www.czasopisma.pan.pl PAN www.journals.pan.pl
POLSKA AKADEMIA NAUK

Polish Journal of Veterinary Sciences Vol. 28, No. 4 (2025), 597–606

DOI 10.24425/pjvs.2025.156087

Original article

Evaluation of comparative efficacy of Prowill disinfectant, Astragalus root extract, and ivermectin against avian coronavirus in chicken embryo model

O. Ardicli[1], F.O. Cöven[2], A. Nalbantsoy[3], F. Cöven[4], J. Abbass[5], H. Babayev[6], K.T. Carli[5]

[1] Division of Food Processing, Milk and Dairy Products Technology Program, Karacabey Vocational School, Bursa Uludag University, TR-16700 Karacabey, Bursa, Türkiye
[2] Department of Bioengineering, Faculty of Engineering and Natural Sciences, Manisa Celal Bayar University, TR-45110 Yunusemre, Manisa, Türkiye
[3] Department of Bioengineering, Faculty of Engineering, Ege University, TR-35040 Bornova, İzmir, Türkiye
[4] İzmir Ceren Animal Health and Laboratory Industry and Trade Inc., TR-35640 Çigli, İzmir, Türkiye
[5] Department of Microbiology, Faculty of Veterinary Medicine, Bursa Uludag University, TR-16059 Nilufer, Bursa, Türkiye
[6] Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, CH-7265 Davos Wolfgang, Switzerland

Correspondence to: K.T. Carli, e-mail: tayfun@uludag.edu.tr, tel.: +90 533 766 35 24

Abstract

Infectious bronchitis virus (IBV) is an avian coronavirus and a primary causative agent of respiratory disease in poultry, representing a significant global economic concern. IBV shares structural and functional similarities with other coronaviruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), making it an invaluable model for studying coronavirus behavior and exploring potential therapeutic strategies. Novel applications could prove valuable in controlling the spread of these pathogens. This study evaluates the antiviral efficacy of various commercial formulations of the probiotic-based disinfectant (Prowill), Astragalus root extract, and ivermectin against the IBV D274 strain (Genotype 1-Lineage 12; GI-12). The agents were applied at different concentrations to specific pathogen-free (SPF) embryonated chicken eggs (9-10 days old) previously inoculated with IBV D274. The key parameters, such as egg mortality, mean hemagglutination (HA) titer, and HA titer (log2) values, were assessed to determine the antiviral efficacy of each treatment. The results revealed that the gel form of Prowill demonstrated superior antiviral activity, including at lower tested concentrations. This gel form showed the lowest egg mortality rates (10% at 0.5%, 1%, and 2% concentrations). This form also exhibited relatively low HA titers (4.7, 3.8, and 3.6 in log2, respectively). In addition, other Prowill formulations demonstrated significant antiviral effects, reducing mortality rates. Astragalus root extract, particularly at a concentration of 5 mg/kg, demonstrated potent antiviral activity by effectively inhibiting viral replication; however, mortality reached 30%. Ivermectin showed remarkably high cytotoxicity. These findings suggest that Prowill disinfectants, especially in their gel form, might represent a promising auxiliary application for managing the spread of IBV and potentially other coronaviruses. Further investigation is required to evaluate the long-term safety and broader applicability of these disinfectants in controlling avian coronaviruses and related pathogens.

Keywords: Astragalus extract, antiviral disinfectants, coronaviruses, infectious bronchitis virus, IBV strain D274, probiotic disinfectants

Introduction

Infectious Bronchitis Virus (IBV) is an avian coronavirus belonging to the Igacovirus subgenus of the Gammacoronavirus genus within the Orthocoronavirinae subfamily of the Coronaviridae family. This virus is a significant cause of respiratory disease in poultry, leading to substantial economic losses in the global poultry industry (Marchenko et al. 2022). Like other members of the coronavirus family, including the human Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), IBV is an enveloped virus with a positive-sense single-stranded RNA genome, a characteristic shared among coronaviruses across various genera (Quinteros et al. 2022). This genetic makeup allows IBV to exhibit similar structural and functional properties to other coronaviruses, making it an important model for studying coronavirus behavior and sensitivity to chemicals and disinfectants, as well as for developing strategies to control their spread.

The high mutation rates of IBV strains complicate efforts to control its transmission, with frequent genetic variations reducing the effectiveness of vaccines. This highlights the need for alternative or complementary control strategies (Zhang et al. 2018, Zhang et al. 2021). Disinfectants are crucial in managing infectious diseases by reducing pathogen load in contaminated environments, thereby preventing viral spread. Effective disinfectants are particularly important in poultry farms, which help mitigate outbreaks and reduce the risk of reinfection (Chima et al. 2012). However, as with any chemical treatment, disinfectants must also be evaluated for their safety, mainly when used in environments with sensitive hosts like poultry. Certain disinfectants, such as iodine-based solutions, have been shown to be effective in controlling poultry viruses, including IBV, but their safety profile must be carefully assessed (Turabekov et al. 2024).

Probiotic disinfectants are innovative cleaning products that are eco-friendly alternatives to traditional chemical disinfectants. They contain beneficial bacteria, such as *Bacillus* spp. and *Lactobacillus* spp., which can effectively reduce harmful pathogens while promoting a healthier microbial environment (Denkel et al. 2024). D'Accolti et al. (2021) demonstrated that formulations of the probiotic-based disinfectant can effectively inactivate a range of enveloped viruses, including human coronaviruses – such as SARS-CoV-2 – and influenza viruses, achieving up to 99.99% efficacy within 1-2 hours of application. These disinfectants also emerge as a viable solution for enhancing biosecurity in poultry farms (Graham et al. 2018). This eco-sustainable approach not only mitigates the risks associated with traditional chemical disinfectants but also addresses concerns related to antimicrobial resistance (AMR) and environmental pollution (D'Accolti et al. 2021). In addition to probiotics, ivermectin, and plant-based compounds like Astragalus root extract have gained attention for their potential to reduce viral load and enhance immune function. Astragalus root extract, a traditional Chinese medicinal herb, has demonstrated potential as an immune booster and antiviral agent in animal models, particularly for its ability to modulate the immune response and reduce viral replication (Zhang et al. 2017, Lu et al. 2019, Wu et al. 2022). Ivermectin, primarily known as an antiparasitic drug, has shown promising antiviral activity in poultry, although its toxicity in avian species requires careful consideration (Azeem et al. 2015). The potential of ivermectin as a therapeutic agent for SARS-CoV-2 underscores the importance of studying this antiparasitic drug in the context of coronavirus treatment (Zaidi and Dehgani-Mobaraki 2022).

Prowill, a commercial disinfectant in Turkey, presents an interesting option for managing the transmission of IBV. This probiotic disinfectant is known for its antibacterial properties, but its antiviral efficacy, particularly against IBV, has not been fully explored. This study aims to fill this gap by evaluating the antiviral activity of various commercial formulations of Prowill against IBV in specific pathogen-free (SPF) embryonated chicken eggs (ECEs). In addition to assessing the antiviral effectiveness, the study also examines the safety of these disinfectants by investigating their lethal effects on embryos. As embryos are among the most sensitive hosts to viral and chemical exposure, this evaluation is crucial for determining the safety of Prowill in poultry settings. Furthermore, the study compares the efficacy of Prowill with that of Ivermectin and Astragalus root extract.

Materials and Methods

Selection of eggs and IBV serotype

In this study, only SPF ECEs aged 9–10 days were used. As SPF ECEs younger than 14 days are not classified as experimental animals under national regulations (https://www.tarimorman.gov.tr), no ethics committee approval was required; however, all procedures were conducted in accordance with institutional and national ethical guidelines. They were obtained from the Veterinary Control Institute, Bornova, Izmir, Turkey. The IBV serotype D274 (Genotype 1, Lineage 12) was selected for this study. The virus strain was obtained from the Poultry Diseases Diagnostic Laboratory, Bornova Diagnostic Institute, Bornova, Izmir, Turkey. To assess its virulence and ensure appropriate experimental conditions, the virus titer was determined and standardized using the Reed and Muench method (Reed and Muench 1938). The virus was diluted to 10^3 Embryo Infective Dose 50% (EID_{50})/ml as recommended by the World Organisation for Animal Health (WOAH) for subsequent use in evaluating the *in ovo* antiviral activity of various disinfectants and substances (WOAH 2018).

Selection and preparation of disinfectants and substances

The disinfectants included are Prowill surface--cleaning disinfectant (*Bacillus* ferment, a fermentation-derived complex produced by *Bacillus* species, 1lt, Efor Group, Turkey), Prowill probiotic hand gel (*Bacillus* ferment, a fermentation-derived complex produced by *Bacillus* species, 250ml, Efor Group, Turkey), Prowill probiotic anti-allergy spray (*Bacillus* ferment, a fermentation-derived complex produced by *Bacillus* species, 300ml, Efor Group, Turkey) (https://prowill.com.tr/). Additionally, Astragalus root extract (250 mg of powdered Astragalus membranaceus root and 225 mg of Astragalus membranaceus extract) (Solgar, NJ, USA) and ivermectin (Merck, NJ, USA) were also used to evaluate their antiviral properties. The dilutions of disinfectants were prepared using phosphate-buffered saline (PBS) to achieve the desired concentrations of 0.5%, 1%, and 2%. The stock solutions of the disinfectants were diluted in PBS to these final concentrations by volume. For ivermectin and Astragalus root extract, stock solutions were prepared by dissolving the compounds in dimethyl sulfoxide (DMSO) to ensure proper solubility. These stock solutions were then diluted in PBS to the following concentrations: 0.01, 0.1, 1, and 5 mg/kg for Astragalus root extract and 0.01, 0.1, and 1 mg/kg for ivermectin.

In ovo virucidal and antiviral activity of disinfectants and substances

The antiviral efficacy of the disinfectants and substances was assessed by inoculating 9-10-day-old SPF ECEs with 10^3 EID_{50}/ml IBV D274 strain mixed with each disinfectant and anti-viral agent at a 1:1 ratio. The mixtures were incubated at room temperature for 1 hour to allow for virucidal activity. An antiviral disinfectant (1% diluted BOREL, Eti Maden, Turkey) was also used to compare the antiviral activity of the disinfectants and antiviral agents. The control groups included: untreated SPF ECEs, vehicle control (virus treated with 5% DMSO), commercial antiviral disinfectant (BOREL 1%), and 10^3 EID_{50}/ml IBV D274 strain alone. Each experimental group consisted of 10 SPF ECEs. After the 1-hour incubation period, 0.1 ml of the virus-disinfectant mixture was inoculated into the chorioallantoic cavity (CAC) of the SPF ECEs. Inoculation points were carefully marked using transmitted light to ensure accurate delivery. The inoculated eggs were sealed and incubated at 37°C with 55% humidity for 48 hours. All inoculation procedures were performed under sterile conditions in a Biosafety Level 2 (BSL-2) cabinet to maintain environmental safety.

Hemagglutination (HA) assay

Hemagglutination (HA) assays were conducted to assess the antiviral activity in the chorioallantoic fluid (CAF). The 1% (v/v) chicken red blood cell (RBC) suspension was prepared from freshly collected chicken blood mixed with Alsever's solution and then washed with PBS. The serial dilutions (1:2 to 1:4096) of CAF were tested using a V-bottom 96-well microplate. The procedure followed standard protocol, adding PBS to all wells, followed by CAF samples, serially diluted across the plate, and RBC suspension (WOAH 2018). The plate was incubated for 45 minutes, and hemagglutination was assessed. A button formation was considered negative, while agglutination or lattice formation was considered positive. The results were analyzed by comparing the endpoint dilution of HA-positive samples to the virus control dilution, determining viral presence and titer (WOAH 2018).

Statistical analysis

Data were expressed as mean values ± standard deviation. Statistical comparisons between groups were performed using non-parametric tests, specifically the Kruskal-Wallis and Mann-Whitney tests. Post hoc comparison was performed using Dunn's test (only virus vs each experimental condition). Differences between experimental groups were considered statistically significant at (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$). All statistical analyses were performed using GraphPad Prism (version 10.2.0).

Results

The results of this experiment provide insightful data on the efficacy of various treatments against viral infection-induced egg mortality and viral replication, measured through both mortality rates and HA titers. The untreated SPF ECEs group, which was not exposed to any treatment or the virus, also showed no mortality, indicating the normal, healthy condition of the embryos under non-infected circumstances. The positive control group, which was inoculated with the virus alone, exhibited a 20% mortality rate (2 out of 10 eggs),

Table 1. Embryo mortality and percent mortality ratio for different dilutions of disinfectant and substances.

Group	Samples	Concentration	No. of eggs	Number of Death		Egg mortality	Mortality%
				Day 1	Day 2		
1	Positive control			1	1	2/10	20%
	(Only virus)						
2	Vehicle control			0	1	1/10	10%
	(Virus treated with %5 DMSO)						
3	Commercial antiviral agent control			0	0	0/10	0%
	(BOREL 1%)						
4	Untreated SPF ECEs			0	0	0/10	0%
5	Astragalus root extract	0.01 mg/kg	10	0	0	0/10	0%
		0.1 mg/kg	10	0	1	1/10	10%
		1 mg/kg	10	2	2	4/10	40%
		5 mg/kg	10	2	1	3/10	30%
6	Ivermectin	0.01 mg/kg	10	1	2	3/10	30%
		0.1 mg/kg	10	2	3	5/10	50%
		1 mg/kg	10	2	3	5/10	50%
7	Surface cleaning liquid	0.5%	10	0	1	1/10	10%
	(Prowill)	1%	10	0	2	2/10	20%
8	Probiotic hand gel	0.5%	10	0	1	1/10	10%
	(Prowill)	1%	10	0	1	1/10	10%
		2%	10	0	1	1/10	10%
9	Probiotic anti-allergy spray	0.5%	10	1	1	2/10	20%
	(Prowill)	1%	10	0	1	1/10	10%
		2%	10	0	1	1/10	10%

providing a baseline to gauge the impact of the virus in the absence of any antiviral intervention. The vehicle control group, which treated the virus with 5% DMSO, showed a slightly lower mortality rate of 10% (1 out of 10 eggs), suggesting that DMSO might have a minimal protective effect or slightly reduce the viral impact, as indicated in Table 1. The commercial antiviral agent control group (BOREL 1%) demonstrated remarkable efficacy, with 0% mortality observed, highlighting the agent's ability to prevent viral-induced egg mortality completely.

Astragalus root extract showed variable results, with its effects differing depending on the concentration. At the lowest concentration (0.01 mg/kg), no mortality was observed (0%). However, at 0.1 mg/kg, the mortality rate increased to 10% (1 out of 10 eggs). As the concentration increased, egg mortality also increased (Fig. 1). At 1 mg/kg, the mortality rate surged to 40% (4 out of 10 eggs), and at 5 mg/kg, the mortality rate was 30% (3 out of 10 eggs). These results suggest that higher concentrations of Astragalus root extract may exert toxic effects, thereby increasing embryo mortality. However, this toxic effect was still less than that caused by ivermectin.

Ivermectin, tested at concentrations of 0.01 mg/kg, 0.1 mg/kg, and 1 mg/kg, showed a dose-dependent impact on egg mortality. At the lowest concentration (0.01 mg/kg), the mortality rate was 30% (3 out of 10 eggs), indicating a modest toxic effect. However, at both 0.1 mg/kg and 1 mg/kg, the mortality rate increased to 50% (5 out of 10 eggs) at each concentration, suggesting that ivermectin at higher concentrations might lead to toxicity, as reflected in the increased mortality rates (Fig. 1).

In Prowill gel, the mortality rate was consistent across all three doses, indicating that increasing doses of Prowill gel did not cause any toxic effects. For Prowill spray, the lowest dose of 0.5% resulted in a 20%

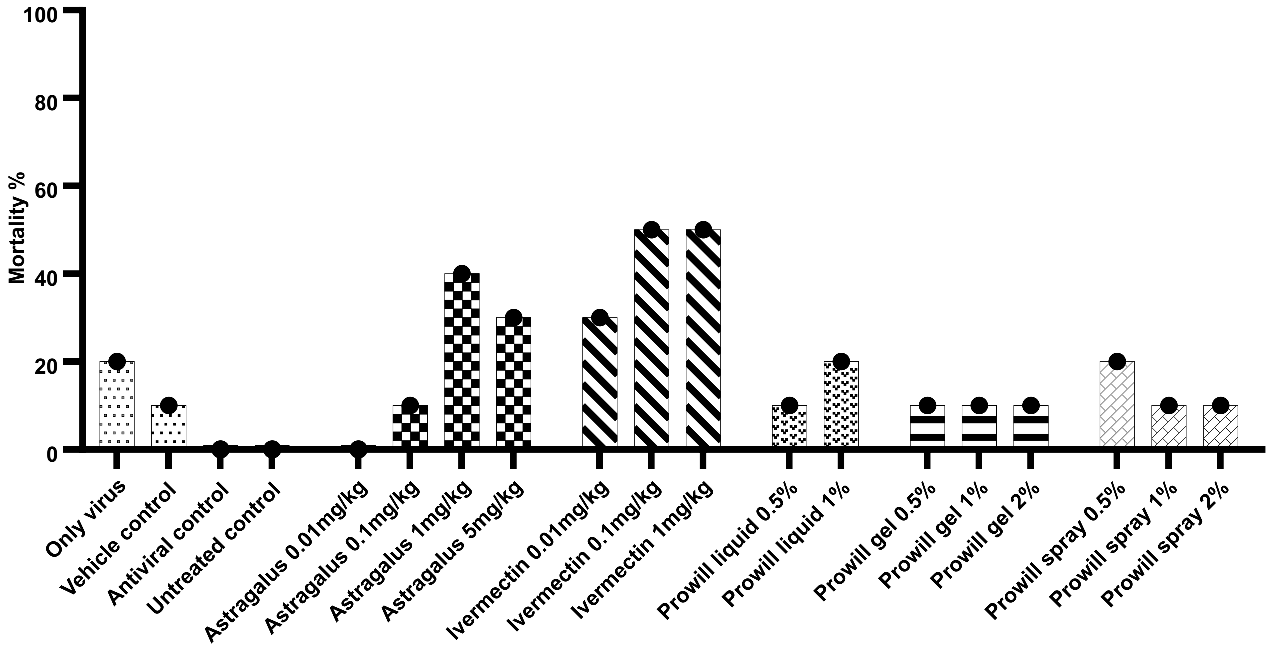

Fig 1. Embryo mortality rates in embryonated chicken eggs treated with 10^3 EID_{50}/mL infectious bronchitis virus. Mortality rates (%) were assessed following infection with 10^3 EID_{50}/mL IBV and treatment with various substances, including Prowill probiotic-disinfectant products (0.5–2%), Astragalus root extract (0.01–5 mg/kg), and ivermectin (0.01–1 mg/kg). Control groups consist of only virus, vehicle control, antiviral control, and untreated control.

mortality rate, whereas higher doses caused only a 10% mortality rate. This further suggests that increasing doses of Prowill spray are not toxic. However, contrary to these observations, the 1% dose of liquid Prowill appeared to be slightly more toxic than the 0.5% dose (Fig. 1).

In terms of viral replication, measured by HA titers, the positive control group (virus only) showed a high mean HA titer of 10.8 (titer in log2), confirming active viral replication. The vehicle control group (virus + 5% DMSO) also showed a mean HA titer of 10.7 (titer in log2), indicating no remarkable interference from DMSO in viral replication. However, the commercial antiviral agent BOREL 1% completely inhibited viral replication (Fig. 2), resulting in an HA titer of 0 (titer in log2), further validating its antiviral efficacy (Table 2).

The results for Astragalus root extract showed a clear dose-dependent reduction in viral replication (Fig. 3). At 0.01 mg/kg, the mean HA titer was 10.9 (titer in log2), similar to that in the positive control group, indicating no antiviral effect. At 0.1 mg/kg, the mean HA titer dropped to 9.8 (titer in log2), suggesting some antiviral activity. At 1 mg/kg, the mean HA titer decreased further to 8 (titer in log2), and at 5 mg/kg, the mean HA titer reached 0 (titer in log2), suggesting complete viral inhibition at this highest concentration. These findings align with the increased mortality rates observed at higher concentrations of Astragalus root extract, indicating a dose-dependent antiviral effect, with the highest concentration providing the most significant reduction in viral replication.

For ivermectin, the HA titers also showed a dose-dependent decrease. At 0.01 mg/kg, the mean HA titer was 8.8 (titer in log2), reflecting moderate antiviral activity. At 0.1 mg/kg, the mean HA titer decreased to 8 (titer in log2), and at 1 mg/kg, it dropped further to 5.9 (titer in log2), suggesting a stronger antiviral effect at higher concentrations. However, as seen with the egg mortality data, the higher concentrations also correlated with increased toxicity to the embryos.

The Prowill disinfectants displayed varying levels of antiviral activity. The surface cleaning liquid at 0.5% had a mean HA titer of 7.8 (titer in log2), which decreased to 6 (titer in log2) at 1%, indicating a mild antiviral effect. The probiotic hand gel at 0.5% exhibited a mean HA titer of 4.7 (titer in log2), which decreased to 3.8 and 3.6 (titer in log2) at both 1% and 2%, respectively, indicating good antiviral activity even at lower concentrations. The probiotic anti-allergy spray at 0.5% had a mean HA titer of 5.8 (titer in log2), which dropped to 2.8 and 2.4 (titer in log2) at both 1% and 2%, respectively, demonstrating significant antiviral activity at the higher concentrations (Table 2). Although significant antiviral effects were observed across all dilutions of the Prowill disinfectants, the probiotic hand gel demonstrated remarkable efficacy in reducing both egg mortality (Fig. 1) and viral load (Fig. 2).

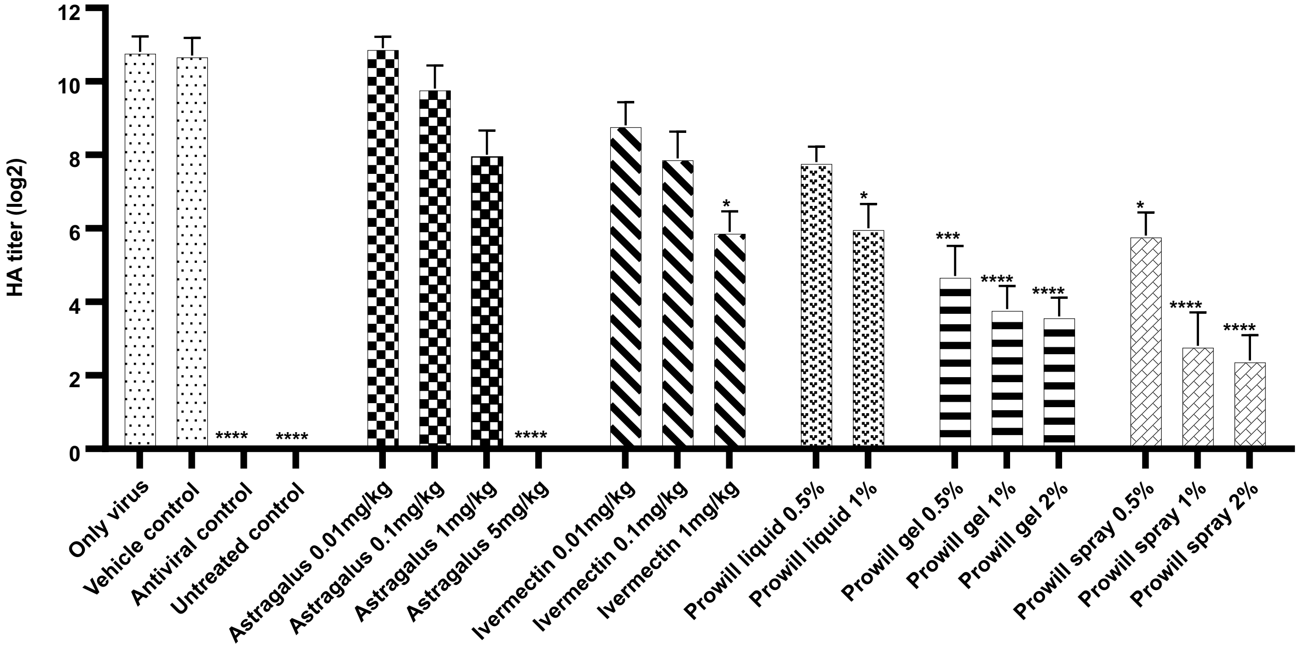

Fig 2. Hemagglutination (HA) activity in embryonated chicken eggs (ECEs) treated with 10^3 EID$_{50}$/mL of IBV after 48 hours. HA activity (log$_2$) was measured in ECEs 48 hours post-infection with 10^3 EID$_{50}$/mL of IBV and treatment with various substances, including Prowill probiotic-disinfectant products (0.5–2%), Astragalus root extract (0.01–5 mg/kg), and ivermectin (0.01–1 mg/kg). Control groups consist of only virus, vehicle control, antiviral control, and untreated control. Data are presented as mean values ± standard deviation. Statistical analysis was performed using the Kruskal-Wallis test with Dunn's test as a post hoc comparison. *p<0.05, ***p<0.001, ****p<0.0001

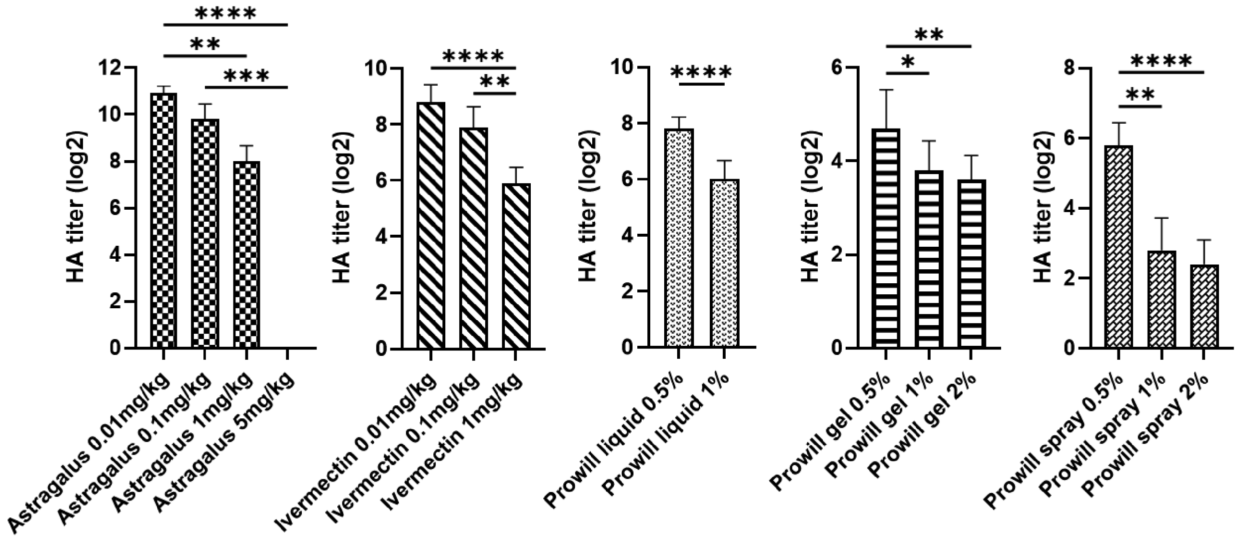

Fig. 3. Comparison of haemagglutination (HA) activity in embryonated chicken eggs treated with 10^3 EID$_{50}$/ml of IBV after 48 hours at different concentrations. Data are presented as mean values ± standard deviation. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Discussion

IBV remains a major threat to global poultry production, causing significant economic losses through respiratory disease, renal complications, and decreased egg production. Despite ongoing vaccination programs and stringent biosecurity measures, IBV's high mutation rate and extensive genomic diversity have complicated its control (Zhang et al. 2021). Alternative strategies, such as novel disinfectants and adjunct antiviral agents, have received growing attention. This study evaluated probiotic-based disinfectants, Astragalus root extract, and ivermectin for their antiviral effects in an ECE model. The results point to a promising role for certain probiotic-based agents in reducing IBV replication while highlighting the dose-dependent benefits and

Table 2. Hemagglutination (HA) titers for specific pathogen-free embryonated chicken eggs following 48-h incubation with IBV.

Group	Samples	Concentration	No. of eggs (n)	HA titer										Mean HA titer	Mean HA titer ($\log_2$)
				ECE1	ECE2	ECE3	ECE4	ECE5	ECE6	ECE7	ECE8	ECE9	ECE10		
1	Positive control			2048	2048	2048	1024	2048	2048	2048	1024	2048	2048	1782	10.8
	(Only virus)														
2	Vehicle control			2048	1024	2048	2048	2048	2048	1024	1024	2048	2048	1663	10.7
	(Virus treated with %5 DMSO)														
3	Commercial antiviral agent control			0	0	0	0	0	0	0	0	0	0	0	0
	(BOREL 1%)														
4	Untreated SPF ECEs			0	0	0	0	0	0	0	0	0	0	0	0
5	Astragalus root extract	0.01 mg/kg	10	2048	2048	1024	2048	2048	2048	2048	2048	2048	2048	1910	10.9
		0.1 mg/kg	10	1024	512	1024	1024	1024	512	1024	2048	1024	512	891	9.8
		1 mg/kg	10	512	256	128	256	256	256	128	256	516	256	256	8
		5 mg/kg	10	0	0	0	0	0	0	0	0	0	0	0	0
6	Ivermectin	0.01 mg/kg	10	256	512	1024	512	512	512	256	512	512	256	445	8.8
		0.1 mg/kg	10	256	128	256	512	256	128	256	256	512	128	256	8
		1 mg/kg	10	64	32	64	64	32	64	64	128	64	64	60	5.9
7	Surface cleaning liquid	0.5%	10	256	256	256	128	256	256	256	128	256	256	223	7.8
	(Prowill)	1%	10	32	64	128	64	64	32	64	128	64	64	64	6
8	Probiotic hand gel	0.5%	10	32	16	32	32	32	8	32	16	64	32	26	4.7
	(Prowill)	1%	10	16	8	16	16	8	16	16	32	8	16	14	3.8
		2%	10	16	16	16	8	16	8	8	16	16	8	12	3.6
9	Probiotic anti-allergy spray	0.5%	10	64	64	64	32	32	64	128	32	64	64	56	5.8
	(Prowill)	1%	10	8	16	4	8	8	8	2	4	8	16	7	2.8
		2%	10	8	8	4	8	4	2	4	8	4	8	5	2.4

toxicity profiles of potential antivirals like Astragalus extract and ivermectin.

Probiotic-based cleaning products have drawn substantial interest because of their ability to offer sustained antimicrobial activity without many of the detrimental ecological and health-related side effects associated with conventional chemical disinfectants (Caselli et al. 2016, D'Accolti et al. 2021). Indeed, prior research has shown that probiotic disinfectants can inhibit a variety of enveloped viruses, including human coronaviruses and influenza viruses (D'Accolti et al. 2021, Ramos and Frantz 2023). In this study, all three formulations of the probiotic-based disinfectant (liquid, hand gel, and anti-allergy spray) demonstrated significant antiviral effects, though the hand gel consistently showed particularly low mortality and HA titers. The high efficacy of this gel formulation may be due to better adherence to surfaces or localized prolonged release of beneficial bacterial metabolites that interrupt the viral membrane (D'Accolti et al. 2021). Furthermore, probiotic-based products have been associated with reduced antimicrobial resistance and lower environmental pollution in poultry farms (Lutful Kabir 2009, Leistikow et al. 2022, Yaqoob et al. 2022), an important consideration given the intensive farming conditions and repeated administration of disinfectants.

Astragalus root extract is derived from an herb widely employed in traditional Chinese medicine and is reputed for its immunomodulatory and antiviral properties (Zhang et al. 2018). Several lines of evidence suggest that Astragalus polysaccharides and flavonoids can boost systemic immunity and decrease viral replication in models of influenza and coronaviruses (Lu et al. 2019, Zhang et al. 2021). In the current experiment, Astragalus extract exerted clear dose-dependent inhibition of IBV replication, as reflected by a substantial reduction in viral HA titers at higher concentrations. However, increased embryotoxicity was observed in parallel, aligning with earlier studies demonstrating that excessively high doses of plant-based compounds can induce cytotoxic effects (Yen et al. 2001, Askin Celik and Aslanturk 2010). The immunostimulatory capacity of Astragalus extracts (such as enhanced T-cell activity and reduced pro-inflammatory cytokines) might reinforce local and systemic defenses against IBV (Kim et al. 2014, Wang et al. 2022).

Ivermectin, an antiparasitic drug with recognized *in vitro* activity against a variety of viruses, has garnered interest as a potential inhibitor of coronaviral replication (Azeem et al. 2015, Zaidi and Dehgani-

-Mobaraki 2022). Nevertheless, the embryonated egg model used in this study revealed that while ivermectin indeed diminished IBV titers, higher doses significantly increased embryo mortality. Similar toxicity concerns have been reported in avian models (Azeem et al. 2015, Reynolds and Simpson 2022), reflecting the narrow therapeutic window for ivermectin use in poultry. This drug has been hypothesized to disrupt viral protein transport within the host cell by binding importin α/β, thus blocking essential viral replication events (Wagstaff et al. 2012). Nonetheless, the persistent toxic effects in higher concentrations highlight the need for caution. Chemical disinfectants have long served as a mainstay in poultry biosecurity and disease control, but repeated use has raised apprehensions surrounding pathogen resistance, operator safety, and environmental hazards (Davies and Wales 2019). Indeed, past reports on ammonia–glutaraldehyde and chlorine-based disinfectants note partial efficacy against avian viruses and highlight the significance of organic matter in diminishing disinfectant potency (Figueroa et al. 2017, Turabekov et al. 2024). Some of these agents show broad efficacy against enveloped viruses like IBV by inactivating the viral envelope proteins needed for cell entry. Disinfectants that rely exclusively on chemical activity can be undermined by the constant accumulation of organic debris in poultry facilities and by the formation of chemical by products that pose environmental or operator risks (Figueroa et al. 2017, Davies and Wales 2019).

In this context, probiotic disinfectants stand out by combining environmentally friendly properties with potentially sustained antimicrobial activity. Their efficacy likely hinges on multiple modes of action: (1) probiotic microbes outcompete pathogens for space and nutrients, (2) they produce bacteriocins or other metabolites (like lactic acid) that destabilize viral envelopes, and (3) they possibly alter the microbiome of the poultry environment to favor beneficial flora (D'Accolti et al. 2021, Leistikow et al. 2022, Denkel et al. 2024). More research is warranted to understand how probiotic formulations might help mitigate not just IBV but also co-infections, such as avian influenza or *Escherichia coli*, that often exacerbate IB-related respiratory disease (Balta et al. 2020).

Because IBV is known for its continued genetic evolution and the frequent emergence of new serotypes that can evade immune responses, integrative approaches are crucial for successful disease control (Zhang et al. 2021, Quinteros et al. 2022). These integrative strategies include vaccination tailored to local genotypes, rigorous hygiene practices, and the use of disinfectants or antivirals that hamper virus viability outside the host (Bhuiyan et al. 2021). Furthermore, the minimal embryonic toxicity of probiotic gel found in this study suggests it could be applied in hatcheries, where viable embryos are particularly vulnerable to chemical disinfectant residues (Tarpey et al. 2006). Although additional *in vivo* trials in older birds would be necessary to confirm benefits at the farm level, this early evidence positions probiotic-based solutions as a promising addition to comprehensive control measures.

Overall, these findings add to a growing body of work indicating that nature-derived or probiotic-based strategies can effectively address coronaviruses in avian species. By reducing reliance on conventional chemical interventions, poultry producers may mitigate environmental burdens and decrease antibiotic use, both of which are critical goals given the growing concerns over antimicrobial resistance and ecosystem health. Taken together, the present data underscore the value of a broader approach to IBV control in modern poultry systems, in which vaccination, safe disinfectants, and auxiliary antivirals function in synergy. As IBV continues to evolve and cause significant disruptions to poultry health and productivity, integrating low-toxicity, potent antiviral disinfectants, and immunomodulatory supplements appears both prudent and necessary.

From a field application perspective, probiotic-based disinfectants appear to hold the greatest potential among the tested agents due to their favorable safety profile, ease of use, and environmental compatibility. In particular, the probiotic hand gel demonstrated notable antiviral activity with minimal embryotoxicity *in ovo*, suggesting that such formulations could be safely used not only in hatchery hygiene routines but also more broadly across various poultry production areas, including environments where human workers are present. This highlights their potential for providing safe and practical hygiene solutions throughout the poultry sector, supporting both animal and occupational health. Although currently lacking standardized veterinary authorization, similar *Bacillus*- and *Lactobacillus*-based sprays have shown promising results in flock settings by reducing viral load and improving clinical outcomes during experimental avian influenza infections (Rasaei et al. 2023). Astragalus extract, despite its clear antiviral effect *in ovo*, presents a narrower safety margin and variable toxicity, which may hinder its direct application in commercial flocks unless further refined. Ivermectin, although cost-effective and widely available, caused severe embryotoxicity even at low doses in this study, making it unsuitable for antiviral use in developing embryos or hatchery environments. In short, probiotic disinfectants, especially those with topical or surface application formats, may represent a promising adjunct to conventional flock-level antiviral management, though further *in vivo* validation under commercial field conditions remains essential. Such trials in poultry flocks and hatchery settings will be crucial for validating their safety, dosing protocols, and protective efficacy under practical poultry system conditions, ultimately supporting evidence-based integration into commercial biosecurity practices.

Consequently, the present findings demonstrate that probiotic-based disinfectants, particularly in gel form, effectively reduce infectious bronchitis virus replication and mortality in an embryonated chicken egg model. Astragalus root extract likewise confers dose-dependent antiviral effects, although higher concentrations pose risk of embryotoxicity. Ivermectin shows modest antiviral activity at lower concentrations but carries substantial toxicity at higher doses. Each of these formulations could contribute to integrative control strategies for IBV, complementing established biosecurity measures and vaccination programs. To realize their full potential, future research should focus on refining safe usage parameters (especially for ivermectin) and on standardizing dosing regimens to balance antiviral efficacy with embryo and bird welfare. Optimizing disinfectant and antiviral approaches will help mitigate IBV in poultry operations, reducing economic losses and improving animal health.

References

Askin Celik T, Aslanturk OS (**2010**) Evaluation of cytotoxicity and genotoxicity of Inula viscosa leaf extracts with Allium test. J Biomed Biotechnol 2010: 189252.

Azeem S, Ashraf M, Rasheed MA, Anjum AA, Hameed R (**2015**) Evaluation of cytotoxicity and antiviral activity of ivermectin against Newcastle disease virus. Pak J Pharm Sci 28: 597-602.

Balta I, Stef L, Pet I, Ward P, Callaway T, Ricke SC, Gundogdu O, Corcionivoschi N (**2020**) Antiviral activity of a novel mixture of natural antimicrobials, in vitro, and in a chicken infection model in vivo. Sci Rep 10: 16631.

Bhuiyan MS, Amin Z, Rodrigues KF, Saallah S, Shaarani SM, Sarker S, Siddiquee S (**2021**) Infectious bronchitis virus (gammacoronavirus) in poultry farming: vaccination, immune response and measures for mitigation. Vet Sci 8: 273.

Caselli E, D'Accolti M, Vandini A, Lanzoni L, Camerada MT, Coccagna M, Branchini A, Antonioli P, Balboni PG, Di Luca D, Mazzacane S (**2016**) Impact of a probiotic-based cleaning intervention on the microbiota ecosystem of the hospital surfaces: focus on the resistome remodulation. PLoS One 11: e0148857.

Chima IU, Unamba-Opara IC, Ugwu CC, Udebuani AC, Okoli CG, Opara MN, Uchegbu MC, Okoli IC (**2012**) Biosecurity and disinfection controls of poultry microbial pathogen infections in Nigeria. J World Poult Res 2: 5-17.

D'Accolti M, Soffritti I, Bonfante F, Ricciardi W, Mazzacane S, Caselli E (**2021**) Potential of an eco-sustainable probiotic-cleaning formulation in reducing infectivity of enveloped viruses. Viruses 13: 2227.

Davies R, Wales A (**2019**) Antimicrobial resistance on farms: a review including biosecurity and the potential role of disinfectants in resistance selection. Compr Rev Food Sci Food Saf 18: 753-774.

Denkel LA, Voss A, Caselli E, Dancer SJ, Leistner R, Gastmeier P, Widmer AF (**2024**) Can probiotics trigger a paradigm shift for cleaning healthcare environments? A narrative review. Antimicrob Resist Infect Control 13: 119.

Figueroa A, Hauck R, Saldias-Rodriguez J, Gallardo RA (**2017**) Combination of quaternary ammonia and glutaraldehyde as a disinfectant against enveloped and non-enveloped viruses. J Appl Poult Res 26: 491-497.

Graham LE, Teague KD, Latorre JD, Yang Y, Baxter MF, Mahaffey BD, Hernandez-Velasco X, Bielke LR, Hargis BM, Tellez G (**2018**) Use of probiotics as an alternative to formaldehyde fumigation in commercial broiler chicken hatch cabinets. J Appl Poult Res 27: 371-379.

Kim BH, Oh I, Kim JH, Jeon JE, Jeon B, Shin J, Kim TY (**2014**) Anti-inflammatory activity of compounds isolated from Astragalus sinicus L. in cytokine-induced keratinocytes and skin. Exp Mol Med 46: e87.

Leistikow KR, Beattie RE, Hristova KR (**2022**) Probiotics beyond the farm: benefits, costs, and considerations of using antibiotic alternatives in livestock. Front Antibiot 1: 1003912.

Lu CH, Ma YM, Wang HX, Xue L (**2019**) Protective effect of therapeutic and prophylactic administration of Astragalus polysaccharides on influenza virus PR8-induced acute lung injury model. Chin J Immun 35:1699-1707.

Lutful Kabir SM (**2009**) The role of probiotics in the poultry industry. Int J Mol Sci 10: 3531-3546.

Marchenko V, Danilenko A, Kolosova N, Bragina M, Molchanova M, Bulanovich Y, Gorodov V, Leonov S, Gudymo A, Onkhonova G, Svyatchenko S, Ryzhikov A (**2022**) Diversity of gammacoronaviruses and deltacoronaviruses in wild birds and poultry in Russia. Sci Rep 12: 19412.

Quinteros JA, Noormohammadi AH, Lee SW, Browning GF, Diaz-Méndez A (**2022**) Genomics and pathogenesis of the avian coronavirus infectious bronchitis virus. Aust Vet J 100: 496-512.

Ramos AM, Frantz AL (**2023**) Probiotic-based sanitation in the built environment—an alternative to chemical disinfectants. Appl Microbiol 3: 536-548.

Rasaei D, Hosseinian SA, Asasi K, Shekarforoush SS, Khodakaram-Tafti A (**2023**) The beneficial effects of spraying of probiotic Bacillus and Lactobacillus bacteria on broiler chickens experimentally infected with avian influenza virus H9N2. Poult Sci 102: 102669.

Reed LJ, Muench H (**1938**) A simple method of estimating fifty per cent endpoints. Am J Epidemiol 27: 493-497.

Reynolds DL, Simpson EB (**2022**) Evaluation of ivermectin antiviral activity against avian infectious bronchitis virus using a chicken embryo model. Int J Vet Sci Med 10: 19-24.

Tarpey I, Orbell SJ, Britton P, Casais R, Hodgson T, Lin F, Hogan E, Cavanagh D (**2006**) Safety and efficacy of an infectious bronchitis virus used for chicken embryo vaccination. Vaccine 24: 6830-6838.

Turabekov MR, Barakhov BB, Myrzabekov ZB, Tagayev OO, Ibragimov PS, Alpysbayeva GE, Narbayeva DD, Alikhanov KD, Taipova AA (**2024**) Enhancing animal welfare and immune health: a study on hydrogen peroxide and iodine-based disinfectants in farms. Eng Sci 29: 1095.

Wagstaff KM, Sivakumaran H, Heaton SM, Harrich D, Jans DA (**2012**) Ivermectin is a specific inhibitor of importin α/β-mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. Biochem J 443: 851-856.

Wang XY, Wang RC, Qu ZY, Zhu YZ, Li YL (**2022**) Advances on immunoregulation effect of astragalus polysaccharides. Front Nat Prod 1: 971679.

World Organization for Animal Health (WOAH) (**2018**) Avian infectious bronchitis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, pp. 1–15. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.03.02_AIB.pdf

Wu ZB, Shao SR, Chen HY (**2022**) Antiviral effects and mechanism of Astragalus polysaccharide on respiratory syncytial virus induced lung infection in young mice. Med J Chin PLA 47: 346-352.

Yaqoob MU, Wang G, Wang M (**2022**) An updated review on probiotics as an alternative of antibiotics in poultry – a review. Anim Biosci 35: 1109-1120.

Yen GC, Chen HY, Peng HH (**2001**) Evaluation of the cytotoxicity, mutagenicity and antimutagenicity of emerging edible plants. Food Chem Toxicol 39: 1045-1053.

Zaidi AK, Dehgani-Mobaraki P (**2022**) The mechanisms of action of ivermectin against SARS-CoV-2 – an extensive review. J Antibiot (Tokyo) 75: 60-71.

Zhang P, Liu X, Liu H, Wang W, Liu X, Li X, Wu X (**2018**) Astragalus polysaccharides inhibit avian infectious bronchitis virus infection by regulating viral replication. Microb Pathog 114: 124-128.

Zhang P, Wang J, Wang W, Liu X, Liu H, Li X, Wu X (**2017**) Astragalus polysaccharides enhance the immune response to avian infectious bronchitis virus vaccination in chickens. Microb Pathog 111: 81-85.

Zhang Y, Xu Z, Cao Y (**2021**) Host antiviral responses against avian infectious bronchitis virus (IBV): focus on innate immunity. Viruses 13: 1698.