Detection and characterization of chicken anemia virus in Colombia

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Abstract: Objective. Identify the presence of chicken anemia virus (CAV) in poultry farms and backyard chickens from Antioquia, Colombia. Methodology. Blood and feather samples were taken from laying chickens; in each farm, three birds of six different ages (1, 15, 30, 60, 90, and 120 days old) were chosen randomly. Backyard chicken samples were also obtained near the research farms. We used serology and molecular techniques to analyze the samples. Results. By PCR, the 84% of the birds were positive in whole blood and 66% were positive in feather samples. The 60% of backyard chickens tested were positive in blood and 40% in feather follicle. By serology, the 22% of the poultry farm birds presented high antibody titers and 19% moderate antibody titers. In the backyard chickens, 43% of them presented high antibody titers and 29% moderate antibody titers. In addition, results from the RFLP test and sequencing showed that the circulating virus found in this study was different from the Cux-1 vaccine strain used in Colombia.

Materials and Methods

CAV Detection

A fragment of 713 bp corresponding to the gene encoding protein VP2 was amplified to confirm the presence of CAV. PCR products were analyzed and separated on agarose gel 1%, stained with GelRedTM Nucleic Acid Gel Stain (Biotium®) and visualized with a UV (UVP®) transilluminator.

ELISA of CAV

ELISA detection of antibodies against CAV. To identify animals with antibodies against CAV, a commercial kit (IDEXX, United States) was used, following the manufacturer’s instructions. Blood samples were centrifugated at 2000 x g for 5 minutes to obtain the serum fractions, which were diluted 1:100 for the test. The results were read on the spectrophotometer (BioTek. Vermont, United States) at 650 nm wavelength.

PCR of the VP2 (713 bp) Serology for CAV

PCR product was digested with endonucleases in a final volume of 32 µL. The products were analyzed and separated on 3% agarose gel, stained with GelRedTM Nucleic Acid Gel Stain (Biotium®) and visualized with UV (UVP®) transilluminator, determining the number of bands and their approximate weight versus weight marker 100 bp and 50 bp (GeneRuler DNA Ladder, ThermoFisher®).

RFLP of CAV

A fragment of 1390 bp corresponding to the gene encoding protein VP1 (vaccine strain Cux-1) was amplified. The PCR product was digested with endonucleases in a final volume of 32 µL. The products were analyzed and separated on 3% agarose gel, stained with GelRedTM Nucleic Acid Gel Stain (Biotium®) and visualized with UV (UVP®) transilluminator.

ELISA results of the VP2 fragment and Serology for CAV

PCR results of the VP2 (713 bp)

PCR in blood PCR in feather S/N<0.2a S/N 0.8-0.2b S/N>2 S/N>0.2c % of positives

1-day-old 82% (12/15) 100% (N) 10% (1/10) 45% (9/19) 37% (7/19) 2/12
2-day-old 82% (12/15) 75% (7/10) 0% 0 17% (2/19) 100% (4/4) 0 0 17% (2/19)
4-day-old 82% (12/15) 75% (7/10) 0% 0 8% (1/12) 75% (3/4) 0 0 25% (3/12)
6-day-old 82% (12/15) 75% (7/10) 0% 0 5% (1/20) 75% (4/4) 0 0 25% (3/12)
8-day-old 82% (12/15) 75% (7/10) 0% 0 25% (3/12) 75% (4/4) 0 0 25% (3/12)
Total positives 84% (68/81) 66% (19/29) 22% (21/78) 19% (15/78)

ELISA results of antibodies against CAV

ELISA results of antibodies against CAV. To identify animals with antibodies against CAV, a commercial kit (IDEXX, United States) was used, following the manufacturer’s instructions. Blood samples were centrifugated at 2000 x g for 5 minutes to obtain the serum fractions, which were diluted 1:100 for the test. The results were read on the spectrophotometer (BioTek. Vermont, United States) at 650 nm wavelength.

Cytochrome C oxidase

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PCR conditions

PCR conditions: Final volume 25 µL

Amplified gene Segment Primers Conditions

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Results

PCR results of the VP2 (713 bp) and Serology for CAV

PCR in blood PCR in feather S/N<0.2a S/N 0.8-0.2b S/N>2 S/N>0.2c % of positives

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Conclusions:

In this sense, it can be concluded that the CAV circulated in the farms studied over the growth stages with low serocconversion in the animals and that according to the sequences obtained, the virus was not identical to the Cux-1 vaccine strain used for preventing the virus in Colombia.

It is worth mentioning that this constitutes the first report of CAV in Colombia using molecular techniques, where the molecular differences between the Colombian and vaccine strains might be related to immunosuppression patterns that could promote a subclinical course of the infection. It might be more investigated, because as we mentioned, CAV could favor the entry and permanency of secondary agents such as MDV, a poor response to vaccination and economic losses yet to be determined. Finally, more studies are needed to deepen the information known about the strain, its pathogenicity, and the economic consequences of its presence on affected farms.