Molecular epidemiology of chicken infectious anemia virus (CIAV) in Brazilian poultry farms revealed field viral heterogeneity and recombination phenomena

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Chicken infectious anemia virus (CIAV) is a non-enveloped virus and belongs to the *Anelloviridae* family. Its genome is a circular single-stranded DNA and encodes three proteins. CIAV causes immunosuppressive disease and other related symptoms such as poor growth, weight loss, and cases of severe anemia in poultry farms, mainly in young birds. CIAV infections may be complicated by secondary pathogens. The control of CIAV outbreaks is through commercial vaccines. Nevertheless, its effectiveness could decrease due to genetic mutations and recombination events. In this study, we performed an epidemiological survey of the CIAV and concomitant infections in Brazilian poultry farms; and the characterization of CIAV strains by analysis of the complete genome sequences. A total of 104 tissue samples of sick chickens from 40 commercial Brazilian poultry farms were included in this study. These farms presented distinct pathologies and were also suspicious of immunosuppression. Nucleic acid extraction and real-time PCR were performed to detect CIAV and other avian pathogens. Nine positive CIAV samples with sufficient viral load were conducted for genome sequencing. Posterior procedures included phylogenetic inferences, comparative genomics, and recombination analysis. Fifty-two samples (50.0%) belonging to 19 poultry farms tested positive for CIAV. These infections were present in farms of broilers (7/19), layers (10/19), and breeders (2/19). Concomitant infections included enteric pathogens (Chicken Parvovirus, Avian Nephritis Virus, Fowl Adenovirus, Chicken Astrovirus, Avian Reovirus), respiratory (Infectious Bronchitis Virus, Mycoplasma spp.), neoplastic (Marek´s Disease Virus), epitheliotropic (Fowlpox Virus), and immunosuppressive (Reticuloendotheliosis Virus). Identity analysis reveals that none of our CIAV sequences was a vaccinal strain (identities range: 92.7 - 99.1%, which equivales from 154 to 17 different nucleotides), neither identical to each other (identities range: 96.0 - 99.6%). Phylogenetic analysis situated our nine Brazilian strains in four polyphyletic genotypes. Moreover, six of these strains showed potential recombination events. Comparative analysis of the capsid protein (VP1) revealed infrequent polymorphisms H22Q, H22E, V75I, M97L, K139Q, E144Q, V157M, S287T, S287A, A290P, and G370S. These analyses showed that some of our strains and polymorphisms are related to previously reported Egyptian strains, which produced clinical signs such as emaciation, ruffled feathers, and atrophic thymus in experimental infection assays. Although six of these polymorphisms are present in CIAV strains isolated from chickens, there are reports that they also appear in CIAV strains isolated from cats and humans which could act as reservoirs. Surprisingly, were identified two non-reported mutations P146A, and R150A in the dual-specificity protein phosphatase (VP2) and one in the apoptin protein (VP3), S110R. Our results show the high prevalence of CIAV in the Brazilian poultry industry. Furthermore, the simultaneous circulation of heterogeneous field strains can facilitate recombination events leading to the generation of new genotypes of concern in poultry. The phenotypic effect of the infrequent polymorphisms, as well as the novel detected mutations, requires further investigation. Finally, the presence of this

immunosuppressive virus (CIAV) in sick birds exposes the risk of hesitating or neglecting the application of vaccination strategies.

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