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INVESTIGATION OF AN OUTBREAK OF ACUTE RESPIRATORY DISEASE IN CÔTE D'IVOIRE IN APRIL 2007

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Abstract

Background: This study aim was to investigate an outbreak of human cases of unexplained influenza-like illness and fatal acute respiratory infection (ARI), with simultaneous poultry illness and high mortality raising concerns of possible influenza A (H5N1), virus in Cote d'Ivoire in February and March 2007.

Materials and Methods: To investigate the outbreak, we conducted active surveillance in the community and reviewed health registries. Persons meeting the case definition were asked to provide nasopharyngeal specimens. On the basis of clinical and epidemiological information, specimens were tested using conventional RT-PCR for the M gene of the influenza viruses and hemagglutinin H5 of avian influenza A (H5N1), virus; negative samples were tested for other respiratory viruses. Specimens from healthy animals were also collected.

Results: Between October 2006, and February 2007, 104 suspected cases of Acute Respiratory Disease that included; 31 deaths recorded. We collected and tested 73 nasopharyngeal specimens; of which, 2, were positive for human *Coronavirus* OC43 and 1 for influenza C virus. No pathogens were identified in animal specimens.

Conclusions: The investigation quickly ruled out influenza A (H5N1), virus as the cause and found laboratory-confirmed cases of influenza C virus and human *Coronavirus* OC 43 for the first time in both Côte d'Ivoire and in a Sub-Saharan African country. However we were not able to show that these viruses caused the outbreak. Monitoring of influenza viruses must be a priority but other respiratory viruses and non-viral causes may be of interest too.

Key words: influenza virus, human Coronavirus OC 43, epidemic, respiratory viruses.

Introduction

Acute respiratory infections (ARI), is among the leading causes of death in children under five years. Forty-two percent of these ARI-associated deaths occur in Africa (Williams, 2002). Despite its importance in regard to morbidity as well as childhood mortality, the epidemiology and pathogenesis of ARI, particularly in Africa, remains understudied and consequently underappreciated. In Sub-Saharan Africa countries, data on the epidemiology of ARI are sparse and focuses mainly on lower respiratory tract infections LRTI (Williams, 2002). ARI are little documented in Côte d'Ivoire. Indeed, influenza was registered on the list of diseases under surveillance within the framework of the integrated disease surveillance and response (IDSR), since the year 2008 (Ministry of Health, 2008).

The recent discovery of avian influenza A (H5N1) virus in Asia sparked concern of a possible epidemic. The pathogenic H5N1 subtype first crossed the species barrier to infect humans in Hong Kong in 1997 (Chan, 2002) and since then has also infected other mammals (Keawcharoen, 2004; Klopfleisch, 2007; Payungporn, 2006; Thanawongnuwech, 2005). Côte d'Ivoire is a West African country, in which outbreaks of avian influenza A (H5N1) virus occurred in poultry in 2006 (Couacy-Hymann, 2008). The first case of high pathogenic avian influenza H5N1 virus in a West African country occurred in Nigeria in January 2006, in the state of Kaduna, and the virus subsequently was detected in Ghana, Burkina Faso, Côte d'Ivoire, and Sudan (Cattoli, 2009). Following confirmation of an outbreak of H5N1 in poultry in Abidjan in May 2006 (Couacy-Hymann, 2008), active surveillance for influenza viruses in humans was established for the early detection of cases of H5N1.

In April 2007, the Department of epidemiological disease surveillance (INHP/MoH), was informed of several human deaths from acute respiratory distress in the town of Diobala, located in the north of the country, during February and March 2007. These deaths were preceded by high mortality in local poultry houses. According to information collected from local authorities of the village, during the months of October, November and December 2006, high mortality rate was observed in chickens, sheep and goats. The animals were reported to have had runny nose, cough, sneezes and diarrhoea. Approximately 90% of the poultry (chickens) and 500 sheep and kids died and mortality peaked at day-3 to day-4 of illness. Around the third week of December, several villagers presented with an illness characterized by intense headaches, fever, cervical and thoracic pains, cough, difficulty breathing and hearing loss. The disease quickly progressed and death occurred between day-3 to day-5; people of all ages were affected. A team of investigators was sent to that site to investigate the cases and identify the agent potentially responsible for this outbreak.

Materials and Methods

Case definition

We defined a suspected case of Acute Respiratory Disease as any person presenting with high fever >38°C, and at least one respiratory

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symptom (e.g., cough, rhinorrhea or difficulties breathing), with onset between October 2006 and February 2007. In addition, persons that reportedly died of a respiratory disease were also considered as suspect cases. A confirmed case was defined as a suspect case with virus identified in a clinical specimen by the Pasteur Institute Influenza laboratory and/or a World Health Organization Collaborating Centre.

Active surveillance

Diobala has a population of about 1500 inhabitants. It is located about 4km from the health centre and about 44km from the administrative centre of the Department of Seguela. To identify patients meeting the case definitions we conducted several types of data collection. With the assistance of the community health workers, and volunteers of Diobala, we interviewed persons in households where we were told there was a case in order to identify persons who met the case definition. In addition, we reviewed the registries of health centres; the names of the patients who met the case definition were recorded on the investigation forms. For cases identified in the registers we visited each household and verified the information details and searched for contacts. The village of these patients was also recorded in order to determine the localities involved. For persons who died, the age, date of the death, size of the household, and information on the clinical features were recorded in order to establish a link between the death and the outbreak. The search for cases and contacts included the neighboring health area of Tiémassoba. All the cases and contacts found were from Diobala. We used standard questionnaires to screen persons who met the case definition or families of deceased patients. People living in the same household as the deceased case were questioned on their health status. Only nasopharyngeal specimens were collected from all living persons meeting the case definition and from persons with or without any clinical symptom but who had lived in the same household as the deceased therefore identified as close contact. No specimens were collected from persons who recovered from their illness and no post mortem specimens were available for testing.

Human and animal samplings

Nasopharyngeal swab samples were kept at 4°C and sent to the Pasteur Institute of Côte d'Ivoire for analysis. We did not collect specimens from healthy controls. A convenience sample was taken from sick animals in the village; specimens included cloacal and nasal swabs and autopsy tissue from sick chickens, and faeces from ill sheep and goats. These samples were kept on ice and then sent to the Central Laboratory for Animal Diseases of Bingerville for testing. All specimens were sent within 8 hours of collection.

RNA preparation

Specimens from persons were transported in viral transport media to the Pasteur Institute of Cote d'Ivoire. In the influenza and respiratory virus laboratory, samples were stored at -80°C until processed. RNA was extracted from 140 µl of each sample using a commercial reagent (QIAamp viral RNA mini kit®, Qiagen), as described by the manufacturer. RNA was eluted in 60 µl of nuclease-free water and 10 µl was used as the template for reverse transcriptase polymerase chain reaction (RT-PCR).

RT-PCR amplification

We performed conventional RT-PCR assay using Invitrogen One Step RT-PCR kits as recommended by the manufacturer. Briefly, RNA was reverse transcribed and amplified in a 50 µl reaction containing 25 µl of 2X RT-PCR buffer (Invitrogen), 4 U RNase inhibitor (Promega), 1 µl RT-PCR enzyme mix (Invitrogen), 0.6 µM each primer. We simultaneously amplified, by conventional RT-PCR, a 245 base pair fragment on the M gene of influenza A viruses using the following primers: M/+52 (5'-CTT CTA ACC GAG GTC GAA ACG -3') and M/-253 (5'-AGG GCA TTT TGG ACA AAK CGT CTA -3') and a 230 base pair fragment of the hemagglutinin H5 gene using the following primers: H5/-NML (5'-TGA CCT TAT TGG TGA CTC C-3') and H5/+NML (5'-GCC CCA AAT ATG TGA AAT C-3'), from the nasopharyngeal swabs. These primers were supplied by the Pasteur Institute of Cambodia. Reactions were first incubated at 50 °C for 30 min, followed by 94 °C for 3 min and were then thermal-cycled for 40 cycles (94 °C for 30 sec, 55 °C for 1 min, and 72 °C for 1 min). For specimens those were negative for the M gene amplicon, three multiplex hemi-nested RT-PCR assays were carried out as described by Bellau-Pujol et al. (2005). This molecular detection consisted of three multiplex RT-PCR reactions targeting 12 RNA respiratory viruses simultaneously: influenza viruses A, B and C, human respiratory syncytial virus (hRSV), human *metapneumovirus* (hMPV), parainfluenza viruses types 1–4 (PIV-1, -2, -3 and -4), human *Coronaviruses* OC43 and 229E (HCoV) and *rhinovirus* (hRV). All amplified products were visualized after electrophoresis on an ethidium bromide-stained 2 % agarose gel.

Confirmation and sequencing of amplified products

Amplified product and samples were sent to the WHO Collaborating Centre and the respiratory virus diagnostics laboratory, at CDC Atlanta, USA, for confirmation and sequencing.

Data analysis

For the statistical analyses, the data were entered and analyzed using the software Epi-Info CDC Atlanta version 6 to estimate the averages and the frequencies for the clinical and socio demographic variables and to describe morbidity and mortality.

Results

In the village of Diobala (population approximately 1500), between December 18, 2006 and February 18, 2007 we identified 104 suspect cases including 31 (29.8%), deaths. Thirty percent of the cases were children <5 years old and 46% were in persons aged 15 years and above. About 60% of the patients were male (sex ratio = 1.47). The case-fatality ratio and attack rates are extremely high (Table 1).

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Table 1: Number of suspected cases and deaths in persons with respiratory illness in Diobala, district of Séguela (North of Côte d'Ivoire), from October 2006 to February 2007 (n = 104).

Age group (years)	Case		Dead	Case-fatality ratio (%)	Population	Attack rate case/1000
	female	male				
0-4	13	18	7	22.6	261	118.8
5-9	7	4	4	36.4	229	48.0
10-14	8	6	3	21.4	198	70.7
≥ 15	14	34	17	35.4	812	59.1
Total	42	62	31	29.8	1500	69.3

The deaths were observed in all the age groups. The lethality of the respiratory illness during this period was high in this population. There were almost as many cases in the 0-4 years old group as there were in the ≥ 15 years old group. The attack rate in the 0-4 year's old group was the highest (118.8/1000).

The weekly number of deaths of persons with respiratory illness ranged from 0 to 10. The weekly number of respiratory deaths ranged from 1 to 5 until the week of January 29 through February 04, 2007 when there were 10 deaths. No deaths occurred after February 5, 2007 (Figure 1). The useful information on baseline levels of morbidity, mortality and respiratory illness in this community, in order to establish whether the outbreak represents an increase in disease and to what extent, were not available. However, according to the health authorities and through the review of the registers of the health center of Diobala, the mortality by ARI in this locality during the previous years is lower than that observed in our investigation. Between December 18, 2006, to February 4, 2007, the mortalities recorded in the registers showed 31 deaths by respiratory infection in 49 days in this locality with about 1500 inhabitants. Under this consideration, the Department of epidemiological disease surveillance (INHP/MoH) declared the outbreak. There is an epidemiological link between some deaths and the outbreak. Indeed, in two families' mother and son died with same clinical features, the two deaths occurred in a week interval.

A total of 73, nasopharyngeal specimens were collected and analyzed by conventional RT-PCR, 49 were from persons reporting a respiratory illness and the rest were from their contacts. All specimens were simultaneously negative for influenza A viruses (targeting the M gene protein) and for the H5. Testing for other respiratory viruses showed three (4%) specimens were positive for a respiratory virus; one sample was positive for influenza C virus and 2 for HCoV OC43 (Figure 2). The three positive specimens were collected on February 15, 2007; two were from ill persons and one was from a person without any symptom. The positive specimen for influenza C virus was collected from a malnourished male child of 17-months old who had close contact with a suspected case that had died. The child presented with fever, cough and myalgia on February 07, 2007 (specimen was collected 8, days after onset of symptoms). The two positive specimens for HCoV OC43 were collected respectively from a male farmer aged 65-years old without any clinical symptom but who had close and constant contact with two suspected cases (his wife and his daughter) who died, and from a female child aged 2 years old who had fever, cough, myalgia, rhinorrhea and was also malnourished. According to the relatives of this little girl, she had no contact with any suspected cases that died and had no known contact with animals. The date of her illness onset was estimated to be January 23, 2007 (specimen was collected 23 days after onset). Our molecular results were confirmed by sequencing. Because a bacterial etiology was also considered, the two ill children were treated with antibiotics. They recovered from their illness within a week.

Testing of the 63 animal samples at the Central Laboratory for Animal Diseases in Bingerville was negative for high pathogenic avian influenza A (H5N1) virus as well as for aspergillum and avian streptococcus.

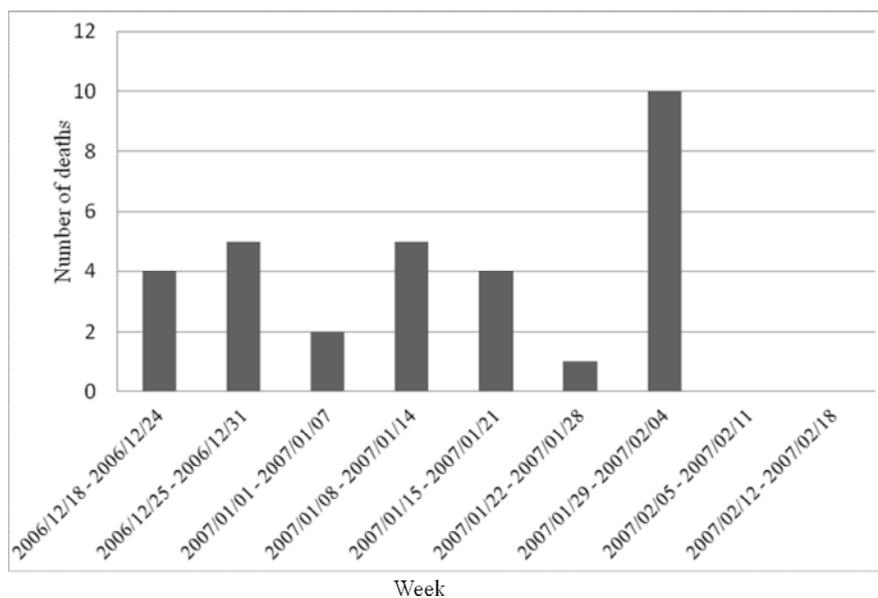


Figure 1: Weekly death of persons with acute respiratory infection in the village of Diobala from December 2006 to February 2007.

Thirty-one deaths with respiratory diseases arose since January 18, 2006 and continued until February 04, 2007. Three peaks of death were observed: with 5 deaths each week from 25 till 31 December 2006 and from 08 till 14 January 2007 and with 10 deaths during the week of January 29 through February 04, 2007.

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Discussion

On the basis of clinical and epidemiological information, three hypotheses were formulated as possible cause of such an outbreak such as anthroozoonosis (Avian influenza, antavirus pulmonary syndromes), seasonal flu, and atypical pneumonia (syndrome of severe acute respiratory infection). Symptoms were mainly respiratory and the disease quickly progressed and death occurred around day-3 to day-5; people of all ages were affected. Several animals died in the village before the occurrence of human cases. With the support of the healthcare workers and volunteers, persons corresponding to the case definition were looked for in the village of Diobala, and in the nearby localities of Tiémassoba. Questions were asked to cases and potential close contacts about possible risk factors for disease such as animal contact, contact with cases or dead person, circumstances within which the illness occurred, proportion of households, sources of food and water, history of travels made. All registered cases were from Diobala but the data were not able to establish the link between the source of exposure and the real mode of transmission.

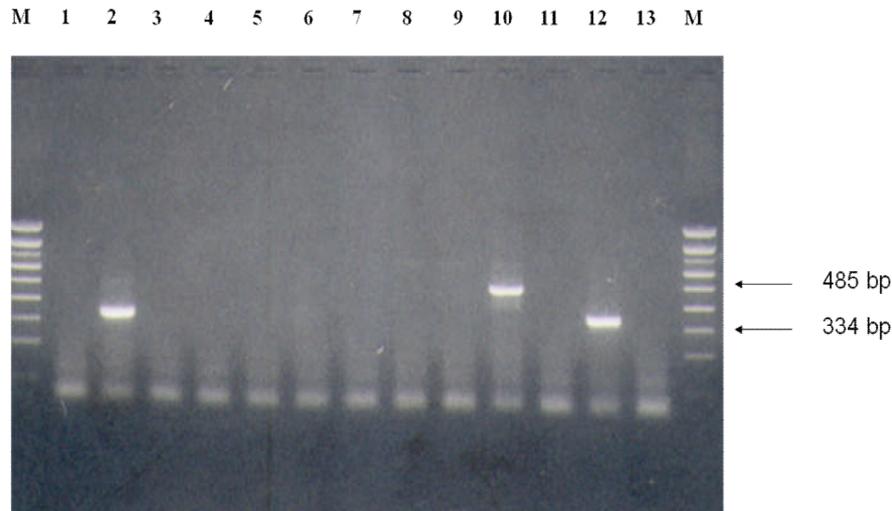


Figure 2: RT-PCR analysis of amplified products from nasopharyngeal swabs.

Electrophoretic analysis on a 2% agarose gel of the multiplex amplified products for simultaneous detection in only one tube of the nucleic acids of the following viruses: the Picornaviruses (Rhinovirus), *Coronavirus* 229E and OC43, and the influenza C virus. Each lane contains the human sample. Lanes 2 and 10 contain nucleic acids of the *Coronavirus* OC43 and lane 12 contains nucleic acids of the influenza C virus. Lanes M: molecular weight (Smart Ladder SF, Eurogentec). Molecular sizes at the right correspond to bands of the amplified products.

Influenza A (H5N1) virus was identified in a sub-Saharan African country in 2006, and in the same year, Côte d'Ivoire was the sixth African country to identify the virus (Couacy-Hymann, 2008). After introduction of H5, Côte d'Ivoire implemented a passive surveillance program where specimens from sick and dead birds of all species as well as those from humans ill with respiratory disease, were sampled. After a reported increase in human respiratory illnesses and deaths and poultry deaths, in the locality of Diobala, we investigated but found no evidence of H5N1 virus in either humans or poultry. The diversity of the human and animal species affected and the severity of the outbreak suggested to us that it could have been due to the highly pathogenic avian influenza A (H5N1) virus. However, this virus was not identified in the specimens analyzed. Besides H5, we tested for certain influenza types A, B and C would they have been able to identify less common influenza subtypes including H7 because primers sequences for this hemagglutinin were not available in our laboratories.

The low yield of pathogens from the specimens collected may be because cases had already recovered from the illness by the time the investigation was conducted. During our laboratory investigation for other respiratory viruses, specimens from humans yielded two viruses: HCoV OC43 and the influenza C virus. The RT-PCR technique used published sets of primers and the results were validated through repeat testing at the WHO Collaborating Centre at CDC Atlanta where sequencing was also conducted. This is the first time that HCoV OC43 and the influenza C virus have been described in a sub-Saharan African country. The influenza C virus is generally considered to cause non-severe disease. Its frequency is likely underestimated, as shown by the presence of anti-influenza C virus antibodies in a large proportion of the French population (Manuguerra, 1992). Before the emergence of SARS-CoV and the global outbreak in humans (Rota, 2003), there were two prototypes of human *Coronaviruses*, OC43 and 229E, both etiologic agents of the common cold (McIntosh, 1974). Studies suggest that HCoV-229E and HCoV OC43 cause 5 to 30% of human respiratory tract infections (McIntosh, 1970). The HCoV OC43 virus, which is generally considered to cause colds, has been described to cause lower respiratory tract infections (pneumonia and bronchitis) in infants and elderly persons (Vabret, 2003). HCoV illness can be accompanied by multiple respiratory and systemic symptoms, and has been associated with hospitalization (Gorse, 2009). Few data are known about HCoV because most laboratories do not test for it and it is probable that its pathogenic role is underestimated (Vabret, 1998). *Coronaviruses* cause acute and chronic respiratory, enteric, and central nervous system diseases in many species of animals, including humans (McIntosh, 1974; Vabret, 2009). The sequencing analysis showed 100% identity between our strains and a reference strain of human *Coronavirus* OC43 (dated not shown). HCoV OC43, usually causes a mild illness and would be unlikely to cause an outbreak of severe respiratory illness with deaths. There may have been something different about this *Coronavirus* or about the population. For example, the affected populations lived in a close community. There could also have been some kind of synergy with another pathogen, bacterial or viral. However, we can only speculate, as we do not have data to support *Coronavirus* as the primary cause of this outbreak. The same can be said of the influenza virus C finding.

The difficulties in confirming the cause/s of this outbreak show the limits of this investigation. The late notification of disease cases to health authorities was at the origin of the high number of registered deaths. Having attributed origin of deaths to mystics, the villagers did not spread the information, and the reporting, which should have been initiated earlier accused a delay. Several human deaths from acute respiratory distress and animals' deaths occurred between January and March 2007 and our investigation was conducted in April 2007. This delay was due to unstable socio-political situation in Côte d'Ivoire. In the registers, the clinical features of cases were dominated by a feverish respiratory symptom, which were observed by healthcare workers. The ARI diagnosis was then established under clinical arguments. No further details of the

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clinical features of cases were available. There were no signs or syndromes described in favor of another pathology. We did not focus on the testing done for non-viral causes of the outbreak such as bacterial or fungal culture on human specimens because we only collected nasopharyngeal specimen. Sometimes diseases such as septicaemia or meningitis can lead to signs and symptoms overlapping with those of respiratory illnesses. Not taking into account this possibility is a limitation for this investigation and this must be considered for future outbreaks. Some of these tests were done for animal samples for aspergillum and avian streptococcus, but these were all negative. No pathogens were identified in animal specimens. The end of the epidemic could be bound to a better organization of the response. Indeed, health authorities led an important intervention associating education and sensitization of the populations on rapid consultation and the measures of cough and hand washing hygiene as well as free treatment with antibiotics, antipyretics and of anti-cough medicines. Indeed, the investigation was practically made at the end of outbreak. An earlier investigation would have allowed collecting good quality information, data and specimens from cases and deaths in particular the nature and the type of exposure, the realization of several sampling types on sick persons. This information would certainly have contributed to establish the diagnosis.

In Côte d'Ivoire, until recently limited epidemiological data have demonstrated the circulation of influenza viruses (Heraud, 2012; Kadjo, 2013), but they are generally under diagnosed in medical practice in our country. In most respiratory illnesses, particularly in young children, treatment is usually for presumptive bacterial diseases and there are no data to describe morbidity and mortality from respiratory viruses. The investigations carried out in Diobala were conducted over concerns for avian influenza. The analyses carried out in the laboratory showed the circulation of two respiratory viruses: human *Coronavirus* OC43 and influenza C virus. Although it is unlikely that they were the cause of the outbreak, our findings highlight the importance of a rapid outbreak investigation with good laboratory diagnostics. This is the first description of the circulation of these viruses in Côte d'Ivoire and in any sub-Saharan African country. Our study shows that we have the surveillance capacity to identify an unexpected event (e.g., an increase in illness or deaths) and resources to collect specimens and laboratory capacity for diagnosis. Because of the strong epidemic potential of many respiratory viruses, it is important to increase the monitoring of respiratory disease outbreaks in Côte d'Ivoire and elsewhere in Africa.

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Conflict of interest: No conflict of interest to declare.

Ethical approval: Since this was an outbreak investigation it was considered an emergency public health response and did not require ethical approval.

References

- Bellau-Pujol, S., Vabret, A., Legrand, L., Dina, J., Gouarin, S., Petitjean-Lecherbonnier, J., Pozzetto, B., Ginevra, C., and Freymuth, F. (2005). Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses. *J Virol Methods*, 126: 53–63.
- Cattoli, G., Monne, I., Fusaro, A., Joannis, T.M., Lombin, L.H., Aly, M.M., Arafa, A.S., Sturm-Ramirez, K.M., Couacy-Hymann, E., Awuni, J.A., Batawui, K.B., Awoume, K.A., Aplogan, G.L., Sow, A., Ngangnou, A.C., El Nasri Hamza, I.M., Gamatié, D., Dauphin, G., Domenech, J.M., and Capua, I. (2009). Highly pathogenic avian influenza virus subtype H5N1 in Africa: a comprehensive phylogenetic analysis and molecular characterization of isolates. *PLoS One*, 4: e4842.
- Chan, P. K. (2002). Outbreak of avian influenza A (H5N1) virus infection in Hong Kong in 1997. *Clin. Infect. Dis.*, 34(Suppl. 2): 58–64.
- Couacy-Hymann, E., Danho, T., Keita, D., Bodjo, S.C., Kouakou, C., Koffi Y.M., Beudje, F., Tripodi, A., de Benedictis, P., and Cattol, G. (2008). The First Specific Detection of a Highly Pathogenic Avian Influenza Virus (H5N1) in Ivory Coast. *Zoonoses Public Health*. 56 (1), 10-15.
- Gorse, G.J., O'Connor, T.Z., Hall, S.L., Vitale, J.N., and Nichol, K.L. (2009). Human coronavirus and acute respiratory illness in older adults with chronic obstructive pulmonary disease. *J Infect Dis.*, 199 (6): 847-857.
- Heraud, J.M., Njouom, R., Rousset, D., Kadjo, H., Caro, V., Ndiaye, M.N., Victoir, K., Collard, J.M., Orelle, A., Yekwa, E.L., Ekaza, E., Razanajatovo, N.H., Adamou, L., Biscornet, L., Enouf, V., van der Werf, S., and Diop, O.M. (2012). Spatiotemporal circulation of influenza viruses in 5 African countries during 2008-2009: a collaborative study of the Institut Pasteur International Network. *J Infect Dis.*, 206 (S1): S5-S13.
- Kadjo, H.A., Ekaza, E., Coulibaly, D., Kouassi, D.P., Nzussouo, N.T., Kouakou, B., Ouattara, A., Adjougou, E.V., Akoua-Koffi, C.G., Elia, G.A., Victoir, K., Bretin-Dosso, M.C., and Mott, J.A. (2013). Sentinel surveillance for influenza and other respiratory viruses in Côte d'Ivoire, 2003-2010. *Influenza Other Respi. Viruses.*, 7 (3): 296-303.
- Keawcharoen, J., Oraveerakul, K., Kuiken, T., Fouchier, T., Amonsin, A., Payungporn, S., Noppornpanth, S., Wattanodorn, S., Theamboorniers, A., Tantilertcharoen, R., Pattanarangsarn, R., Arya, N., Ratanakoen, P., Osterhaus, D. M.E., and Poovorawan, Y. (2004). Avian influenza in tigers and leopards. *Emerg. Infect. Dis.*, 10: 2189–2191.
- Klopfleisch, R., Wolf, P.U., Uhl, W., Gerst, S., Harder, T., Starick, E., Vahlenkamp, T.W., Mettenleiter, T.C., and Teifke, J.P. (2007). Distribution of lesions and antigen of highly pathogenic avian influenza virus A/Swan/Germany/R65/06 (H5N1) in domestic cats after presumptive infection by wild birds. *Vet. Pathol.*, 44: 261–268.
- Manuguerra, J.C., Hannoun, C., and Aymard, M. (1992). Influenza C virus infection in France. *J. Infect.*, 24: 91–99.
- McIntosh, K., Kapikian, A.Z., Turner, H.C., Hartley, J.W., Parrott, R.H., and Chanock, R.M. (1970). Seroepidemiologic studies of coronavirus infection in adults and children. *Am. J. Epidemiol.*, 91: 585–592.
- McIntosh, K. (1974). Coronaviruses: a comparative review. *Curr. Top. Microbiol. Immunol.*, 63: 85–129.
- Ministry of Health, Côte d'Ivoire. (2008). Technical guide for integrated disease survey and response in Côte d'Ivoire. Abidjan, 293 p.
- Payungporn, S., Chutinimitkul, S., Chaisingh, A., Damrongwattanapokin, S., Buranathai, C., Amonsin, A., Theamboonlers, A., and Poovorawan, Y. (2006). Single step multiplex real-time RT-PCR for H5N1 influenza A virus detection. *J. Virol. Methods.*, 131: 143–147.
- Rota, P.A., Oberste, M.S., Monroe, S.S., Nix, W.A., Campagnoli, R., Icenogle, J.P., Penaranda, S., Bankamp, B., Maher, K., Chen, M.H., Tong, S., Tamin, A., Lowe, L., Frace, M., DeRisi, J.L., Chen, Q., Wang, D., Erdman, D.D., Peret, T.C., Burns, C., Ksiazek, T.G., Rollin, P.E., Sanchez, A., Liffick, S., Holloway, B., Limor, J., McCaustland, K., Olsen-Rasmussen, M., Fouchier, R., Gunther, S., Osterhaus, A.D., Drosten, C., Pallansch, M.A., Anderson, L.J., and Bellini W.J. (2003). Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Sci.*, 300: 1394–1399.
- Thanawongnuwech, R., Amonsin, A., Tantilertcharoen, R., Damrongwattanapokin, S., Theamboorniers, A., Payungporn, S., Nanthapornphiphat, K., Ratanamungklanon, S., Tunak, E., Songserm, T., Vivathanavanich, V., Lekdumrongsak, T., Kesdangakonwut, S., Tunhikorn, S. and Poovorawan, Y. (2005). Probable tiger-to-tiger transmission of avian influenza H5N1. *Emerg. Infect. Dis.*, 11: 699–701.
- Vabret, A., Brouard, J., Petitjean, J., Eugene-Ruellan, G., and Freymuth, F. (1998). Human coronavirus infections: importance and diagnosis. *Presse Med.*, 27: 1813-1817.
- Vabret, A., Mourez, T., Gouarin, S., Petitjean, J., and Freymuth, F. (2003). An outbreak of coronavirus OC43 respiratory infection in Normandy, France. *Clin. Infect. Dis.*, 36: 985–9.
- Vabret, A., Dina, J., Brison, E., Brouard, J., and Freymuth, F. (2009). Human coronaviruses. *Pathol. Biol. (Paris)*, 57: 149-160.
- Williams B. G. (2002). Estimates of World-Wide Distribution of Child Deaths from Acute Respiratory Tract Infections. *Lancet Infect Dis.*, 2:25–32.