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Research Article

Clinical Trials of Common Infectious Bronchitis Vaccines in Commercial Poultry of Pakistan

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ABSTRACT Infectious Bronchitis Virus (IBV) is a major threat to poultry health and production. The disease causes respiratory illness, poor growth, and increased mortality in broiler flocks. Control depends mainly on vaccination, but protection varies with strain matching. The Massachusetts (Ma5) strain and the 4/91 strain are widely used vaccines in Pakistan. Both vaccines induce immune responses, but their field performance has not been compared systematically. This study evaluated production outcomes and antibody responses in broiler flocks vaccinated with these two strains under commercial conditions. A total of 50 commercial broiler flocks were included. Twenty-five flocks were vaccinated with the 4/91 strain and 25 with the Ma5 strain. Flock size ranged from 22,000 to 31,000 birds. Performance parameters recorded at slaughter included bodyweight, feed conversion ratio (FCR), and mortality. Blood samples were collected to determine ELISA geometric mean titers (GMT) and coefficient of variation (CV%). Data were analyzed using descriptive statistics, non-parametric tests, and regression models. Results showed that bodyweight did not differ significantly between the two groups i.e. 2,189 g in the 4/91 group and 2,145 g in the Ma5 group. Feed conversion ratio was significantly lower in the 4/91 group (1.58) compared with the Ma5 group (1.69) ($p < 0.01$). Mortality was also significantly reduced in the 4/91 flocks (5.2%) compared with 12.0% in the Ma5 group. ELISA GMTs were higher in the Ma5 group (10,595) compared with the 4/91 group (8,143). CV% was higher in the 4/91 group, though the difference was not significant. Logistic regression confirmed that the 4/91 strain reduced the odds of high mortality (OR 0.04, CI 0.00 - 0.23, $p < 0.01$) indicating a strong protective effect. Linear regression showed that FCR was significantly improved by the 4/91 strain. ELISA GMT had a small positive association with FCR, while CV% showed no effect. This study demonstrates that the 4/91 strain provided superior flock performance compared with the Ma5 strain. Lower mortality and better feed efficiency were achieved with 4/91 strains. These findings also highlight the importance of strain matching over antibody levels alone.

KEYWORDS Infectious Bronchitis Virus (IBV), Broiler performance, Vaccine efficacy, 4/91 strain, Ma5 strain

Introduction

Infectious Bronchitis (IB) is a highly contagious viral disease of poultry (Cook *et al*, 2012). The disease primarily affects the respiratory system of chickens. It can also impact the renal and reproductive systems (Raj and Jones, 1997). Broilers often present with coughing, sneezing, and tracheal rales. Layers show decreased egg production and poor egg quality. Mortality rates can be high, especially in young flocks (Junghans *et al*, 2022). Even when mortality is low, the economic losses are considerable due to reduced weight gain and poor feed conversion. The disease also compromises flock uniformity at slaughter age. Secondary infections often follow IB outbreaks. These co-infections worsen the overall health status of poultry houses (Liu *et al*, 2024). The virus spreads rapidly through aerosols and contaminated equipment (De Herdt *et al*, 2001; Abozeid, 2023). Once introduced, it is difficult to eradicate from poultry-dense areas. For the poultry industry, IB remains one of the most persistent economic threats.

Vaccination is the main tool for controlling IB. Live and inactivated vaccines are used worldwide (Alsakini *et al*, 2024; Eid *et al*, 2024). Vaccines help reduce clinical signs and viral shedding. They do not always prevent infection completely. Protection depends on the match between the vaccine strain and field strain (Hassan *et al*, 2024). Infectious Bronchitis Virus (IBV) is highly variable (Abozeid, 2023; Rafique *et al*, 2024). The virus belongs to the family Coronaviridae and genus Gammacoronavirus which is further

divided into several genotypes and lineages. The most common classification uses the GI grouping system (Rafique *et al*, 2024). Within these, multiple variants circulate in different countries. The GI-13 lineage, includes multiple strains such as the 4/91 strain, is widespread in Europe, the Middle East, and Asia (Alhafufi *et al*, 2025). Other variants, such as GI-1 (Massachusetts) and GI-19 (QX-like), also cause major outbreaks (Wang *et al*, 2024). Continuous evolution of IBV makes vaccination strategies complex. This diversity underlines the importance of selecting appropriate vaccines.

This study was designed to compare the performance of broiler flocks vaccinated with 4/91 and Ma5 strains. The comparison focused on bodyweight, feed conversion ratio, antibody titers, coefficient of variation, and mortality. Previous studies have examined IB vaccines in controlled conditions. However, few have assessed their impact in field conditions in Pakistan. This study provides real-world evidence from commercial broiler farms. It evaluates both production outcomes and immune responses. The findings highlight the relationship between immune titers and flock performance. The study also shows that high antibody levels do not always equal better protection. This adds to current understanding of IB vaccine efficacy. The results contribute to the knowledge on strain matching and vaccine selection. They also emphasize the need for region-specific vaccination strategies. By comparing two widely used vaccines, this research offers practical insights for poultry producers. The outcomes will support evidence-based decision making in IB control programs.

Materials and Methods

Study design and flock selection

This clinical trial study was conducted on commercial broiler farms located in Pakistan between January 2024 and December 2024. A total of 50 broiler flocks were included, comprising 25 flocks vaccinated with the 4/91 strain vaccine and 25 flocks vaccinated with the Ma5 strain vaccine. Each flock consisted of 22,000–31,000 birds raised under intensive commercial production systems with standardized management, feeding, and biosecurity practices. Farms were selected to represent comparable production conditions in order to minimize confounding due to management variability

Vaccination Protocol

The 4/91 strain vaccine was administered at hatchery level following the manufacturer's instructions, whereas other flocks received the Ma5 strain vaccine under the same conditions. Each farm followed standard commercial vaccination protocols with no additional infectious bronchitis vaccines administered during the production cycle.

Data collection

Performance data were collected from flock records at slaughter age. The parameters measured included final bodyweight (g), feed conversion ratio (FCR), and mortality (%). Immunological response was assessed by collecting serum samples from each flock at 30–35 days of age. ELISA antibody titers were determined using the IDVet commercial ELISA kit, and results were expressed as geometric mean titers (GMT). The coefficient of variation (CV%) of antibody responses and the number of individual samples tested per flock were also recorded.

Data Analysis

Descriptive statistics (mean, standard deviation, median, interquartile range, and range) were calculated for each parameter. Differences between vaccine groups were analyzed using the Wilcoxon rank-sum test, with $p < 0.05$ considered statistically significant. To further evaluate predictors of flock performance, regression analyses were conducted. Logistic regression was used to assess the effect of vaccine strain, ELISA GMT, and CV% on the risk of high mortality (>10%). Linear regression was applied to evaluate the influence of vaccine strain, ELISA GMT, and CV% on FCR outcomes. Odds ratios with 95% confidence intervals (CI) were reported for logistic regression, while beta estimates with 95% CI were reported for linear regression. All analyses were performed using R statistical language (version 4.3.1).

Results

Distribution of parameters

Distribution of all the parameters showed higher variation in the groups (Fig. 1). Bodyweight distributions appeared similar, with slightly higher central values for the 4/91 strain. Feed conversion ratio (FCR) showed a marked difference, with the 4/91 strain producing consistently lower values, indicating better efficiency. The Ma5 strain displayed wider variation in FCR and higher averages. ELISA GMT values were higher in flocks vaccinated with Ma5, while the 4/91 strain showed lower but adequate titers. The spread of GMT values in the Ma5 group was broad, suggesting variability in antibody responses. In contrast, the 4/91 group exhibited more compact distributions. Coefficient of variation (CV%) showed no consistent difference, although the 4/91 strain group displayed a wider spread with some higher outliers. Mortality patterns were distinct between the two vaccines. The 4/91 strain was associated with lower and more tightly clustered mortality rates. The Ma5 strain demonstrated higher mortality with a broader spread, including extreme outliers.

Comparison with different parameters

The comparative analysis between broiler flocks vaccinated with the 4/91 strain and those vaccinated with the Ma5 strain revealed notable differences in performance (Table 1). The mean bodyweight was slightly higher in the 4/91 group (2189 g) compared with the Ma5 group (2145 g), though this difference was not statistically significant ($p = 0.5$). FCR showed a significant improvement in the 4/91 strain group, with a mean of 1.58 compared to 1.69 in the Ma5 group ($p < 0.001$) which indicated superior feed efficiency in the 4/91 group. ELISA GMT values were significantly lower in the 4/91 strain group (mean 8143) compared to the Ma5 group (mean 10,574) ($p = 0.049$). The coefficient of variation (CV%) did not differ significantly between groups,

with means of 31% for the 4/91 strain and 20% for the Ma5 strain ($p = 0.2$). Mortality showed a marked difference, with the 4/91 strain group recording a mean of 5.2% compared with 12.2% in the Ma5 group ($p < 0.001$).

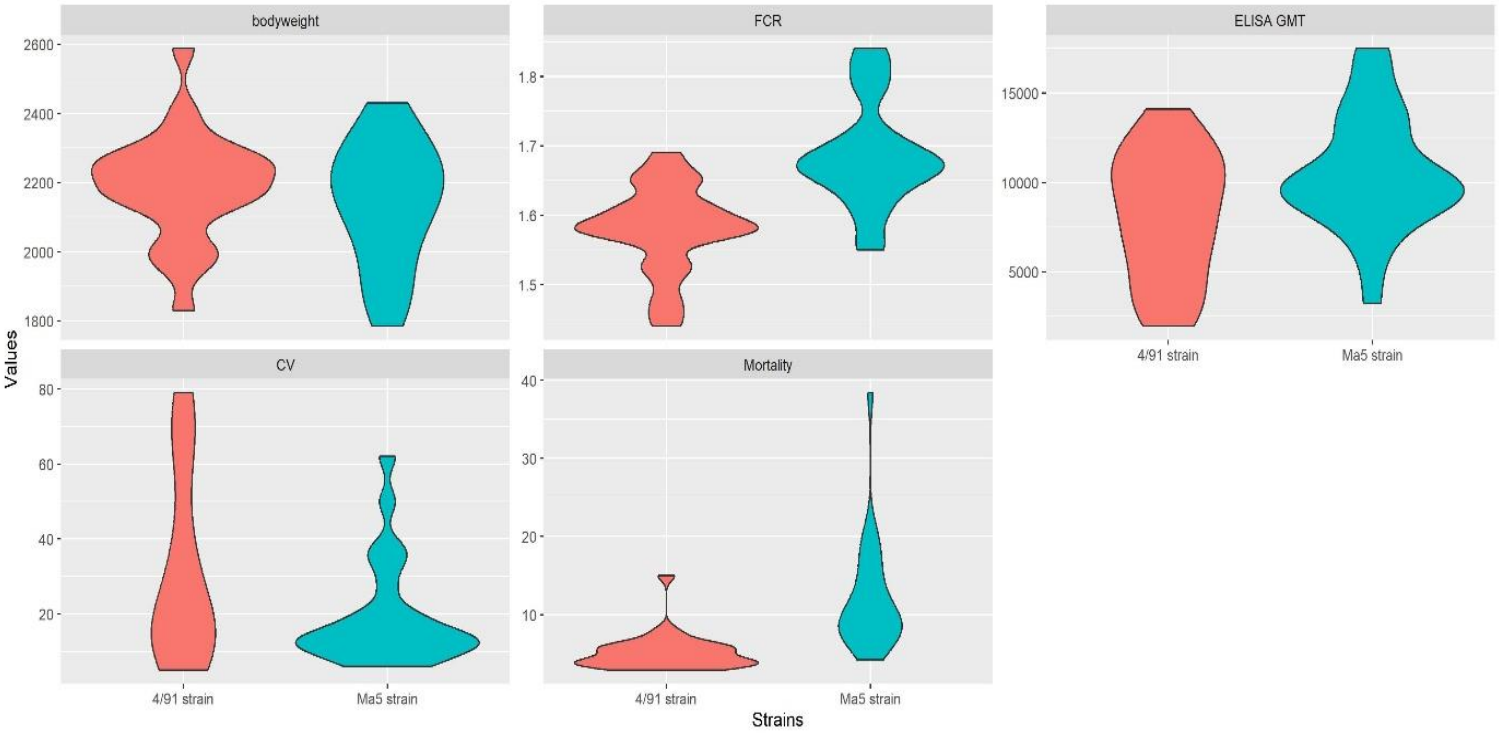

Fig. 1: Violin plots showing the distribution of all the parameters to understand the variation.

Table 1: Comparison of parameters between both vaccine groups using non-parametric tests.

Characteristic	4/91 strain	Ma5 strain	p-value
Bodyweight	2,189 ± 157	2,143 ± 178	0.5
FCR	1.58 ± 0.06	1.69 ± 0.08	<0.001
ELISA GMT	8,143 ± 3,551	10,595 ± 3,332	0.045
CV	31 ± 25	19 ± 14	0.13
Mortality	5.2 ± 2.5	12.0 ± 7.1	<0.001

Effect of predictors on mortality and FCR

The majority of respondents regarded vaccination of the regression analysis examined the predictors of high mortality and feed conversion ratio (FCR) in broiler flocks vaccinated with different IB strains (Table 2). For mortality, the odds ratio for the 4/91 strain compared to Ma5 was 0.04, indicating a strong protective effect. This reduction in odds of high mortality was statistically significant ($p < 0.001$). The confidence interval ranged from 0.00 to 0.23, confirming the robustness of the effect. Neither ELISA GMT nor CV% were significant predictors of mortality in the model. The overall model explained 28.6% of the variation in mortality risk. For FCR, the 4/91 strain again showed a significant improvement compared with Ma5. The estimate was –0.10, with a confidence interval between –0.14 and –0.06 ($p < 0.001$). ELISA GMT also had a small but significant positive effect on FCR ($p < 0.05$). CV% did not show a significant association with FCR ($p = 0.168$). The FCR model explained 42.7% of the variation, with an adjusted R^2 of 0.39. The 4/91 strain provided superior outcomes in both mortality and feed efficiency compared with the Ma5 strain.

Table 2: Regression analysis results revealed the association of vaccination with Mortality and FCR

	Mortality High			FCR		
Predictors	*Odds Ratios*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	1.08	0.49 – 2.41	0.842	1.59	1.49 – 1.69	**<0.001**
Strains [4/91 strain]	0.04	0.00 – 0.23	**0.003**	-0.10	-0.14 – -0.06	**<0.001**
ELISA GMT				0.00	0.00 – 0.00	**0.049**
CV				0.00	-0.00 – 0.00	0.168
Observations	50			50		
R^2 Tjur	0.286			0.427 / 0.390		

Discussion

This study compared the performance of broiler flocks vaccinated with the 4/91 strain and the Ma5 strain under commercial field conditions. One of the key observations in this study was the difference in antibody titers. Flocks vaccinated with Ma5 produced higher ELISA GMTs compared with 4/91. This finding aligns with previous reports where Ma5 was associated with strong humoral responses (Eid *et al*, 2024; Parvin *et al*, 2024). However, higher titers did not correspond to better flock performance. Mortality and FCR were worse in Ma5 flocks despite the elevated antibody levels. Protection is not only determined by the magnitude of antibody titers (Al-Rasheed *et al*, 2021; Huang *et al*, 2025). The 4/91 strain appears to be better matched to the field variants present in Pakistan. The compact antibody distributions in the 4/91 group suggest more uniform protection (Houta *et al*, 2024; Liu *et al*, 2024). By contrast, the wider spread in Ma5 titers suggests variability in immune responses. These findings emphasize that serological titers should be interpreted cautiously in vaccine evaluation.

The methodology used in this study allowed both direct comparisons and deeper evaluation of predictors. The Wilcoxon rank-sum test identified significant differences in FCR, GMT, and mortality. Logistic regression confirmed the protective effect of the 4/91 strain on mortality. Linear regression demonstrated that FCR was influenced by both vaccine type and antibody levels. CV% was not a significant predictor in regression, although extreme values may still carry risk. Together, these methods strengthen the reliability of the findings when compared with previous studies (Hassan *et al*, 2024; Parvin *et al*, 2024; Wang *et al*, 2024). The combination of field data and advanced analysis adds novelty to the study.

The superiority of the 4/91 strain observed here is consistent with studies from other regions (Rafique *et al*, 2024). Research in the Middle East has reported strong cross-protection by 4/91 against local variants (Wang *et al*, 2024). Similar findings were observed in Europe, where 4/91 reduced mortality compared with Mass-based vaccines (Yang *et al*, 2023). Ma5 has been shown to produce strong immune responses but sometimes less consistent protection. The results of this study mirror those trends under Pakistani field conditions. The poor correlation between high antibody titers and flock performance has also been reported elsewhere (Al-Rasheed *et al*, 2021; Yang *et al*, 2023; Wang *et al*, 2024). These findings reinforce the idea that protection depends on strain matching rather than antibody levels alone. The observation of higher variability in Ma5 titers is consistent with other field trials (Sultan *et al*, 2019). This research therefore provides new and locally relevant evidence.

The findings of this study have direct implications for poultry health management in Pakistan. The 4/91 vaccine demonstrated better performance outcomes. This suggests it may be more effective against circulating field strains in the region. Ma5 provided strong immune stimulation but was linked with higher mortality and worse feed efficiency. A combined vaccination strategy using both Ma5 and 4/91 may provide broader protection as suggested in previous studies (Cuadrado *et al*, 2024; Huang *et al*, 2025; Shao *et al*, 2025). Prime-boost programs could help balance immune strength with effective protection.

In conclusion, the 4/91 vaccine showed superior field protection, and its use should be prioritized in commercial flocks. Future studies should test this strategy under controlled and field conditions. Molecular characterization of circulating IBV variants is also needed. This would confirm the match between vaccine strains and field viruses. The results also highlight the limitations of relying on ELISA GMT alone to judge vaccine efficacy. Performance indicators such as mortality and FCR should be integrated into vaccine evaluations.

Declaration of Competing Interest

The authors declare that they have no competing or conflict of interests.

Author Contributions

MKJ: Conceptualization, Methodology, formal analysis, Writing—original draft preparation. **UB:** Methodology, Formal analysis. **AKT:** Formal analysis, Writing—review and editing. **MA:** Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

References

1. Abozeid, H. H. (2023). *Global Emergence of Infectious Bronchitis Virus Variants: Evolution, Immunity, and Vaccination Challenges*. Transboundary and emerging diseases 2023(1):1144924. doi: https://doi.org/10.1155/2023/1144924
2. Al-Rasheed, M., C. Ball, and K. Ganapathy. (2021). *Route of infectious bronchitis virus vaccination determines the type and magnitude of immune responses in table egg laying hens*. Veterinary Research 52(1):139. doi: 10.1186/s13567-021-01008-7
3. Alhafufi, A. N., S. Kasem, F. N. Almajhdi, H. A. Albaqshi, F. A. Alaql, E. A. Rihan, E. M. Abd-Allah, A. A. Alyousaf, Y. K. Aljasem, N. D. Aljehani, M. A. Haridy, A. R. Alhimaidi, and A. S. Abdel-Moneim. (2025). *Full-length genome reveals genetic diversity and extensive recombination patterns of Saudi GI-1 and GI-23 genotypes of infectious bronchitis virus*. Virology Journal 22(1):1. doi: 10.1186/s12985-024-02614-5
4. Alsakini, K., F. O. Coven, and A. Nalbantsoy. (2024). *Adjuvant effects of novel water/oil emulsion formulations on immune responses against infectious bronchitis (IB) vaccine in mice*. Biologicals 85:101736. doi: 10.1016/j.biologicals.2023.101736
5. Cook, J. K. A., M. Jackwood, and R. C. Jones. (2012). *The long view: 40 years of infectious bronchitis research*. Avian Pathology 41(3):239-250. doi: 10.1080/03079457.2012.680432
6. Cuadrado, C., C. Breedlove, E. van Santen, K. S. Joiner, V. L. van Santen, and H. Toro. (2024). *Protection Against Infectious Bronchitis Virus Vaccine Recombinants and Chicken-Selected Vaccine Subpopulations*. Avian Dis 68(2):89-98. doi: 10.1637/aviandiseases-D-23-00064
7. De Herdt, P., A. R. Ducatelle, A. E. Uyttebroek, A. Sneep, and R. Torbeyns. (2001). *Infectious bronchitis serology in broilers and broiler breeders: correlations between antibody titers and performance in vaccinated flocks*. Avian Dis 45(3):612-619.
8. Eid, A. A. M., A. M. Mahmoud, E. E. Hamouda, M. Metwally, R. M. M. Ezz-Eldin, and R. M. ElBakrey. (2024). *The efficacy of simultaneous successive classic and variant infectious bronchitis virus vaccines versus circulating variant II Egyptian field virus*. Open Vet J 14(1):90-107. doi: 10.5455/OVJ.2024.v14.i1.9
9. Hassan, M. S. H., A. Ali, M. E. Mahmoud, D. Altakrouni, S. M. Najimudeen, and M. F. Abdul-Careem. (2024). *Protection of laying chickens against the Canadian DMV/1639 infectious bronchitis virus infection through priming with heterologous live vaccine and boosting with heterologous or homologous inactivated vaccine*. Virus Res 339:199281. doi: 10.1016/j.virusres.2023.199281
10. Houta, M. H., K. E. Hassan, W. H. Kilany, S. A. S. Shany, A. A. El-Sawah, M. F. ElKady, A. S. Abdel-Moneim, and A. Ali. (2024). *Evaluation of different heterologous-homologous vaccine regimens against challenge with GI-23 lineage infectious bronchitis virus*. Virology 598:110193. doi: 10.1016/j.virol.2024.110193
11. Huang, B., S. Chen, Z. Wang, K. Feng, Y. Teng, R. Li, G. Shao, J. Rao, X. Zhang, and Q. Xie. (2025). *Development and immunoprotection assessment of novel vaccines for avian infectious bronchitis virus*. Virol Sin 40(3):462-476. doi: 10.1016/j.virs.2025.03.008
12. Junghans, A., L. Deseniß, and H. Louton. (2022). *Data evaluation of broiler chicken rearing and slaughter—An exploratory study*. Front. Vet. Sci. 9:957786. doi: 10.3389/fvets.2022.957786
13. Liu, K., Z. Li, Q. Li, S. Wang, R. Curtiss, 3rd, and H. Shi. (2024). *Salmonella typhimurium Vaccine Candidate Delivering Infectious Bronchitis Virus S1 Protein to Induce Protection*. Biomolecules 14(1)doi: 10.3390/biom14010133
14. Parvin, M. K., M. E. Haque, M. A. Haque, M. M. Kamal, M. Sadekuzzaman, S. Hayat, M. T. Rahman, M. P. Siddique, S. S. Nahar, A. K. M. Khasruzzaman, M. T. Hossain, and M. A. Islam. (2024). *Immunogenicity and protective efficacy of an inactivated infectious bronchitis virus vaccine candidate from a local isolate of Bangladesh*. J Adv Vet Anim Res 11(3):592-600. doi: 10.5455/javar.2024.k809
15. Rafique, S., Z. Jabeen, T. Pervaiz, F. Rashid, S. Luo, L. Xie, and Z. Xie. (2024). *Avian infectious bronchitis virus (AIBV) review by continent*. Front. Cell. Infect. Microbiol. 14:1325346. doi: 10.3389/fcimb.2024.1325346
16. Raj, G. D., and R. C. Jones. (1997). *Infectious bronchitis virus: Immunopathogenesis of infection in the chicken*. Avian Pathology 26(4):677-706. doi: 10.1080/03079459708419246
17. Shao, G., J. Fu, Y. Pan, S. Gong, C. Song, S. Chen, K. Feng, X. Zhang, and Q. Xie. (2025). *Development of a recombinant infectious bronchitis virus vaccine expressing infectious laryngotracheitis virus multiple epitopes*. Poult Sci 104(1):104578. doi: 10.1016/j.psj.2024.104578
18. Sultan, H. A., A. Ali, W. K. El Feil, A. H. I. Bazid, M. A. Zain El-Abideen, and W. H. Kilany. (2019). *Protective Efficacy of Different Live Attenuated Infectious Bronchitis Virus Vaccination Regimes Against Challenge With IBV Variant-2 Circulating in the Middle East*. Volume 6 - 2019doi: 10.3389/fvets.2019.00341
19. Wang, M., Z. Bo, C. Zhang, M. Guo, Y. Wu, and X. Zhang. (2024). *Deciphering the Genetic Variation: A Comparative Analysis of Parental and Attenuated Strains of the QXL87 Vaccine for Infectious Bronchitis*. Animals (Basel) 14(12)doi: 10.3390/ani14121784
20. Yang, C. Y., P. Peng, X. Liu, Y. Cao, and Y. Zhang. (2023). *Effect of monovalent and bivalent live attenuated vaccines against QX-like IBV infection in young chickens*. Poult Sci 102(4):102501. doi: 10.1016/j.psj.2023.102501