Seroprevalence of infectious bronchitis virus in Nigerian chicken flocks

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SEROLOGICAL EVIDENCE OF INFECTIOUS BRONCHITIS VIRUS IN COMMERCIAL CHICKEN FLOCKS IN NIGERIA

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SUMMARY

Serum samples of 52 flocks from poultry farms in Nigeria were tested for the presence of Infectious Bronchitis Virus (IBV) antibodies using a commercial ELISA kit. Samples were collected between 1999 and 2004 from 1 to 7 days old baby chicks from 9 different breeders, 11 broiler, 19 pullet, 3 layer, 8 broiler breeder, 1 pullet breeder and 1 cockerel flocks. Forty seven out of the fifty two tested flocks (90%) were positive for IBV antibodies. All flocks of more than eight weeks of age had a seroprevalence above 94%. Seroprevalence of maternally derived antibodies in baby chicks ranged from 67 to 100%. The older chickens became infected as soon as they became susceptible after waning of maternal antibodies, suggesting an internal source of infection on most farms. This is the first seroprevalence study of IBV in West-Africa. Further studies are necessary to assess economic losses due to IBV and the cost-benefit of countermeasures.

INTRODUCTION

Infectious Bronchitis Virus (IBV) causes an acute, highly contagious respiratory disease in chickens and is associated with tracheal rales, coughing and sneezing. Poultry farmers observe significant economical losses due to lower productivity rather than bird mortality. The disease represents a diagnostic challenge with respect to other respiratory diseases caused by *Mycoplasma gallisepticum* (chronic respiratory disease), Infectious Laryngotracheitis Virus, *Haemophilus paragallinarum* (Infectious Coryza) and Newcastle Disease Virus. Infectious Bronchitis Virus is a Group 3 Coronavirus (*Coronaviridae*) (Cavanagh, 2000, Enjuanes et al., 2000) and distinct from the group 1 and 2 coronaviruses infecting humans. The recent emergence of a variant causing severe acute respiratory syndrom (SARS) in human coronaviruses have renewed

interests in these viruses. This envelopped virus was first reported in the United States in 1930 and since has been observed in most countries throughout the world: in America (Johnson and Marquardt, 1975), Europe (Capua et al., 1994, Cavanagh and Davis, 1993, Gough et al., 1992), Asia (Wang et al., 1997), Australia (Ignjatovic and McWaters, 1991, Lohr, 1976). Although IBV has also been detected and isolated in Morocco (El-Houadfi et al., 1986), little is known about the prevalence of this virus throughout most of Africa. Despite a large poultry industry, no evidence of IBV has been reported from Nigeria. The aim of this study was to investigate the seroprevalence of IBV in commercial poultry farms in this country.

MATERIALS AND METHODS

The flocks

Day old baby chicks were bled by cardiac puncture. Otherwise, blood samples were obtained by jugular venipuncture from 3 to 98 randomly selected apparently healthy birds, although many of the flocks experienced bouts of respiratory distress that were clinically diagnosed as CRD. Serum was stored at –20°C and/or –70°C until tested. Flocks included broilers (n=11), pullets (19), cockerels (1), layers (3), pullet breeders (1), broiler breeders (8) and baby chicks (9) from different flocks of commercial farms in Ogun, Oyo and Lagos states in southwest Nigeria. The age of the flocks varied between 1 day and 65 weeks; flock sizes ranged from 189 to 22,000 birds, most of the flocks containing above 4000 chickens. In total, 914 serum samples were tested for IBV antibodies.

ELISA

A commercial test kit was used to detect specific antibodies against IBV (Synbiotics Corporation, ProFLOCK®, San Diego, USA) following the instructions of the manufacturer. The kit consisted of IBV-antigen pre-coated plates, positive and negative IBV antibody control sera, sample diluent, washing buffer, anti-IBV horseradish peroxidase conjugate, substrate and stop solutions. Serum was used at a dilution of 1:100. Optical density was read at 405 nm using a SPECTRAmax® ELISA reader (Molecular Devices, Sopachem, Brussels).

RESULTS

The overall seroprevalence among all birds tested was 84% (766/914). The percentage of positive birds was highest among breeders and layers and was lowest among broilers and cockrels (Figure 1). Forty seven of the fifty two flocks sampled (90%) were positive, with a seroprevalence ranging from 15 to 100%. Sixty four percent (7/11) of the broiler flocks, 100% (19/19) of pullet flocks, of the breeder flocks (8/8) and of the baby chick flocks (9/9) were seropositive. The three layer flocks were also positive, while the only cockerel flock was negative.

Most of the baby chicks were positive as a result of maternally derived antibodies, and essentially all flocks became positive shortly after having lost maternal antibodies.

Figure 2 suggests this may be as early as at 7 weeks of age. Flocks above 8 weeks of age invariably have a seroprevalence above 94%. Even at a similar age, pullet flocks tend to have a higher overall seroprevalence (90%) than broiler flocks (57%).
Among the antibody-positive birds, 11% of the chickens had low titers (<600), 28% intermediate titers (600<titers<5000), 15% high titers (5000<titers<10000) and 29% very high titers (titers>10000). Titers were independent of age, type of bird, origin of antibodies and size of flock.

DISCUSSION

Antibodies against IBV were previously reported in poultry from South Africa (Thekisoe et al., 2003), Zimbabwe (Kelly et al., 1994) and the virus was detected in Morocco (El-Houadfi and Jones, 1985, El-Houadfi et al., 1986). Our study appears to be the first seroepidemiological study of IBV in West-Africa. In Nigeria, almost all participating farms were affected and 84% of all chickens had antibodies against IBV. These results are very similar to those in Zimbabwe (Kelly et al., 1994) but almost twice as high as in free-ranging chickens in South Africa (Thekisoe et al., 2003). The high seroprevalences are due to infection by wild-type virus since Nigerian farmers vaccinate only breeder flocks. The local practice of vaccinating the breeders with a single dose at 10 days of age is probably not sufficient to explain the high seroprevalences in the breeder flocks. The single vaccination normally does not prevent breeders from wild-type IBV infection nor warrant seropositivity in 1-7 days old chicks. These results indicate that the vaccination of breeder flocks should be improved. At an age of 2-7 weeks, seroprevalence sometimes drops to 0% before most flocks become infected by wild-type virus (Figure 2), but so far symptoms have not been attributed to IBV.

Serological evidence of an early and high rate of infection in pullet and layer flocks suggest that in most cases the virus is transmitted from one generation of flocks to the next, by direct contact between flocks or by contaminated infrastructure. Contacts between overlapping susceptible and infected flocks could be avoided by proper timing and spacing of flocks and/or periodic decontamination.

Infectious bronchitis has been associated with losses in productivity either due to the disease itself or as a result of secondary infections (Cavanagh and Naqi, 2003). Our results show that IBV is ubiquitous and infects all susceptible poultry flocks in this part of Nigeria and probably across most of Africa. Rearing losses in poultry flocks are high in Nigeria and are caused by a number of pathogens (Owoade et al., 2004a, Owoade et al., 2004b) and this study shows that infectious bronchitis is yet another one of them. Further studies are necessary to assess economic losses due specifically to IBV and the cost-benefit of countermeasures. Furthermore, farmers need to be educated about the signs and the importance of IBV.

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REFERENCES

Capua, I., Gough, R. E., Mancini, M., Casaccia, C., and Weiss, C. (1994). A 'novel' infectious bronchitis strain infecting broiler chickens in Italy. Zentralbl Veterinarmed B *41*, 83-89.

Cavanagh, D. (2000). Coronaviruses and toroviruses. In Principles and practiceb of clinical virology, J. E. B. A.Z. Zuckerman, and J.R. Pattison, ed. (Chichester, United Kigdom, John Wiley and Sons), pp. 345-356.

Cavanagh, D., and Davis, P. J. (1993). Sequence analysis of strains of avian infectious bronchitis coronavirus isolated during the 1960s in the U.K. Arch Virol *130*, 471-476.

Cavanagh, D., and Naqi, S. (2003). Infectious Bronchitis. In Diseases of poultry, Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald, and D. E. Swayne, eds. (Iowa, Iowa State Press), pp. 101-119.

El-Houadfi, M., and Jones, R. C. (1985). Isolation of avian infectious bronchitis viruses in Morocco including an enterotropic variant. Vet Rec *116*, 445.

El-Houadfi, M., Jones, R. C., Cook, J. K. A., and Ambali, A. G. (1986). The isolation and characterization of six avian infectious bronchitis viruses isolated in Morocco. Avian Pathology *15*, 93-105.

Enjuanes, L., Brian, D., Cavanagh, D., Holmes, K., Lai, M. M. C., Laude, H., Masters, P., Rottier, P., Siddell, S., Spaan, W. J. M., *et al.* (2000). Coronaviridae. In Virus Taxonomy, F. A. Murphy, C. M. Fauquet, D. H. L. Biship, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. A. Mayo, and M. D. Summers, eds. (New York, Academic Press), pp. 835-849.

Gough, R. E., Randall, C. J., Dagless, M., Alexander, D. J., Cox, W. J., and Pearson, D. (1992). A 'new' strain of infectious bronchitis virus infecting domestic fowl in Great Britain. Vet Rec *130*, 493-494.

Ignjatovic, J., and McWaters, P. G. (1991). Monoclonal antibodies to three structural proteins of avian infectious bronchitis virus: characterization of epitopes and antigenic differentiation of Australian strains. J Gen Virol *72 (Pt 12)*, 2915-2922.

Johnson, R. B., and Marquardt, W. W. (1975). The neutralizing characteristics of strains of infectious bronchitis virus as measured by the constant-virus variable-serum method in chicken tracheal cultures. Avian Dis *19*, 82-90.

Kelly, P. J., Chitauro, D., Rohde, C., Rukwava, J., Majok, A., Davelaar, F., and Mason, P. R. (1994). Diseases and management of backyard chicken flocks in Chitungwiza, Zimbabwe. Avian Dis *38*, 626-629.

Lohr, J. E. (1976). Serologic differences between strains of infectious bronchitis virus from New Zealand, Australia, and the United States. Avian Dis *20*, 478-482.

Owoade, A. A., Mulders, M. N., Kohnen, J., Ammerlaan, W., and Muller, C. P. (2004a). High sequence diversity in infectious bursal disease virus serotype 1 in poultry and turkey suggests West-African origin of very virulent strains. Arch Virol *149*, 653-672.

Owoade, A. A., Oluwayelu, D. O., Fagbohun, O. A., Ammerlaan, W., Mulders, M. N., and Muller, C. P. (2004b). Serologic evidence of chicken infectious anemia in commercial chicken flocks in southwest Nigeria. Avian Dis *48*, 202-205.

Thekisoe, M. M., Mbati, P. A., and Bisschop, S. P. (2003). Diseases of free-ranging chickens in the Qwa-Qwa District of the northeastern Free State province of South Africa. J S Afr Vet Assoc *74*, 14-16.

Wang, H. N., Wu, Q. Z., Huang, Y., and Liu, P. (1997). Isolation and identification of infectious bronchitis virus from chickens in Sichuan, China. Avian Dis *41*, 279-282.