Introduction: A infection causes substantial mortality and morbidity worldwide, particularly in infants, the elderly, and the immunocompromised. The efficacy of current vaccines is highly dependent on the magnitude of the hemagglutinin (HA) and neuraminidase (NA) surface proteins of the vaccine virus with currently circulating strains. Should mutations accumulate rapidly that could result in increased transmissibility, a rapid global pandemic could result. Current vaccines are based on inactivated virus (ID) or recombinant viral antigens that may elicit less cross-protection. CLDC is a unique adjuvant that is particularly promising for its ability to induce both humoral and cellular immunity, even in the absence of an adjuvant. Recent studies have indicated that CLDC is effective in a non-naïve chicken model, with the injection of 1 µg vaccine and a boost with 1 µg vaccine, resulting in better humoral and cellular immunity than control groups. This suggests that CLDC may be a promising adjuvant for use in human vaccines.

Methods: To determine the effect of CLDC vaccination on humoral and cellular immunity, a non-naïve chicken model was used. Chickens were divided into four groups, with each group receiving a different vaccine: Group 1 received 1 µg vaccine + 1 µg Alum (control), Group 2 received 1 µg vaccine + 1 µg CLDC (test), Group 3 received 1 µg vaccine + 1 µg CLDC (test), and Group 4 received 1 µg vaccine + 1 µg CLDC (test). Blood samples were collected at day 14 and day 28 post-vaccination. Serum samples were assayed for hemagglutinin (HA) and neuraminidase (NA) antibodies using the Hemagglutination Inhibition Assay (HAI) and Neuraminidase Inhibition Assay (NIA), respectively. Splenocytes were isolated from each group and restimulated with either 1 µg vaccine or 2 µg vaccine, followed by analysis of cytokine levels and IFN-γ production using the Cytokine ELISA kit. In addition, the effect of CLDC on the expression of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) was evaluated using qRT-PCR. The levels of IL-6, TNF-α, and IL-1β were determined by the recognition of the best-fit line that fits the data points.

Results: Vaccination with CLDC induced higher serum antibody levels and IFN-γ production compared to the control groups. The geometric mean titers (GMTs) of HA antibodies in the CLDC group were significantly higher than those in the control groups. The GMTs of NA antibodies in the CLDC group were also higher than those in the control groups. The IFN-γ production was significantly higher in the CLDC group compared to the control groups. The expression levels of IL-6, TNF-α, and IL-1β were also significantly lower in the CLDC group compared to the control groups. These results suggest that CLDC vaccination may provide improved humoral and cellular immunity compared to conventional vaccines.

Conclusions: The results of this study indicate that CLDC vaccination may provide improved humoral and cellular immunity compared to conventional vaccines. CLDC vaccination may be a promising adjuvant for use in human vaccines.