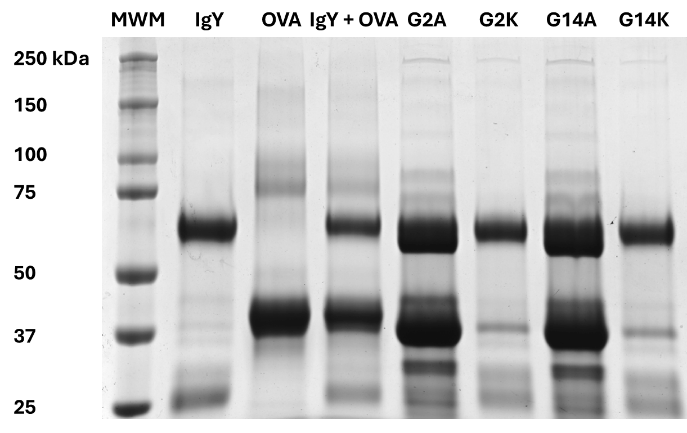
**Supplementary**

***Figures***

|  |  |  |
| --- | --- | --- |
| A)  A plastic container with black writing on it  Description automatically generated | B)  A close-up of a round object  Description automatically generated | C) |

**Figure S1.** Evaluation of Virus Neutralization in a 96-Well Plate Format. (A) 96-well plate with wells annotated for different dilutions and controls, displaying colorimetric readouts indicative of cytopathic effects. (B) Positive cytopathic effect evidenced by extensive cell lysis and viral replication, denoting a lack of neutralizing activity. (C) Negative cytopathic effect with a majority of intact cells, indicating effective viral neutralization by the tested antibodies or compounds.



**Figure S2**. Comparative SDS-PAGE Analysis of IgY Purification and Protein Complexes: The lanes from left to right include the molecular weight marker (MWM), pure IgY, ovalbumin (OVA), a combination of IgY and OVA, and samples from groups G2 and G14 subjected to two different purification processes, labeled as G2A (in-house method) and G2K (Pierce Chicken IgY Purification Kit) for group 2, and similarly G14A and G14K for group 14. The protein bands were stained with Coomassie Blue.

***Tables***

**Table S1.** Comparative Analysis of Egg Yolk Extracts Using Different Extraction Methods. This table summarizes the total volume, purity, total protein, and IgY concentrations of extracts obtained using the proprietary In-house Method and the IgY Purification Kit (Pierce Chicken IgY Purification Kit). The results highlight variations in IgY yield and purity between the two methods, demonstrating the efficiency of the in-house technique in generating higher IgY concentrations despite lower purity levels compared to the commercial kit.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ID | IgY Extraction Method | Total Protein  (mg/ml) | IgY  (mg/ml) | Purity (%) |
| G2/K 081213 | IgY Purification Kit | 6,5 | 5,33 | 82 |
| G2/A 081213 | In-House Method | 18,9 | 10,21 | 54 |
| G2/K 091214 | IgY Purification Kit | 4,9 | 4,17 | 85 |
| G2/A 091214 | In-House Method | 14,7 | 6,32 | 43 |
| G2/K 100911 | IgY Purification Kit | 5,7 | 4,79 | 84 |
| G2/A 100911 | In-House Method | 21,5 | 8,82 | 41 |
| G14/K 081213 | IgY Purification Kit | 7,2 | 6,045 | 84 |
| G14/A 081213 | In-House Method | 25 | 9,75 | 39 |
| G14/K 091214 | IgY Purification Kit | 5,1 | 4,39 | 86 |
| G14/A 091214 | In-House Method | 16,5 | 6,60 | 40 |
| G14/K 100911 | IgY Purification Kit | 5,2 | 4,37 | 84 |
| G14/A 100911 | In-House Method | 22,2 | 7,99 | 36 |
| G18/K  0912 | IgY Purification Kit | 5 | 4,15 | 83 |
| G18/A  0912 | In-House Method | 15 | 6,30 | 42 |
| G18/K 100911 | IgY Purification Kit | 7,6 | 6,38 | 84 |
| G18/A 100911 | In-House Method | 24,9 | 9,96 | 40 |

**Table S2.** Body Weight Changes in Syrian Golden Hamsters Over a 7-Day Period Post-Experiment. The table presents the mean body weights (in grams) and percentage changes from Day 0 for three groups: Negative control, IgY treated, and positive control group. Standard deviations are provided for each measurement to indicate variability within groups. Data are represented to reflect weight dynamics across the experimental timeline, highlighting the physiological impact of treatments on animal weight.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Treatment Group** | **Day 0** | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** | **Day 7** |
| **Mean** | Negative Control | 88.2  (100 %) | 89.3  (101 %) | 89.8  (102 %) | 89.5  (101 %) | 87.9  (99.7 %) | 90  (102 %) | 90.1  (102 %) | 91  (103 %) |
| IgY Treated | 84  (100 %) | 84.2  (100 %) | 84.3  (100 %) | 84.5  (101 %) | 84.6  (101 %) | 84.4  (100 %) | 84.7  (101 %) | 85.7  (102 %) |
| Positive Control | 85.1  (100 %) | 84.6  (99.6 %) | 84.3  (99.1 %) | 83  (97.7 %) | 82.9  (97.6 %) | 82.1  (96.6 %) | 81.8  (96.3 %) | 82.7  (97.3 %) |
| **Standard Deviation** | Negative Control | 4.48  (5.08 %) | 4.71  (5.34 %) | 4.35  (4.93 %) | 4.16  (4.72 %) | 5.55  (6.29 %) | 4.77  (5.41 %) | 4.98  (5.65 %) | 6.08  (6.89 %) |
| IgY Treated | 5.36  (6.38 %) | 5.35  (6.37 %) | 5.32  (6.33 %) | 5.38  (6.4 %) | 5.68  (6.76 %) | 5.95  (7.08 %) | 6.13  (7.3 %) | 5.9  (7.02 %) |
| Positive Control | 6.92  (8.13 %) | 5.91  (6.94 %) | 6.12  (7.19 %) | 6.1  (7.17 %) | 5.99  (7.04 %) | 5.77  (6.78 %) | 6.28  (7.38 %) | 6.92  (8.13 %) |



**Table S3.** The virus copy numbers by ddPCR and body weight related to hamster individuals.



**Table S4.** Descriptive Statistics of ddPCR Viral Copy Numbers Over Time by Treatment Group. This table presents the mean ddPCR viral copy numbers for each day of the experiment across three different treatment groups: Negative Control, IgY Treated, and Positive Control. The mean values indicate the average viral load detected in each group, while the standard deviations reflect the variability within each group. The Negative Control group consistently showed no viral presence, while the IgY Treated and Positive Control groups demonstrated varying levels of viral RNA copies, with the Positive Control group displaying significantly higher means and variability in viral loads (Table S2).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Treatment Group | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
| **Mean** | Negative Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| IgY Treated | 0 | 1 | 1273 | 1116 | 354 | 507 | 526 | 440 |
| Positive Control | 0 | 182 | 4411 | 4377 | 1656 | 1292 | 857 | 442 |
| **Standard deviation** | Negative Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| IgY Treated | 0 | 2 | 3040 | 2539 | 598 | 1050 | 999 | 1182 |
| Positive Control | 0 | 238 | 5275 | 5259 | 1185 | 1027 | 586 | 323 |

**Table S5.** Daily Viral Load Evaluation by ddPCR in Three Experimental Hamster Groups. The results from the Kruskal- Wallis test provide a comprehensive overview of viral load variances among the Negative Control, IgY Treated, and Positive Control groups over a span of seven days. The table delineates the chi-square (χ²) values, degrees of freedom (df), p-values, and effect sizes (ε²), with the latter quantifying the magnitude of observed differences. Statistically significant deviations are marked with asterisks (\*) where the inter-group differences surpassed the threshold of α = 0.05, signifying notable changes in viral load that could be attributed to treatment impact.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | χ² | df | p | ε² |
| **Day 0** | NaN | 2 | NaN | NaN |
| **Day 1** | 30.8 | 2 | < .001\* | 0.88 |
| **Day 2** | 25.9 | 2 | < .001\* | 0.76 |
| **Day 3** | 22.4 | 2 | < .001\* | 0.641 |
| **Day 4** | 23.5 | 2 | < .001\* | 0.673 |
| **Day 5** | 20.9 | 2 | < .001\* | 0.597 |
| **Day 6** | 19.7 | 2 | < .001\* | 0.563 |
| **Day 7** | 19.9 | 2 | < .001\* | 0.584 |

**Table S6.** Daily Pairwise Comparison of Viral Load in Hamster Groups Using Dwass-Steel-Critchlow-Fligner Method. This table presents the pairwise comparison results between the Negative Control, IgY Treated, and Positive Control groups across eight days. Asterisks (\*) indicate days where the differences in viral loads were statistically significant at the 5% level (α = 0.05).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | Group | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
| **Negative Control** | **IgY Treated** | NaN | 0.577 | 0.286 | 0.083 | 0.083 | 0.083 | 0.083 | 0.083 |
| **Negative Control** | **Positive Control** | NaN | < .001\* | < .001\* | < .001\* | < .001\* | < .001\* | < .001\* | < .001\* |
| **IgY Treated** | **Positive Control** | NaN | < .001\* | 0.001\* | 0.009\* | 0.004\* | 0.026\* | 0.057 | 0.038\* |

**Table S7.** The table presents the results of a Kruskal-Wallis test and subsequent pairwise comparisons for the consolidation to total lung area ratio data. The top section shows the Kruskal-Wallis test results, indicating a chi-square value (χ²) of 14.11, with 2 degrees of freedom (df), and a highly significant p-value (< 0.001). The effect size (ε²) is 0.4032, categorized as large based on the provided criteria (small effect: ε² < 0.01, medium effect: 0.01 ≤ ε² < 0.06, large effect: ε² ≥ 0.06). The bottom section details the pairwise comparisons using the W statistic and p-values for three group pairs: Negative Control vs. IgY treated, Negative Control vs. Positive Control, and IgY treated vs. Positive Control.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Kruskal-Wallis Test** | | | | | | |
|  | | **χ²** | | **df** | **p** | **ε²** |
| **T/L ratio** | | 14.11 | | 2 | < .001\* | 0.4032 |
| **Pairwise comparisons - Consolidation/Total Lung ratio** | | | | | | |
|  | |  | |  | **W** | **p** |
| Neg | vs | IgY |  |  | 0.898 | 0.801 |
| Neg | vs | Pos |  |  | 5.062 | 0.001\* |
| IgY | vs | Pos |  |  | 3.919 | 0.015\* |
| \*p<0.05 indicates statistical significance. | | | | | | |

