

## Review

# Diagnosis and epidemiology of chicken infectious anemia in Africa

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Accepted 19 February, 2010

Chicken infectious anemia (CIA) has recently emerged as an important disease problem in some of Africa's major poultry-producing countries. Economic losses due to this disease arises from poor growth, increased mortality, carcass condemnations and the cost of antibiotics used to control secondary bacterial infections. Thus, it constitutes a significant threat to the continent's food security efforts. Published studies were reviewed to obtain data on techniques used for serological detection as well as biological and molecular diagnosis of CIA virus (CIAV) in Africa. In the African countries where CIAV has been reported, diagnosis involved the use of serological, biological and nucleic acid-based detection techniques. While serological investigations detected CIAV antibodies in chickens in South Africa, Egypt and Nigeria, monoclonal antibody reactivity showed that CIAV isolates from Nigeria were antigenically related to the reference Cuxhaven-1 virus. The polymerase chain reaction detected CIAV DNA in tissues, blood and sera of infected chickens while restriction endonuclease analysis indicated the circulation of a mixed population of CIAV strains among chickens in Africa. Analysis of sequenced isolates revealed that the amino acid composition of African CIAV strains was highly conserved. The implications of these findings for the epidemiology and control of CIA in African poultry are discussed.

**Key words:** Chicken infectious anemia virus, chickens, serology, virus isolation, molecular diagnosis, Africa.

## INTRODUCTION

Livestock, including poultry, are the major capital asset and income generation source of smallholder farmers in many developing countries where they often contribute up to 50% of agricultural gross domestic product and provide between 20-60% of household income on smallholder farms (ILRI, 2009). In particular, poultry are a good source for meeting the animal protein needs of many

African countries. However, there are some factors which constitute major constraints to the development and improvement of the poultry industry in Africa. These include lack of selection, low genetic potential, poor nutrition, low productivity of some indigenous stock and high incidence of infectious diseases, all of which constitute a great setback to the continent's food security efforts. Some of these infectious diseases include Newcastle disease, Marek's disease, infectious bursal disease (IBD), fowl pox, infectious bronchitis, fowl cholera, fowl typhoid, avian leukosis, mycoplasmosis and avian influenza (Hamid and Sharma, 1990; Adene, 1997; Joannis et al., 2006).

In the last three decades, chicken infectious anemia (CIA) has emerged as a new economically important disease that causes severe anemia and hemorrhages in young chickens. This disease is associated with immunosuppression due to subclinical illness, leading to enhanced infections by secondary viral, bacterial or fungal agents. Immunosuppression resulting from CIA virus (CIAV)

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**Abbreviations:** CIA, Chicken infectious anemia; CIAV, chicken infectious anemia virus; IBD, infectious bursal disease; IBDV (bold), infectious bursal disease virus (unbold); SPF, specific-pathogen-free; IIF, indirect immunofluorescence; VNT, virus neutralization test; ELISA, enzyme-linked immunosorbent assay; MBE, monoclonal blocking enzyme-linked immunosorbent assay; CPE, cytopathic effect; AGPT, agar gel precipitin test; PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; MAbs, monoclonal antibodies; RE, restriction endonuclease.

infection could also lead to vaccination failures.

This disease is caused by CIAV which is a small, non-enveloped, icosahedral virus measuring 25 nm in diameter with a negative-sense, single-stranded circular DNA genome of about 2.3 kilobase pairs (Gelderblom et al., 1989). It has been classified as the only member of the genus *Gyrovirus* in the family *Circoviridae* (Pringle, 1999). The CIAV genome encodes proteins of about 50, 30 and 16 kDa, which have been designated VP1, VP2 and VP3, respectively. VP1 is the viral capsid protein while VP3 or apoptin causes apoptosis in chicken thymocytes and chicken lymphoblastoid cells. It has been proposed that VP2 acts as a scaffold protein, ensuring that VP1 folds in the proper way (Schat, 2003).

Diagnosis of CIAV infections can be made by detecting infectious virus, virus antigen, virus DNA, or virus-specific antibodies (McNulty, 1998). The purpose of this article is to define the current state of knowledge concerning the serological, biological and molecular diagnosis of CIA in Africa and also emphasize the need to eradicate this disease from the continent.

## HISTORY AND ECONOMIC IMPACT OF CHICKEN INFECTIOUS ANEMIA

Chicken infectious anemia was first recognized as a new disease in young chickens caused by a novel virus agent. The virus was isolated unexpectedly from commercial chickens during investigation of a Marek's disease vaccine accident caused by reticuloendotheliosis virus in Japan in 1974 (Yuasa et al., 1979). The newly described condition was associated with increased mortality in very young chickens that was characterized by severe anemia, lymphoid depletion, yellow to white bone marrow, atrophy of the thymus and bursa of Fabricius and hemorrhage (Yuasa et al., 1979).

Infection with CIAV poses a serious economic threat especially to the broiler industry and the producers of specific-pathogen-free (SPF) eggs. Economic losses due to CIAV infections arise from poor growth, increased mortality and the cost of antibiotics used to control secondary bacterial infections (McNulty, 1991). It has been reported that net income per 1000 birds, feed conversion ratio and average weight per bird were lower in flocks with antibodies to CIAV compared to those without antibodies (McNulty et al., 1991). In addition, McIlroy et al. (1992) reported a loss of net income of about 18.5% due to decreased weight at processing and increased mortality in CIAV-infected birds.

## DIAGNOSIS

A definitive diagnosis of CIA can be made by virus isolation, demonstration of virus antigen in impression smears and cryostat sections of tissues, detection of

serum antibodies to the virus and detection of CIAV nucleic acid in tissues from diseased birds and infected Marek's disease virus-transformed chicken lymphoblastoid (MDCC-MSB1) cell line (McNulty, 1998).

## Serological diagnosis

Serological testing of poultry flocks for CIAV infections is necessary to determine whether breeder flocks have seroconverted during the rearing period, whether vaccination is warranted and to monitor its outcome and lastly, to monitor the CIAV status of SPF flocks especially those used to provide eggs for vaccine production (Todd et al., 2001). CIAV antibodies are produced post-infection and post-vaccination and can be detected by indirect immunofluorescence (IIF) and immunoperoxidase tests and virus neutralization test (VNT). However, these tests have been superseded by the enzyme-linked immunosorbent assay (ELISA) (McNulty, 1998). While several ELISAs have been developed for CIAV antibody detection (Todd et al., 1990; Pallister et al., 1994; Todd et al., 1999), commercial ELISAs for serodiagnosis of CIAV are now also available.

In Egypt, serological investigations showed that commercial chickens were greatly exposed to CIAV (Zaki and El-Sanousi, 1994; Amin et al., 1998). Using commercial ELISA kits, Owoade et al. (2004) found that 55% of 20 chicken flocks and 86% of seven farms tested in southwestern Nigeria were positive for CIAV antibodies. Subsequently, Emikpe et al. (2005) presented the first evidence that indigenous chickens in Africa were susceptible to CIA when they reported a CIAV seroprevalence of 88.9% among apparently healthy Nigerian indigenous chickens in four communities in southwestern Nigeria. Recently, Oluwayelu et al. (2009) reported the development and use of a monoclonal blocking ELISA (MBE) for the serodiagnosis of CIA in Nigeria. Performance evaluation of the MBE using Nigerian and Northern Ireland commercial chicken sera revealed 99.3 and 86.1% concordance respectively, between the MBE and the commercial IDEXX CIAV ELISA (IDE) (IDEXX, Maine, USA) and between the MBE and the indirect ELISA. Compared with the IDE and the indirect ELISA, there were substantial reductions in reagent costs, time and labor input with the MBE, thus making it a rapid and more economical option for surveillance and serodiagnosis of CIAV infections. The MBE will therefore be applicable in most of Africa's developing economies where commercial ELISA kits are expensive and largely unavailable for routine diagnostic use. In an earlier study which evaluated the performance of the IIF for CIAV serodiagnosis (Oluwayelu et al., 2007), it was observed that similar to the MBE, the IIF is cheap in terms of equipment cost, time and labor input and can also be used for surveillance and serodiagnosis of CIA in developing countries.

## BIOLOGICAL CHARACTERISTICS OF CIAV ISOLATES

### Virus isolation

CIAV has been isolated from chickens in Japan, West Germany, the United Kingdom, Sweden, the Netherlands, France, Australia, the United States, Brazil, New Zealand, Argentina, China and Chile. Apart from one-day-old chicks and chicken embryos, several cell lines including the MDCC-MSB1, MDCC-JP2, LSCC-1104B1 and MDCC-CU147 have been used for isolating the virus (Schat, 2003). However, the MDCC-MSB1 or MDCC-CU147 cell lines are preferred for virus isolation and propagation (Calnek et al., 2000). Cultures showing cytopathic effect (CPE), which is characterized by cell death, enlarged swollen cells, alkaline medium and inability to subculture after 1 - 6 (but sometimes up to 10) subcultures, are suggestive of CIAV infection (McNulty, 1991).

In Africa, CIAV was first isolated from broiler chickens in South Africa (Wicht and Maharaj, 1993) and later from broilers in Egypt (Aly, 2001). This was followed by the report of Hussein et al. (2002) who isolated the virus by inoculating 1-day-old SPF chicks with tissue suspensions from clinically and subclinically infected broiler breeders in Egypt. Recently, Oluwayelu et al. (2005) isolated CIAV from birds that died in disease outbreaks tentatively diagnosed as IBD based on positive results obtained with the agar gel precipitin test (AGPT). Following inoculation of MDCC-MSB1 cells with tissue suspensions positive for CIAV DNA by the polymerase chain reaction (PCR), four samples caused CPE after 11 - 14 passages. Presence of CIAV antigens in these samples was demonstrated by IIF using monoclonal antibodies (MAbs) 3B1 and 2A9 (McNulty et al., 1990b). Virus isolation was unsuccessful in two other PCR-positive samples.

### Antigenic relationship of CIAV isolates

CIAV isolates from different geographical areas of the world are antigenically indistinguishable by serum neutralization tests (Yuasa and Imai, 1986). However, it is possible to differentiate them using immunofluorescent staining patterns with MAbs (McNulty et al., 1990b), restriction endonuclease (RE) analysis (Todd et al., 1992) and sequence comparisons (Islam et al., 2002). Specifically, MAb reactivity has revealed antigenic differences between the reference Cuxhaven-1 (Cux-1) virus and Japanese, UK, USA and Australian isolates (McNulty et al., 1990b; Connor et al., 1991).

A limiting-dilution method with a panel of four MAbs prepared against the CIAV isolate was used in reactions with antigens of the four Nigerian and Cux-1 isolates by the IIF. The Nigerian isolates were found to be antigenically closely related to each other and to the Cux-1 virus as MAbs 3B1, 2A9 and 1H1 were reactive with the

Nigerian isolates at dilutions similar to those reactive with the Cux-1 virus (Oluwayelu et al., 2005).

## MOLECULAR CHARACTERIZATION

Virus isolation in SPF chicks or MDCC-MSB1 cells is laborious and time-consuming as it requires many passages. However, the development of the PCR has made rapid detection of the virus possible. In addition, RE mapping of the virus genome permits characterization of the virus.

### Polymerase chain reaction (PCR)

The PCR has proven to be specific and more sensitive than cell culture isolation of CIAV and facilitates sequence and RE analysis. It is the assay of choice for detecting CIAV DNA in infected cell cultures, chicken tissues, archived formalin-fixed paraffin-embedded tissues, or vaccines (Schat, 2003). A real-time PCR assay has also been developed for the quantitation of CIAV DNA and RNA (Markowski-Grimsrud et al., 2002).

Using the PCR for molecular diagnosis of CIAV in Egypt, Hussein et al. (2002) detected a 418 base pair (bp) CIAV-specific band in blood and tissue samples from infected broiler breeder chicks. In Nigeria, Oluwayelu et al. (2005) used a sensitive PCR assay to confirm the presence of CIAV DNA in tissues of necropsied birds. Agarose gel electrophoresis revealed a 733 bp DNA fragment which was the same size as that produced using DNA specific to the Cux-1 CIAV. Furthermore, Ducatez et al. (2006) used the PCR (nested and semi-nested formats) in establishing the molecular epidemiology of CIAV in Nigeria. In a recent report (Oluwayelu, 2009), CIAV DNA was demonstrated by PCR in archived bursa of Fabricius samples from outbreaks that had been tentatively diagnosed as IBD based on pathological findings and positive AGPT results. This study which is the first PCR detection of CIAV DNA in archived chicken tissues, also established the fact that CIAV has been circulating, undiagnosed, in the Nigerian poultry population since, at least, 1999.

### Restriction endonuclease (RE) analysis

Todd et al. (1992) reported the use of RE analysis of amplified DNA fragments to differentiate CIAV isolates from diverse geographical locations. They showed that the 14 CIAV isolates studied can be assigned to seven distinct groups. Recently, PCR-restriction endonuclease analysis was employed to characterize six CIAVs from Nigerian commercial chickens (Oluwayelu et al., 2005). Using *CfoI* enzyme, three different RE patterns were obtained. Three samples yielded fragments with

estimated sizes of 288, 244, 150 and 51 bp as expected for the reference Cux-1 virus while two had fragments with estimated sizes of 340, 190, 160 and 45 bp. The last sample had a unique profile also resembling that of Cux-1 but with additional fragments of approximately 340 and 190 bp in submolar amounts. These findings indicate the circulation of more than one CIAV strain among the Nigerian commercial chicken population and infer that the sample that had two additional fragments (340 and 190 bp) in submolar quantities contained a mixed population of CIAV strains. Since CIAV has not been routinely diagnosed in indigenous chickens, Oluwayelu and Todd (2008) used the PCR-RE analysis also for the detection and characterization of CIAV in Nigerian indigenous chickens as a step to elucidating their role in the epidemiology of CIAV infections. Digestion of DNA from the sera of these indigenous chickens with *CfoI* yielded a RE pattern similar to that of the Cux-1 but also with the same additional fragments (340 and 190 bp). This suggests that the indigenous chickens also contained a mixed population of CIAV strains.

### Sequence and phylogenetic analysis

DNA sequence determination can be used to differentiate CIAV isolates. Since the genome sequence of CIAV was first published (Noteborn et al., 1991), several low- and high-passage isolates have been sequenced (Meehan et al., 1992; Renshaw et al., 1996; Islam et al., 2002). The extent of genetic diversity in these sequences determines the biological behavior of individual virus strains. In a molecular epidemiological study of CIAV in Nigeria, Ducatez et al. (2006) reported that the nucleotide and amino acid diversities of the VP1 sequences of 30 Nigerian CIAVs were 4.4 and 2.5%, respectively. When the nucleotide sequences of the VP1, VP2 and VP3 genes of these CIAV strains were compared, they found that VP1 was more diverse than the other two CIAV genes. This is consistent with the findings of van Santen et al. (2001) and Islam et al. (2002) who reported that VP1 was more variable at the amino acid level than VP2 and VP3.

Furthermore, in a recent study to determine the extent of genetic diversity between CIAVs circulating in commercial and indigenous chickens, Oluwayelu et al. (2008) reported the partial sequencing of the VP1 gene of three Nigerian commercial chicken CIAV strains (designated NGR-1, NGR-4 and NGR-5) and the cloning and sequencing of CIAV DNA extracted from the sera of Nigerian indigenous chickens. NGR-1, and NGR-4 and NGR-5 were more variable (maximum diversity of 6%) than the six indigenous chicken cloned strains (designated NGR/CI-1, NGR/CI-2, NGR/CI-5, NGR/CI-7, NGR/CI-8 and NGR/CI-9) that had a maximum diversity of 4%. The sequences of two indigenous chicken strains, NGR/CI-8 and NGR/CI-9, were almost identical and evolutionarily closely related to the commercial chicken strains NGR-1,

NGR-4 and NGR-5, respectively (Oluwayelu et al., 2008). A 98% nucleotide identity existed between NGR-1 and a Malaysian strain (SMSC-1) and between NGR-4 and the Bangladeshi virus (BD-3). Similar to the findings of van Santen et al. (2001) and Ducatez et al. (2006) on the existence of CIAV mixed infections or quasispecies and the earlier report of Oluwayelu and Todd (2008) that indigenous chickens probably harbor a mixed population of CIAV strains, co-infection of at least two CIAV strains was confirmed in the indigenous chickens in this study.

### PERSPECTIVE

In selecting a test for serodiagnosis of CIA, especially when large number of sera are involved, consideration should be given to cost and time factors. Serological evidence of CIAV presence in South Africa, Egypt and Nigeria was provided using commercial ELISA kits, which are not easily available and affordable for routine diagnosis. On the other hand, for serodiagnosis of CIA, the MBE has been shown to be comparable in performance to the IIF (Oluwayelu et al., 2007) and to have advantages in terms of cost, time and labor input over the IDE and the indirect ELISA (Oluwayelu et al., 2009). Thus, large scale seroepidemiological investigations of CIAV should be carried out to evaluate the full potential of the MBE and to determine the distribution of CIAV in other African countries. Since vaccination against CIAV is currently not practiced in Egypt and Nigeria, the high CIA seroprevalence obtained in apparently healthy commercial chickens in these countries is an indication of subclinical exposure to the virus. In view of the fact that CIAV causes immunosuppression, the detection of its presence in commercial chickens suggests that inadequate response to vaccinations with other agents seen in these chickens may be due to CIAV infections. In addition, since vaccination and other disease preventive measures are not practiced in indigenous chickens in Africa, the detection of CIAV DNA and antibodies in the sera of these free-roaming chickens (Emikpe et al., 2005; Oluwayelu et al., 2009) indicates natural exposure to the virus and implicates them as a potential source of the infection to commercial chickens. Indigenous chickens should therefore be taken into consideration in future reviews of national vaccination and disease elimination policies in all African countries.

Results of virus isolation studies (Oluwayelu et al., 2005) showed that, unlike other isolates elsewhere (McNulty et al., 1989, 1990a), the four Nigerian CIAV isolates that replicated in cells seem to be slow-growing strains of the virus, as it took a minimum of 11 passages before they could produce CPE. Also, the finding that two samples did not grow in MDCC-MSB1 cells is consistent with previous reports that not all field strains of CIAV replicate in these cells (Yuasa et al., 1983; Soine et al., 1994; Islam et al., 2002). It is possible that the CIAV strains in these two samples may be cytopathogenic in

other cell lines that have been observed to be permissive for some CIAV strains (Calnek et al., 2000). Moreover, it has been suggested that certain amino acid exchanges in the VP1 hypervariable region (amino acid 139-151) probably contribute to differences in efficiency of CIAV replication *in vitro* (Renshaw et al., 1996). In addition, van Santen et al. (2001) suggested an association between the occurrence of glutamine on position 22 of the VP1 protein of field isolates of CIAV with poor replication kinetics and slow replication in cell culture. It is possible that the two Nigerian CIAVs that did not replicate in cell culture contained these amino acid polymorphisms.

The IIF performed with four MAbs prepared against the Cux-1 isolate revealed the existence of antigenic similarity between the Cux-1 and Nigerian isolates (Oluwayelu et al., 2005). This agrees with reports that all CIAVs are antigenically related (Yuasa and Imai, 1986; McNulty et al., 1990a) and indicates that the Nigerian isolates belong to the same serotype as those from other geographical areas of the world. However, an antigenically different isolate (CIAV-7), which is regarded as a putative second serotype, has been reported in the USA (Spackman et al., 2002a, b). This report underscores the possibility that, although only one serotype of CIAV has been identified so far, additional serotypes may emerge in the future. This could have important consequences for vaccine efficacy and serodiagnosis. In Africa, efforts should be made to isolate and serotype CIAV from chickens in those countries where no information on CIA is currently available in order to determine their antigenic relatedness to existing isolates of the virus.

The detection of CIAV DNA in chicken tissues, blood and sera in Egypt and Nigeria (Hussein et al., 2002; Ducatez et al., 2006; Oluwayelu et al., 2008) using PCR underscores the increasing importance of this diagnostic technique as a rapid, highly sensitive and specific method of diagnosing infectious agents. The high sensitivity of the PCR in detecting CIAV DNA suggests that it will be valuable for detecting extraneous CIAV in poultry vaccines being marketed in the continent. Moreover, the PCR detection of CIAV DNA in archived chicken tissues that were positive for IBDV antigen (Oluwayelu, 2009) suggests co-infection of IBD and CIA. This is consistent with reports of synergistic interaction between IBDV and CIAV (Imai et al., 1999) and indicates that CIA should be considered as a differential diagnosis in cases that present with 'IBD-like' clinical signs and pathology.

The finding of Ducatez et al. (2006) and Oluwayelu et al. (2008) that the nucleotide and amino acid sequences of Nigerian CIAV strains are conserved is consistent with the report that CIAV isolates show extremely limited genetic variability worldwide (van Santen et al., 2001). However, biological differences exemplified by ability to replicate in cell culture exist among Nigerian CIAV strains despite low genetic variability of isolates. The report of a high level of genetic relatedness between Nigerian indigenous and commercial chicken CIAV strains

(Oluwayelu et al., 2008) is an indication that CIAV infection is not restricted to the farm premises. The virus may have spread from the farm to the indigenous chickens, or vice versa. The practice of culling and selling spent layers, some of which may be harboring the virus and ultimately end up as backyard chickens, may contribute to dissemination of this virus in the field. Furthermore, the finding of high level (98%) of nucleotide identity between NGR-1 and Malaysian SMSC-1 and between NGR-4 and BD-3 show that NGR-1 and SMSC-1 on one hand and NGR-4 and BD-3 on the other, share common evolutionary origins. Since vaccination against CIAV is not done in Nigeria at present, it is likely that these two CIAV strains were introduced into the Nigerian poultry population through importation of infected poultry, poultry vaccines or other biologicals. It is advocated that importation of day-old chicks by poultry farmers should be discouraged by governments across Africa since such chicks are a potential source of introduction of the virus. In addition, poultry biologicals especially vaccines imported into the continent should be regularly screened for contamination with CIAV.

The detection of six distinct CIAV variants in Nigerian indigenous chickens using molecular cloning and sequence analysis (Oluwayelu et al., 2008) confirmed the findings of RE analysis which suggested that both commercial and indigenous chickens contain mixed populations of CIAV (Oluwayelu et al., 2005; Oluwayelu and Todd, 2008). Other workers (van Santen et al., 2001; Ducatez et al., 2006) made similar findings of mixed infections or quasispecies among CIAV strains. Van Santen et al. (2001) suggested that this phenomenon could be due to a recombination event. Recently, He et al. (2007) provided evidence that homologous recombination occurred between two CIAV strains and resulted in a novel CIAV genotype. It has also been reported that virulent variants of some other viruses have been generated by homologous recombination (Worobey et al., 1999; Anderson et al., 2000). Since the virulence of CIAV is mainly determined by VP1 (Yamaguchi et al., 2001) and the recombination between CIAVs occurred in VP1, there is a need to further investigate the existence of homologous recombinants among African CIAV strains and subsequently evaluate the effect of homologous recombination on the virulence of these African strains.

Furthermore, genetic typing which has been used as a tool for tracing the spread of CIAV (Natesan et al., 2006; Simionatto et al., 2006), can be used by epidemiologists to trace the origin of CIAV in commercial and indigenous poultry populations in Africa and to monitor its spread. The above findings, coupled with the fact that African indigenous chickens comprise a mixture of different breeds, suggest that these chickens can provide a rich milieu for the generation of novel genotypes of CIAV that may alter the epidemiologic picture of this virus in future. It can be concluded that lower productivity and poor performance generally associated with African indigenous

chickens, among other factors, are also related to CIAV infections.

Control measures for CIA should include vaccination and good poultry health and management practices. CIAV immunization of breeder flocks during rearing, which has been used successfully elsewhere (Vielitz et al., 1987; Steenhuisen et al., 1994), is therefore advocated to ensure more protective levels of passive immunity for the progeny chicks during the first weeks of life. This will minimize vertical transmission of the virus. However, consideration should be given to the nature and immunopathogenesis of CIAV infection in relation to other agents before the introduction of CIAV vaccination programme. In particular, since co-infection with IBDV enhances the pathogenicity of CIAV, control of IBD should be an integral part of any measures to control CIAV. The presence of high level of genetic similarity between indigenous and commercial chicken CIAV strains in Nigeria underlines the need for improved hygiene and biosecurity on poultry farms across Africa to prevent cross-contamination between flocks and introduction of virus from indigenous to commercial chickens, or vice versa. Moreover, contacts between overlapping susceptible and infected flocks should be avoided. In addition, periodic decontamination and proper timing of flocks should be practiced.

There are still many gaps in our knowledge of the pathology, pathogenesis and epidemiology of CIAV in Africa, especially in indigenous chickens. With the availability of reagents and modern technologies such as CIAV-specific monoclonal antibodies, immunohistochemical methods, DNA probes and PCR-based molecular detection techniques, it should be possible in the near future to answer questions about CIAV persistence, the pathogenesis of subclinical infections, the role of CIAV in immunosuppression and its interaction with other viruses and infectious agents in African poultry. In addition, the establishment of an African poultry disease database which will contain basic information about African isolates of poultry disease agents and their sequences is recommended. This will be an invaluable resource that can be accessed for the development of more sensitive, rapid and cheaper diagnostic tests and for future vaccine development purposes. It is further advocated that studies should be conducted to evaluate the economic losses caused by CIA in Africa, especially in indigenous chickens with the aim of determining the impact on the income generation capacity of the average African household which depends on income from small-holder poultry for sustenance.

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