Influenza virus infection in quail (*Coturnix coturnix*): characterization of humoral immune response

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Since quail have emerged as a potential intermediate host in the spread of avian influenza viruses (AIV), new efforts have been made to clarify the epidemiologic role of this species in avian influenza infection. In this study, we sought to better understand the immune response generated by different influenza virus in quail. An experimental infection with a low pathogenic avian influenza virus (LPAIV) H5N2 and a human H1N1 (pH1N1) virus were conducted in quail to evaluate the humoral immune response, at both systemic and local levels.

Forty-eight quails were divided into three groups housed in independent isolation units at the biosafety level 3 (BSL-3) facilities of CReSA. Birds on group 1 (G1; n=20) were inoculated with H5N2 LPAIV (10^6EID_{50}/50µL), birds on group 2 (G2; n=20) were inoculated with pH1N1 (10^6EID_{50}/50µL) and birds on group 3 (G3; n=8) were inoculated with saline solution; thus served as a negative control. Five animals of each infected group and two animals of the control group were euthanized at 3, 6, 10 and 12 days post-inoculation (dpi). Blood, choanal, tracheal and cloacal swab samples were collected. Blood was

used to evaluate seroconversion and antibodies isotype dynamics by competition ELISA (cELISA). Swabs were used to study viral shedding by quantitative RT-PCR and total antibodies at local level by cELISA. Direct ELISA tests were performed in order to determine if quail antibodies could be recognized by commercial antibodies against chicken antibodies.

Quail supported the replication of H5N2 subtype, therefore, seroconversion was present in H5N2 infected animals. On the contrary, only one bird belonging to G2 group (pH1N1) showed viral shedding on both choanal and tracheal swabs at 3 dpi and they did not show seroconversion. Anti-chicken goat IgG polyclonal antibodies recognized IgY and IgM quail antibodies in serum. Quail IgM isotype antibodies against influenza nucleoprotein (NP) were found in serum samples from H5N2 infected quails with a strong humoral immune response. Moreover, total antibodies against NP were detected in swab samples from one animal belonging to H5N2 infected group at 12 dpi.

Most of the animals inoculated with H5N2 LPAIV were capable to develop a humoral immune response in front of the infection. These data correlate well with the replication capability of the viruses tested. We demonstrated that anti-IgY and IgM chicken antibodies cross-reacted with quail antibodies and they can be used for seroresponse studies in quails. In H5N2 infected animals, IgM isotype antibodies could be detected as early as 6 dpi and a poor response by IgY isotype antibodies was detected, indicating that the days sampled in this experimental infection were too early to detect them effectively.