maladie de Newcastle : les résultats ont été positifs pour 531 de ces sérums. La prévalence sérologique constatée s'élevant à 19,8 % confirme la présence endémique de la maladie de Newcastle, bien que ce taux soit probablement inférieur à celui de la prévalence réelle de la maladie en Côte d'Ivoire.

Mots-clés

Amplification en chaîne par polymérase couplée à une transcription inverse – Côte d'Ivoire – Épreuve d'inhibition de l'hémagglutination – Influenza aviaire – Sous-type H5 – Sous-type H7 – Sous-type H9 – Virus de la maladie de Newcastle.

Vigilancia de la influenza aviar y la enfermedad de Newcastle en bandadas caseras de aves de corral de Côte d'Ivoire entre 2007 y 2009

E. Couacy-Hymann, A.V. Kouakou, C.K. Kouamé, A.L. Kouassi, Y.M. Koffi, P. Godji, P. Nana, Z. Tarnagda & C. Akoua-Koffi

Resumen

Entre 2007 y 2009 se estudiaron activamente las bandadas caseras de aves de corral (pollos, pintadas y patos) de cuatro zonas de Côte d'Ivoire, dos de las cuales habían sufrido en 2006 brotes de influenza aviar por el virus H5N1. Todas

las aves fueron sometidas a examen clínico. En total se extrajeron 5.578 muestras séricas, 4.580 traqueales y 5.120 cloacales, además de tejidos de 35 pollos enfermos.
La prueba de inhibición de la hemaglutinación (IH) arrojó resultado positivo para los subtipos H5 y H7 en 277 y 36 muestras séricas, respectivamente, mientras que todas resultaron negativas para el subtipo H9. La reacción en cadena de la polimerasa acoplada a transcripción inversa deparó únicamente resultados negativos. Estos resultados confirman que en las explotaciones avícolas caseras del país circula la influenza aviar de los subtipos H5 y H7. Dado que las aves seropositivas estaban sanas, es posible que los subtipos circulantes correspondan a cepas víricas de escasa patogenicidad.
La mitad de las muestras séricas de pollo (2.680) se sometieron a una prueba de HI para detectar anticuerpos contra el virus de la enfermedad de Newcastle: 531 resultaron positivos. El 19,8% de seroprevalencia que de ahí se sigue confirma el carácter endémico de ese virus, aunque quizá sea una subestimación de su verdadera prevalencia en Côte d'Ivoire.

Palabras clave
Côte d'Ivoire – Influenza aviar – Prueba de inhibición de la hemaglutinación – Reacción en cadena de la polimerasa acoplada a transcripción inversa – Subtipo H5 – Subtipo H7 – Subtipo H9 – Virus de la enfermedad de Newcastle.

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