Research Article

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Effect of Ribavirin on Experimentally Induced Infectious Bronchitis in Broiler Chicks

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Abstract

Infectious bronchitis virus (IBV) causes infectious bronchitis (IB), a highly contagious disease affecting the respiratory, reproductive, and renal systems in chickens. There is no approved drug, and vaccination often fails to protect due to high mutation and recombination rates. This study evaluated the effect of ribavirin on broilers' experimental IB. Diseased broilers with IB were confirmed using reverse transcription polymerase chain reaction (RT-PCR). The IBV was passed in embryonated chicken eggs three times to propagate the virus. The allantoic fluid (AF) from the third viral passage was used to infect broiler chicks. Fifty-four unvaccinated chicks, 17- days -old each, were used to evaluate the antiviral effect of ribavirin. The chicks were divided into infected (36 chicks) and noninfected (18 chicks) groups. The 36 chicks were divided into four equal groups, and the remaining 18 birds were divided into two groups and kept in a separate facility. When the birds reached 17 days of age, 36 birds were infected with IBV by intranasal administration of 0.2 mL AF. The same day the chicks were infected with IBV, ribavirin treatment began and was repeated twice daily for seven days. On the fifth day of infection, we collected tracheal swab samples from the birds for PCR testing, which confirmed the presence of the IBV virus in all of them. However, we observed no mortality. Our experimental data, including PCR test results, chicks' body weight, temperature, feeding, water consumption, clinical signs, postmortem, and histopathological examinations, showed that ribavirin was ineffective against IBV.

Keywords: Disease, Virus, PCR, Ribavirin.

Introduction

The avian infectious bronchitis virus (IBV), which causes avian infectious bronchitis (IB), is an acute and highly contagious chicken disease that significantly impacts the poultry industry and results in significant global economic losses. IBV is a genus of gammacoronaviruses in the family Coronaviridae and the order Nidovirales. It primarily copies itself in cells that line the respiratory tract, causing serious lung diseases in people of all ages (1). IBV can infect various bird species (2), but several variables, including age, genetics, and environmental stress, contribute to birds' vulnerability to the IBV strains (3). The only known natural hosts of the IBV are domestic poultry (*Gallus gallus*) and pheasants (*Phasianus* spp.). Several strains have been recorded and identified in other bird species, such as turkeys, peafowls, teals, pigeons, geese, ducks, quails, and parrots. Additionally, reports have been made on IBV isolates in penguins and Guinea fowl (4).

IBV is distributed worldwide (5). Morbidity with the incidence of infection is almost always 100%, and the mortality rate is between 20 and 30 percent, whereas secondary infection might raise flock mortality (6). The disease affects chickens' eggs and meat type. Young, growing chickens are reported to have respiratory illnesses, although meat-type broilers have reduced weight gain and feed efficiency. Broiler chickens with IBV are at risk for subsequent bacterial infections, including pericarditis, perihepatitis, and airsacculitis. IBV replicates in the oviducts of layers and breeder chickens, resulting in prolonged damage or reduced egg production. The albumen becomes watery, and the damaged shell's color turns pale (7).

Effective vaccination against IB remains challenging due to the numerous currently co-circulating and continuously evolving genotypes and serotypes of IB, as well as the limited cross-protection that current vaccines provide (8). In order to rationally attenuate IBV and lessen reliance on the supply of embryonated hens' eggs for vaccine manufacture, specific alterations to the viral genome are being pursued. However, many IBV strains, particularly economically significant strains like 4/91(UK), have restricted cell tropism and cannot be multiplied in cell culture. The inability to examine different strains *in vitro* limits the development of an IBV vaccine and ultimately impacts the quick response to newly developing field strains (9).

The viral replication cycle includes attachment, penetration and uncoating, viral RNA transcription and translation, viral genomic RNA replication, and assembly and release (10). Therefore, drugs that are effective against IBV would exert their effects by acting on one of the viral replication cycle steps.

Ribavirin is a purine nucleoside analog antiviral agent (11). The drug inhibits the replication of many RNA and DNA viruses because it inhibits the enzyme inosine monophosphate dehydrogenase, which is necessary to make guanosine triphosphate, even though the exact mechanism of action

is unknown. Lethal RNA genome mutagenesis is the last step in this series of events (12). Ribavirin's broad-spectrum RNA virus suppression was anticipated to make it helpful in treating coronavirus infections. In several investigations, ribavirin has been found to have *in vitro* action against SARS-CoV (13). Based on the above literature review, we assumed that ribavirin would inhibit IBV in experimental infections of birds with IB. Accordingly, this study was designed to test the antiviral efficacy of ribavirin in broiler chickens, hoping that this antiviral may reduce the mortalities caused by IB in broilers and other domestic, exotic, and wild birds kept in captivity.

Materials and Methods

Study design

This study evaluated the antiviral effectiveness of ribavirin on experimentally infected broilers with avian infectious bronchitis. The experimental design included the detection of IBV from natural infection in broilers in Sulaimani province, propagation of the virus in embryonated chicken embryos (ECEs), experimental infection of broilers with infectious bronchitis virus, and testing of the antiviral activity of ribavirin. The study lasted from March until October 2022.

Virus identification

Suspected IB cases were collected at the Biolab Veterinary Clinic for Avian Diseases in Sulaymaniyah, Iraq. The suspected chicks were from two poultry farms and were later confirmed to have been infected with IB. Samples were collected from the kidneys, lungs, tracheas, and respiratory secretions of five chicks and pooled. Collected samples were used to detect the suspected virus with RT-PCR.

The viral RNA was extracted from infected tissues using the viral nucleic acid extraction kit manufactured by Bio-Tech Company (Korea), and the extraction procedure was done following the kit manufacturer's instructions.

The primers used in the detection of IBV were produced by Macrogen® (Korea). The primer sequences are illustrated in Table 1. A master mix was prepared to synthesize cDNA from each RNA of the viruses and amplify the target cDNA pieces in one step. It was done using AccuPower® RocketScript RT-PCR PreMix, manufactured by Bioneer Inc. As directed by the manufacturer, a 20 µL reaction volume master mix was prepared.

Table 1: Primers used to identify the infectious bronchitis virus.

Primer	Sequence	Gene fragment	Amplicon size	Reference
Forward	5`-GTT TAC TAC TAC CAA AGT GCC TT-3`	S1 gene	448bp	(14)
Reverse	5`-GTG TAA ACA AGG TCA CCA TTT A-3`			

The thermocycler was programmed to perform a pre-denaturation cycle at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension at 72°C for 5 minutes finished the PCR.

The analysis of the PCR product was done using the SafeBlue Illuminator/Electrophoresis System produced by Major Science®. With the well row on the cathode's side, the agarose gel was put in the electrophoresis tank. A 1X TBE buffer was poured into the electrophoresis tank until it completely covered the gel. 10 µL of PCR product was loaded into the agarose gel cast, and 10 µL of the ladder was loaded into the agarose gel cast. After that, the electrical current was run at 100 volts and 200 milliamperes for approximately 45 minutes. The agarose gel cast was illuminated by UV light, and SYNGENE Ingenius 3, with a digital camera, documented the DNA bands. The DNA bands were compared with the 100-bp DNA ladder, and the positive PCR products were compared with the positive controls and the DNA ladder.

Viral propagation in embryonated chicken eggs

The tissue samples of infected broilers with IBV were crushed and added separately to phosphate-buffered saline (PBS) that contained 250 µg/mL of amphotericin B, 10,000 µg/mL of streptomycin, and 10,000 IU/mL of penicillin. The mixture was refrigerated for about 48 hours and then centrifuged at 1000 rpm for 10 minutes. The supernatants were used as inoculum. Five nine-day-old embryonated chicken eggs were inoculated with 0.2 mL of each sample into their allantoic cavities (15).

The first group of non-embryonated eggs was collected from a local breeder flock to ensure they were not vaccinated. An egg incubator was used to incubate the eggs at 36.5°C–37.5°C with 30–33% humidity. The eggs were turned every two hours until nine days, the correct age at which they were ready for the first passage of viral inoculation. The eggs were examined daily under a flashlight to ensure that only viable embryos and the air chamber located at the blunt end of the egg were utilized (7).

The IBV was passed in embryonated chicken eggs thrice to ensure the virus's pathogenicity. RT-PCR was used each time to ensure the presence of IBV from harvested AF. The AF from the third viral passage was utilized to infect broiler chicks.

Sixteen birds (each seven days old) were brought and divided into four equal groups. When the birds were 14 days old, we infected them with a prepared virus intranasally. The first group was infected with a mixture of allantoic fluid (150 µL) and normal saline (50 µL), making a final volume of 200 µL. The second group was infected with 100 µL of IBV-containing allantoic fluid and 100 µL of normal saline. The third group was infected with a 200 µL mixture of normal saline (133 µL) and allantoic fluid (67 µL). Also, the fourth group was given a mixture of allantoic fluid (50 µL) and normal saline (150 µL). After another seven days, we did a PCR test for each group, receiving a sample from the tracheal mucus and lung to see the viral activity. The collected allantoic fluid, diluted three times, successfully infected the chicks with the IBV infection.

Preliminary study to determine the virus's infective dose:

Experimental infection of broiler chicks with IBV

Fifty-four non-vaccinated, one-day-old, apparently healthy chicks were brought from a local hatchery and divided into two main groups. The first group of 36 chicks was kept in an isolated room to prevent the spread of infection, and the birds were all experimentally infected with IBV through the nasal route. The second group of 18 chicks was kept in a separate place from the first group, as these birds were used as uninfected control groups. The chicks were numbered by attaching a piece of surgical silk plaster to the shank.

The 36 chicks were divided into four equal groups. Each group was kept in an area of 1.0 m^2 by partitioning the room via pieces of gypsum board. Moreover, the remaining 18 birds were divided into two groups and kept in a separate facility, but the husbandry measures were the same in both localities.

The chicks were fed with broiler feed, which contained 22% protein and 2,950 calories, till they became 17 days old. The feed was changed in its ingredient percentage with 19.5% of proteins and 3,050 calories till the last day of management.

The chicks from four groups were intranasally infected with IBV when they reached 17 days. The process of infecting the chicks was conducted as follows: The allantoic fluid of an infected egg with IBV, frozen at -80 °C, was thawed at room temperature and diluted with normal saline at a ratio of 1:2. Then, 200 µL of the solution was injected into the nasal cavity of each bird using an insulin syringe. Then, the chicks were examined twice daily for clinical signs of IB. The body temperature was measured using a thermometer.

Treatment with ribavirin started the same day the chicks were infected with IBV. Six experimental groups of chicks were used in the experiment, with nine birds per group, for a total of 54 chicks. All treatments were given twice a day and continued for seven days. The treatment groups were as follows: Group 1 (the positive control) was infected with IB intranasally without any drug; Groups 2 to 4 were infected with IB and orally administered 10.0, 20.0, and 30.0 mg/kg ribavirin, respectively; Group 5 (the negative control) was left without infection or treatment and served as control over the other groups; and Group 6 was not infected with IB but given 30.0 mg/kg ribavirin.

The human dose of ribavirin is 500 mg/70 kg twice daily. The dose can be extrapolated for the rabbit by multiplying the human dosage by 3.1, which equals 20 mg/kg (16). The metabolic rate of rabbits and chickens is close since they have relative body weight, body surface area, and resting heart rate (200–300 beats per minute). Hence, the human dose can be changed to chicken by multiplying the former by 3.1. However, since chickens belong to a different class of animal, we also included a higher (30 mg/kg) and a lower (10 mg/kg) dosage.

Observation of clinical signs, morbidity, and mortality

Clinical signs, morbidity, and mortality were observed and compared between infected and treated groups with positive and negative control groups. We compared all treated groups with both negative and positive control groups. Also, PCR was done to confirm infection by taking oral swabs from two birds of each infected and treated group with positive and negative control groups.

Postmortem examination

Suspected birds were subjected to postmortem inspection after eleven days of viral infection and selected drug treatment on the 28th day of their age. The chicks were killed by suffocation with surgical scissors, and their bodies were opened to grossly examine the internal organs, especially those affected by IBV, such as the trachea, kidneys, and pericardium in secondary bacterial infection with peritoneum. During the postmortem examination, the infected and treated groups were compared with both positive and negative control groups.

Histopathological examination

Samples were taken from the chicks' lungs and kidney organs for histopathological tests on the same day of the postmortem examination. We conducted a gross examination to identify any defective portions of the organs, such as hemorrhage, then cut the organs into five-millimeter-diameter pieces and placed them into plastic containers with a 10% phosphate-buffered formalin solution for fixation for approximately 48 hours. The organs were processed to prepare histological sections (17). The tissue was then sectioned with a microtome, advancing the tissue block toward a sharp knife. The tissue was moved up and down to be cut into 5 microns. Then, the slides were put into the oven for about 40 minutes at 60–70 °C. After that, they were stained with an automated slide stainer and covered with mounting media.

Statistical Analysis

Data were analyzed using a one-way analysis of variance (ANOVA), using Statistical Package for Social Sciences (SPSS version 24.0, by IBM, USA), followed by Duncan's post hoc. Differences between groups were considered significant at $p \leq 0.05$.

Results

Virus identification Suspected cases of IB were confirmed by visualizing the 448 bp amplicon of the S1 gene. As a result, four samples were confirmed to be infected with IBV, and one sample was not infected (Figure 1).

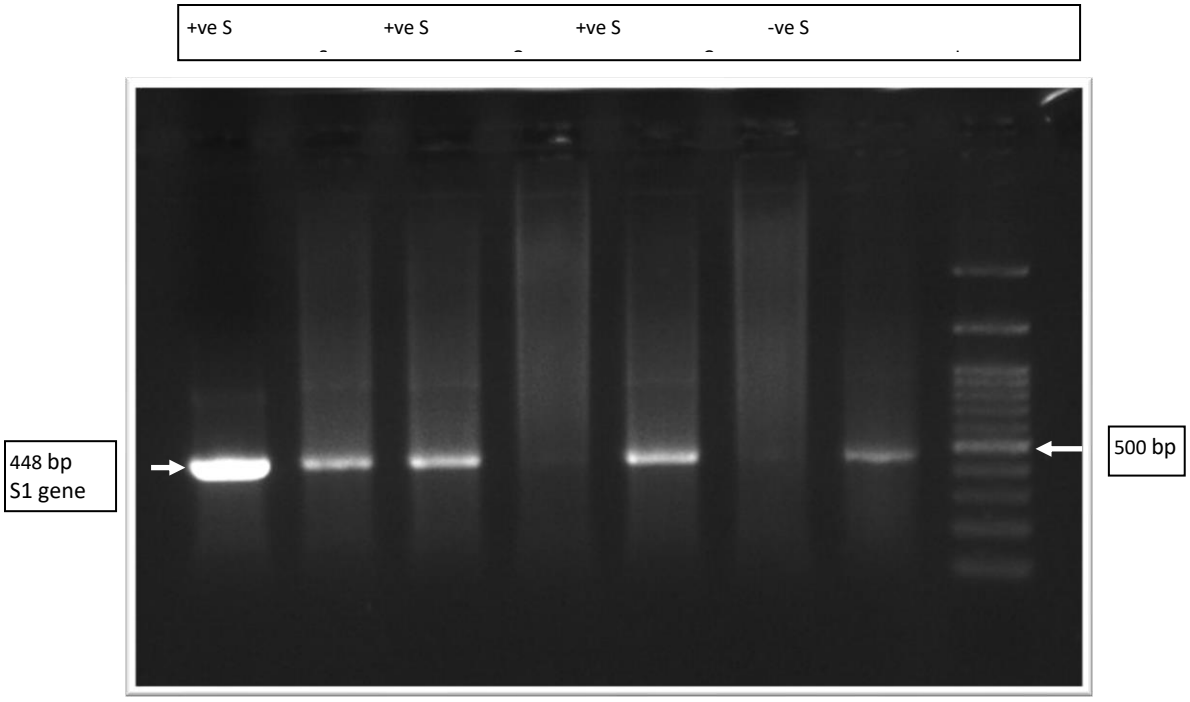

Figure 1. Visualization of the PCR products in samples infected with IB. +ve S = sample infected with IB; -ve S = sample not infected with IB; -ve C = negative control; +ve C = positive control; L = 100 bp ladder.

Viral infection of embryonated chicken eggs

Embryonic chicken eggs were successfully infected by injecting the allantoic cavity with prepared IBV virus, and RT-PCR tests confirmed the presence of the virus in the allantoic fluid in each passage. The virus was passed in ECEs three times to ensure the technique's success and to propagate the virus. At the end of each passage, signs of IBV infection were observed in the embryos, including dwarfism and hemorrhage on the neck, head, and other parts of the dead embryo.

Effect of ribavirin on IB infection

Clinical signs, morbidity, and mortality: The chicks' body weights increased steadily from day nine until 19 without significant differences between the groups. Also, their body temperatures were within the normal ranges until they reached 19 days, with no differences between the groups. The body weights and temperatures in the groups from day 19 to 31 are shown in Tables 4 and 5, respectively.

After six days of infection, clinical signs were evident in groups 1 (positive control) and 2 (ribavirin 10 mg/kg) and less evident

in the other groups. Respiratory signs were detected in the positive control, such as sneezing, coughing, nasal discharge, tracheal rales, gasping between times with watery eyes, depression, watery droppings, ruffled feathers, and increased water intake. Also, clinical signs were evident in groups 3 (20 mg/kg) and 4 (30 mg/kg), with less depression, watery eyes, and tracheal rales. The birds also did not feed well and had a non-normal water intake. RT-PCR ensured that the IBV virus was present in all of the infected chicks. So, morbidity was 100%, and no mortality was seen until then.

All clinical signs were apparent in most of the birds of group 2 (ribavirin 10 mg/kg), with loss of appetite and severe depression. Also, head swelling was present in 10% of the birds. However, clinical signs were evident in groups 3 (20 mg/kg) and 4 (30 mg/kg), with less depression, watery eyes, and tracheal rales (Table 4.2). The birds also had poor feeding and non-normal water intake (Tables 4.3 and 4.4). RT-PCR ensured the IBV virus's presence in all infected chicks. So, morbidity was 100%, and no mortality was seen until then.

Table 2. Clinical Signs, morbidity, and mortality of chicks after experimental infection with IBV.

Group1 **Positive control**	**group 2** **(ribavirin 10 mg/kg)**	**Groups 3 and 4** **(20 mg/kg and 30 mg/kg)**
Respiratory signs (coughing, sneezing, nasal discharge, tracheal rales, gasping between times with watery eyes)	Respiratory signs (coughing, sneezing, nasal discharge, tracheal rales, gasping between times with watery eyes) with 10% head swelling	Lesser respiratory signs with less watery eyes and tracheal rales.
depression and ruffled feather	Severe depression and ruffled feather	Less depression
increased water intake and loss of appetite	increased water intake and loss of appetite	high water intake and not low feeding
watery dropping	watery dropping	Watery dropping
Morbidity=100%	Morbidity=100%	Morbidity 100%
No mortality	No mortality	No mortality

Table 3. Weight of chicks from days 20 to 31.

Gp.	Age (days) 20	21	22	23	24	25	26	27	28	29	30	31
1	528.6^{a} ± 2.9	570.9^{a} ± 4.2	598.4^{a} ± 4.4	659.9^{a} ± 2.9	698.8^{a} ± 4.8	739.1^{a} ± 5.0	789.7^{a} ± 4.2	855.6^{a}± 4.6	914.6^{a} 5.4	± 978.3^{a} ±5.4	1044.7^{a} ±5.1	1111.6^{a} ±5.6
2	536.3^{ab} ± 3.5	583.4^{ab} ± 0.9	630.0^{b} ± 1.5	688.8^{b} ± 2.2	754.8^{bc} ± 2.7	817.6^{b} ± 2.8	879.8^{b} ± 4.2	954.2^{b} ± 4.6	1014.6^{b} ± 5.3	1087.0^{bc} ± 6.0	1160.9^{bc} ± 5.2	1237.6^{bc} ± 4.5
3	530.7^{a} ± 3.2	584.3^{ab} ± 2.4	620.4^{b} ± 2.9	681.6^{b} ± 3.5	745.0^{b} ± 3.7	806.0^{b} ± 4.1	871.3^{b} ± 4.6	937.4^{b} ± 5.3	1010.0^{b} ± 6.1	1070.1^{b} ± 7.4	1142.7^{b} ± 8.7	1230.3^{bc} ± 13.7
4	530.9^{a} ± 2.6	582.0^{ab} ± 2.9	622.3^{b} ± 3.0	678.2^{b} ± 3.3	743.7^{b} ± 4.9	803.0^{b} ± 6.9	865.8^{b} ± 8.9	932.0^{b} ± 8.2	1009.6^{b} ± 10.2	1067.4^{b} ± 9.8	1138.3^{b} ± 10.6	1217.6^{b} ± 10.7
5	555.5^{c} ± 5.8	613.6^{c} ± 7.7	660.1^{c} ± 6.8	717.6^{c} ± 6.3	778.6^{c} ± 5.6	848.3^{c} ± 5.8	924.1^{d} ± 7.0	1012.5^{d} ± 7.3	1086.3^{c} ± 7.2	1158.8^{d} ± 8.4	1230.5^{d} ± 7.6	1313.5^{d} ± 8.1
6	540.0^{b} ± 0.9	590.8^{b} ± 2.2	646.0^{c} ± 5.4	688.2^{b} ± 5.4	750.2^{b} ± 5.5	817.8^{b} ± 3.9	888.8^{c} ± 5.1	959.2^{c} ± 6.5	1024.1^{bc} ± 6.8	1096.2^{c} ± 6.7	1169.8^{c} ± 7.9	1247.6^{c} ± 8.4

Values represent means ± SEM (9 chicks/group). Different superscripts denote significant differences within the column ($p \leq 0.05$). Test = one-way ANOVA (post hoc = Duncan).

Table 4. Body temperature of chicks from day 20 to 31.

Gp.	Age (days) 20	21	22	23	24	25	26	27	28	29	30	31
1	40.5^{b} ± 0.1	40.8^{b} ± 0.1	41.2^{b} ± 0.1	41.5^{b} ± 0.1	41.8^{b} ± 0.1	42.2^{c} ± 0.1	42.4^{b} ± 0.1	42.8^{c} ± 0.1	42.9^{b} ± 0.1	42.8^{b} ± 0.1	42.7^{c} ± 0.1	41.6^{b} ± 0.1
2	40.5^{b} ± 0.1	40.8^{b} ± 0.1	41.1^{b} ± 0.1	41.5^{b} ± 0.1	41.7^{b} ± 0.1	42.0^{b} ± 0.1	42.3^{b} ± 0.1	42.6^{b} ± 0.1	42.8^{b} ± 0.1	42.8^{b} ± 0.1	42.5^{b} ± 0.1	41.7^{b} ± 0.1
3	40.5^{b} ± 0.1	40.8^{b} ± 0.1	41.1^{b} ± 0.1	41.5^{b} ± 0.1	41.7^{b} ± 0.1	42.0^{b} ± 0.1	42.3^{b} ± 0.1	42.6^{b} ± 0.1	42.8^{b} ± 0.1	42.8^{b} ± 0.1	42.4^{b} ± 0.1	41.7^{b} ± 0.1
4	40.5^{b} ± 0.1	40.8^{b} ± 0.1	41.2^{b} ± 0.1	41.5^{b} ± 0.1	41.7^{b} ± 0.1	42.0^{b} ± 0.1	42.3^{b} ± 0.1	42.6^{b} ± 0.1	42.9^{b} ± 0.1	42.8^{b} ± 0.1	42.4^{b} ± 0.1	41.7^{b} ± 0.1
5	40.3^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.6^{a} ± 0.1	40.6^{a} ± 0.1
6	40.4^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.6^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.6^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1	40.5^{a} ± 0.1

Values represent means ± SEM (9 chicks/group). Different superscripts denote significant differences within the column ($p \leq 0.05$). Test = one-way ANOVA (post hoc = Duncan).

Postmortem signs

A small amount of mucous exudate in the trachea with redness, urate deposition, and pericarditis was observed in the positive control birds. There was pericarditis, a large amount of tracheal mucous exudate, tracheal redness, and urate deposition in one of the postmortem bodies of group 2. The birds in groups 3 and 4 had only tracheal mucous exudate. A copious amount of mucous exudate was present in the trachea, with redness, urate deposition, and pericarditis in the positive control group. One bird in group 2 had pericarditis, a large amount of tracheal mucous exudate, tracheal redness, and urate deposition, while the other birds only had tracheal mucous exudate and redness. Birds in groups 3 and 4 had only tracheal mucous exudate.

Histopathological examination

Lung sections of the negative control sample without treatment showed secondary bronchus, parabronchi, atria, air capillaries, and interstitial blood vessels (Figure 2-A). The interstitial blood vessels were congested (blue arrows) with a few numbers of extravasated RBCs (hemorrhage) within the parabronchus (green arrows), some of the atria (red arrows) and air capillaries (black arrows) in the sample treated with 30 mg/kg ribavirin without infection (Figure 2-B).

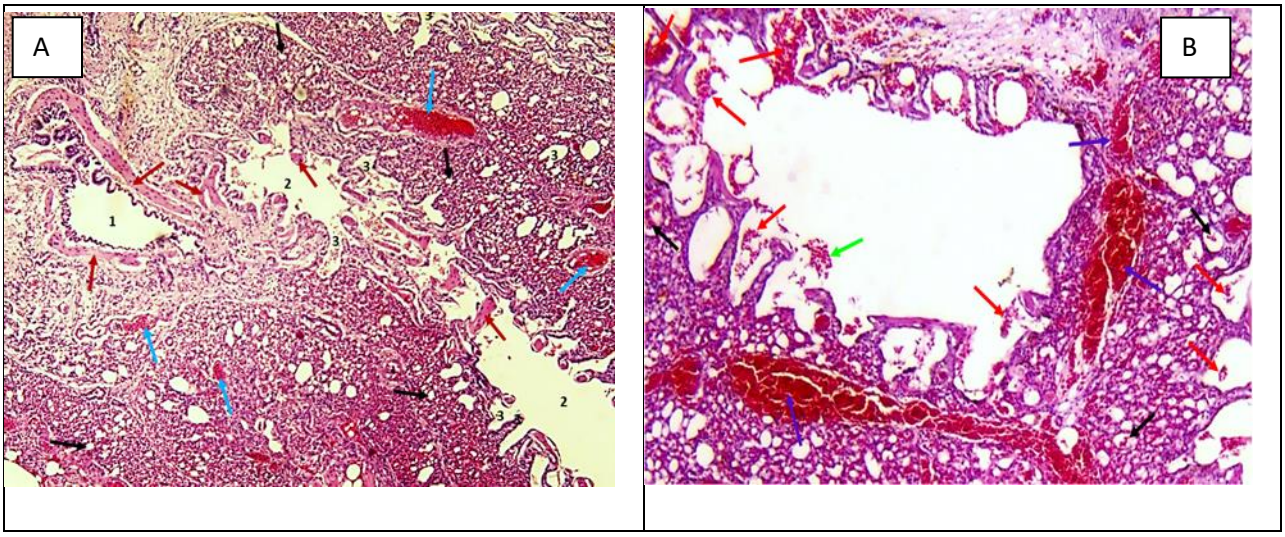

Figure 2. A: Lung section of a negative control sample without treatment showing secondary bronchus (1), parabronchi (2), atria (3), air capillaries (black arrows), and interstitial blood vessels (blue arrows). B: A negative control sample treated with 30 mg/kg ribavirin, showing congestion of interstitial blood vessels (blue arrows) and a few numbers of extravasated RBCs (hemorrhage) within the parabronchus (green arrows), some of the atria (red arrows) and air capillaries (black arrows).

In the lung section of an infected chick treated with 10 mg/kg ribavirin, there were extensive hemorrhages in the parabronchial and atrial lumens, focal epithelial sloughing, and foamy macrophage infiltration in the parabronchial walls (black arrows) (Figure 3-A). Microscopic examination of lung sections from infected chicks treated with 20 mg/kg ribavirin revealed congested interstitial blood vessels and blood capillaries, perivascular and peri-parabronchial hemorrhages, and a few extravasated RBCs in some air capillaries (Figure 3-B). Infected chicks treated with 30 mg/kg ribavirin exhibited moderate numbers of extravasated RBCs in the parabronchial and atrial lumens, as well as some of the air capillaries associated with interstitial blood vessel congestion and blood capillaries (Figure 3-C). Also, there were large blood clots in the secondary bronchial lumen, multiple layers of epithelial sloughing, subepithelial bleeding, and a buildup of mononuclear inflammatory cells, mostly lymphocytes, in the lungs of the positive control chicks (Figure 3-D).

Figure 4: The kidneys of the negative control chicks that were not given any medicine had normal renal corpuscles, Bowman's spaces (BS), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), glomerular blood capillaries, podocytes, and mesangial cells. There was interstitial blood vessel congestion, a slight widening of Bowman's spaces, eosinophilic protein droplets in the lumens of some renal tubules, and cytoplasmic vacuolation associated with nuclear pyknosis in some of the tubular lining epithelial cells in the kidneys of negative control chicks treated with 30 mg/kg ribavirin (Figure 4-B).

Infected chicks that were given 10 mg/kg ribavirin showed tubular epithelial sloughing, vacuolation, interstitial hemorrhages, and eosinophilic protein droplets in the lumens of the tubes (Figure 5-A). In infected chicks treated with 20 mg/kg ribavirin, tubular atrophy was caused by ongoing tubular necrosis with cytoplasmic vacuolation of the tubular epithelium, interstitial hemorrhages, and infiltration of foamy macrophages (Figure 5-B). Infected chicks treated with 30 mg/kg ribavirin also had focal tubular atrophy surrounded by ongoing tubular necrosis, cytoplasmic vacuolation of the tubular epithelium, and interstitial hemorrhages (Figure 5-C). In contrast, the positive control chicks' kidneys showed a large, focal area of hemorrhagic necrosis in which only a few remnants of the renal parenchyma are left. Mononuclear inflammatory cells, mainly macrophages, and large numbers of RBCs are seen within the necrotic area. Interstitial hemorrhages, fibrosis, and lymphocytic infiltration are also seen surrounding the necrotic area. (Figure 5-D).

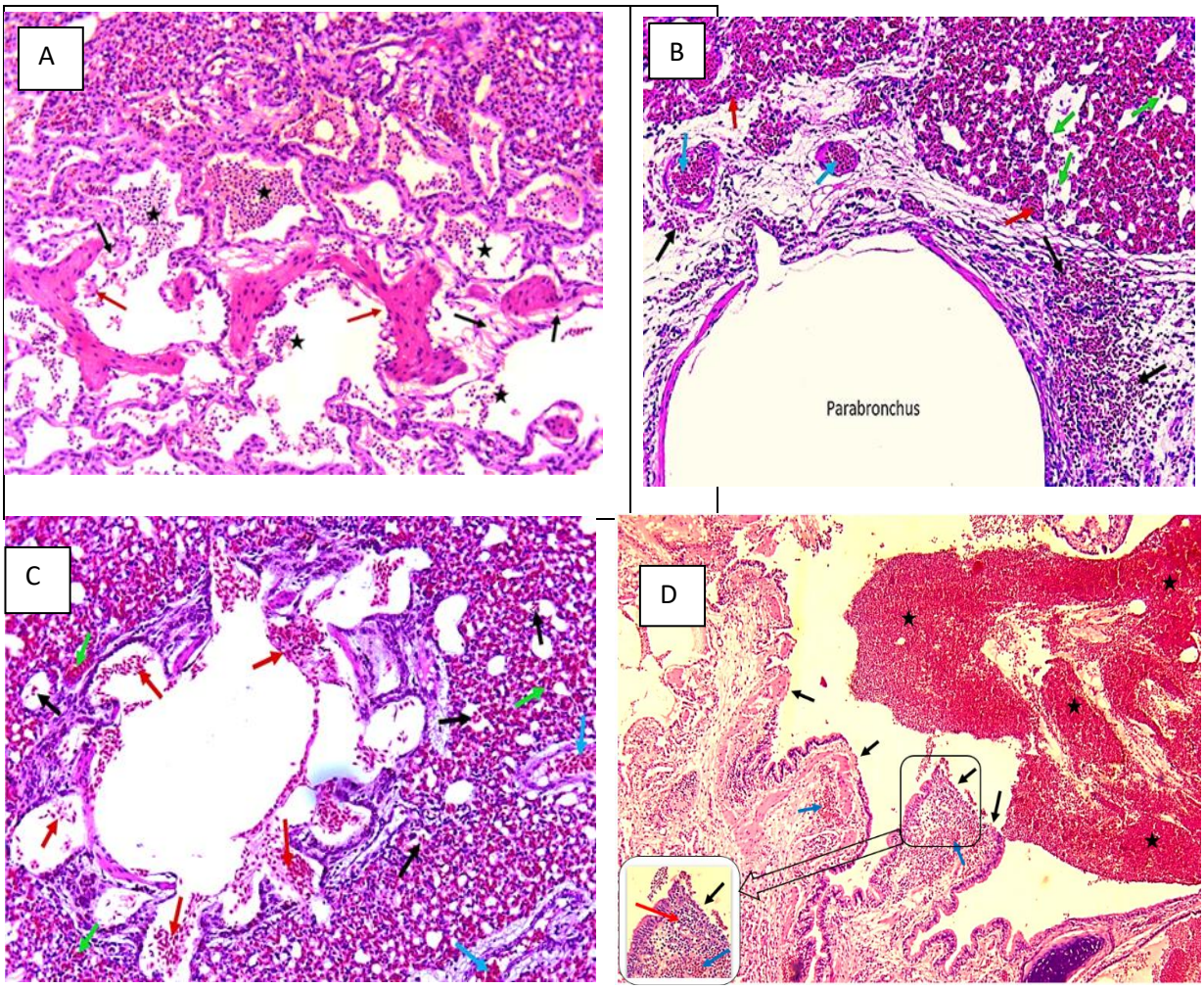

Figure 3. A: A lung section of an infected chick treated with 10 mg/kg ribavirin shows extensive hemorrhages in the parabronchial and atrial lumens (black stars), focal epithelial sloughing (red arrows), and infiltration of foamy macrophages in the parabronchial walls (black arrows). B: A lung section of an infected chick treated with 20 mg/kg ribavirin, interstitial blood vessels (blue arrows), and blood capillaries (red arrow) were congested, and there were perivascular and peri-parabronchial hemorrhages (black arrows), and a few extravasated RBCs in some air capillaries (green arrows). C: A lung section of an infected chick treated daily with 30 mg/kg ribavirin showed moderate numbers of extravasated RBCs in the parabronchial and atrial lumens (red arrows) and in some of the air capillaries (black arrows) associated with congestion of the interstitial blood vessels (blue arrows) and blood capillaries (green arrows). D: A lung section of a chick from the positive control group shows extensive hemorrhages in the secondary bronchial lumen (black stars) associated with multifocal epithelial sloughing (black arrows), subepithelial hemorrhages (blue arrows), and infiltration of mononuclear inflammatory cells, mainly lymphocytes (red arrows).

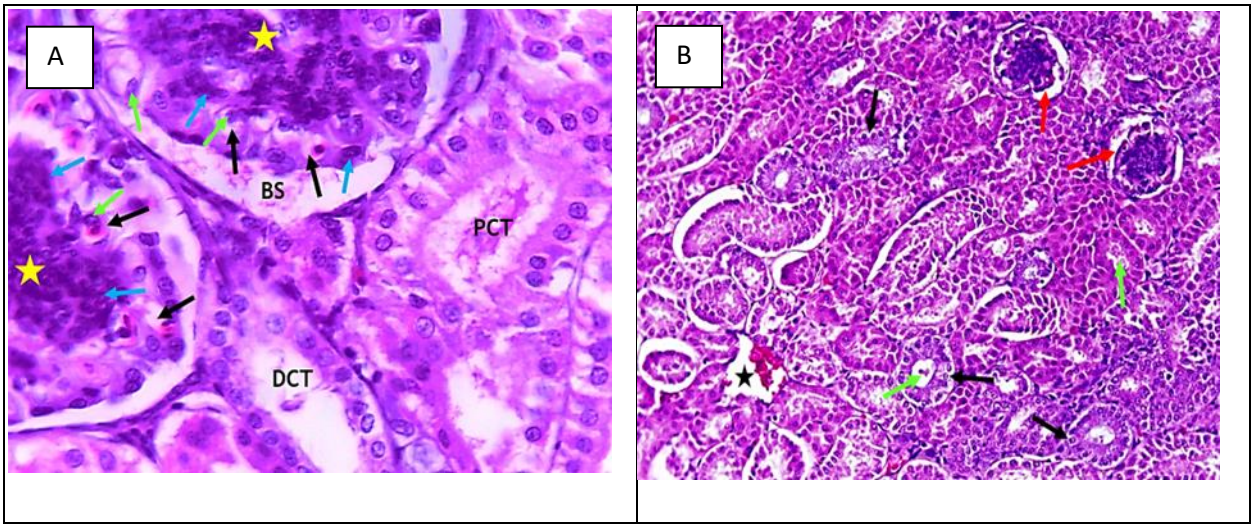

Figure 4. A: The kidney of a negative control chick without treatment shows normal renal corpuscles (yellow stars), Bowman's spaces (BS), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), glomerular blood capillary (black arrows), podocytes (green arrows), and mesangial cells (blue arrows). B: The kidney of a chick treated with 30 mg/kg ribavirin without infection shows congestion of an interstitial blood vessel (black star), slight widenings of Bowman's spaces (red arrows), presence of eosinophilic protein droplets in lumens of some renal tubules (green arrows) and cytoplasmic vacuolation associated with nuclear pyknosis in some of the tubular lining epithelial cells (black arrows).

Discussion

IBV infections cannot be effectively treated with medication, and because there is insufficient cross-protection between various serotypes and genotypes, vaccination is mostly inefficient (18). Hence, this study tested the antiviral activity of ribavirin against IBV infection *in vivo*.

In this study, the chicks were infected when they reached 17 days of age since the severity of disease and mortality are more significant in young chickens compared to adults (19). PCR was done to confirm infection by taking oral swabs from several birds of each infected and treated group with positive and negative control groups. The ribavirin treatment began on the same day as the IBV infection in the chicks. This method is still considered a treatment method since the antiviral agent inhibits the final step of viral assembly and liberation out of the infected host cell (20, 21).

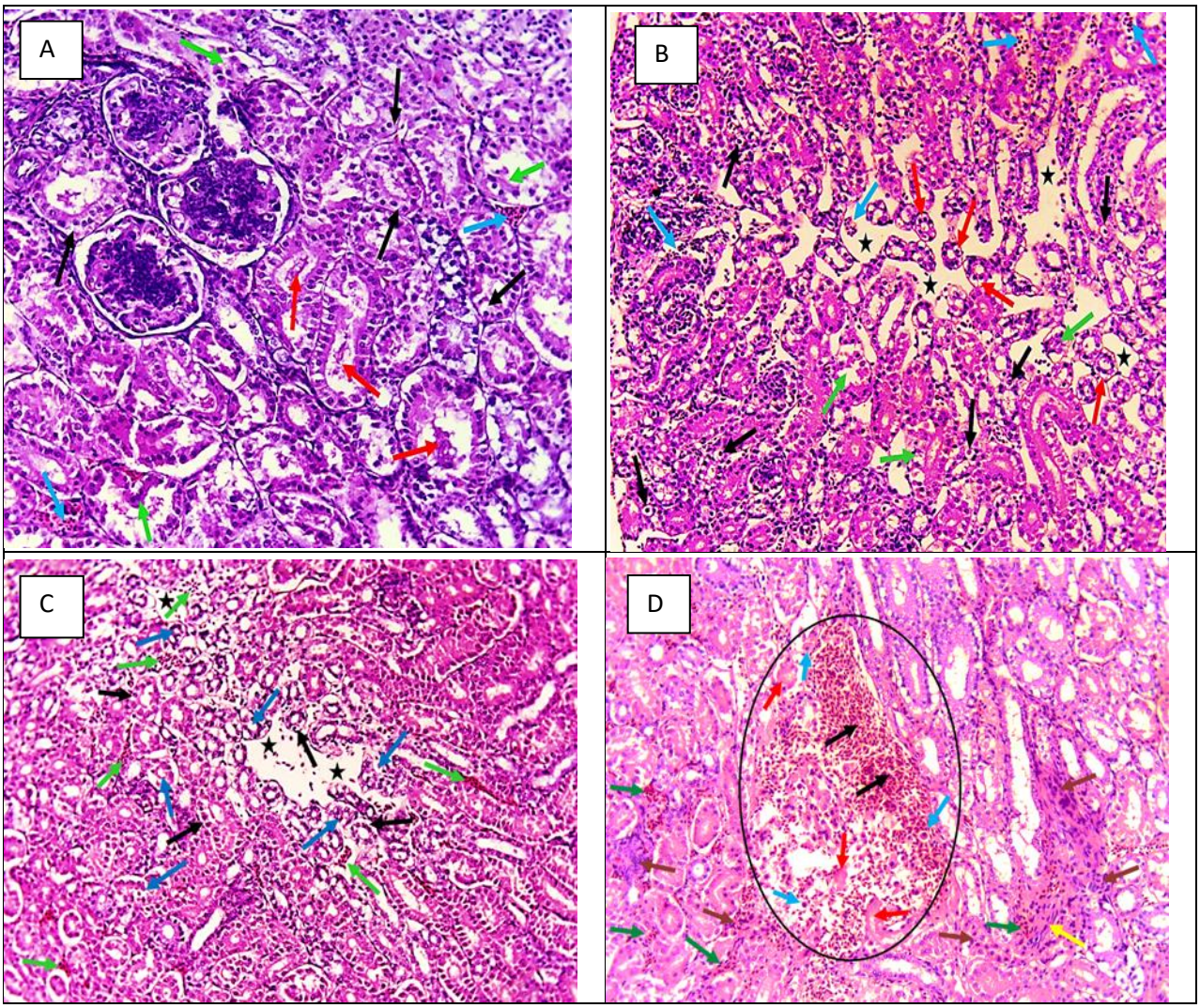

Figure 5. A: The kidney of an infected chick treated with 10 mg/kg ribavirin shows tubular epithelial sloughing (green arrows), vacuolation (black arrows), interstitial hemorrhages (blue arrows), and the presence of eosinophilic protein droplets in tubular lumens (red arrows). B: The kidney of an infected chick treated with 20 mg/kg ribavirin shows tubular atrophy (black stars) due to ongoing tubular necrosis (green arrows) with cytoplasmic vacuolation of the tubular epithelium (red arrows), interstitial hemorrhages (blue arrows), and infiltration of foamy macrophages (black arrows). C: The kidney of an infected chick treated with 30 mg/kg ribavirin shows focal tubular atrophy (black stars) surrounded by ongoing tubular necrosis (blue arrows), cytoplasmic vacuolation of the tubular epithelium (black arrows), and interstitial hemorrhages (green arrows) D: The kidney of a positive control chick shows a large, focal area of hemorrhagic necrosis (the black circle-surrounded area) in which only a few remnants of the renal parenchyma are left (red arrows). Mononuclear inflammatory cells, mainly macrophages (blue arrows), and large numbers of RBCs (black arrows) are seen within the necrotic area. Interstitial hemorrhages (green arrows), fibrosis (yellow arrows), and lymphocytic infiltration (brown arrows) are also seen surrounding the necrotic area.

Infectious bronchitis causes a decline in weight gain (22). The experimental data showed that groups of 2 to 4 were infected and treated with 10.0, 20.0, and 30.0 mg/kg ribavirin, respectively, showing decreasing body weight. Infection with IBV causes an increase in body temperature (23). Experimental data for chicken body temperature in our study from the 19th day shows that the body temperature of groups of 2 to 4 increased day by day, the same as the positive control group.

Another characteristic of IB and other viral infections is that they lead to decreased feed consumption (24). In this study, feed consumption decreased in the infected groups that were treated with different dosages of ribavirin compared to the negative control groups. Also, broiler water consumption increases in IB (25). The experimental data showed that water consumption of groups 2 to 4 (treated with ribavirin) increased, which agrees with previously reported data about the disease.Postmortem signs of infected chickens with IBV primarily include the presence of a copious amount of mucous exudate in the trachea, with redness (26). This organ is the most affected, represented by inflammatory secretions with apparent congestion that may reach the stage of hemorrhage, urate deposition with swelling of the kidneys, and fibrin accumulation around the heart (27). In this study, we detected all IB signs in the internal organs. The occurrence of postmortem lesions in the positive control chicks further supported the idea that the chicks were successfully infected with IBV.

qRT-PCR assays were developed to aid in the accurate and rapid diagnosis of IBV in the field, which could be performed on clinical samples and would rapidly identify particular IBV types. The wide dynamic range, high sequence specificity, high sensitivity, short run times, and functional simplicity of real-time RT-PCR have made it one of the most widely used techniques for gene quantitation. Real-time RT-PCR is useful for identifying the viral agents causing infectious diseases (28). In this study, based on RT-PCR methods for detecting IBV, it was shown that ribavirin was not effective on the detected virus. The RT-PCR test further confirmed the observation of clinical signs, postmortem examination, and histopathological examination between all control groups (infected and noninfected) and treated groups with different doses of ribavirin per single chick's weight.

By infecting blood monocytes and tracheal macrophages, deep respiratory infections, and potentially ascending viral infection from the cloaca, IBV spreads systemically throughout the body. Nephrotropic IBV results in severe kidney disease that includes renal failure, inflammation, and tubular epithelial cell necrosis (29). In this study, lung and kidney tissues of infected and treated chicken with ribavirin showed lesions similar to those of the positive control group. Overall, the experimental data of the RT-PCR test, clinical signs, postmortem examination, and histopathological lesions all led to the conclusion that ribavirin has no significant effect on the virus itself, but it reduces

clinical signs. Ribavirin is a guanosine analog that inhibits viral mRNA capping and metabolism, it inhibits viral growth. It is an monophosphate dehydrogenase. (30). Allergic reactions, RBC breakdown, and liver damage are some complications of ribavirin (31). So, perhaps those complications were reasons that led to weight loss, severe depression, increased temperature, and increasing clinical signs of the disease. Also, human body metabolism completely differs from avian body metabolism (32), which might be another cause of ribavirin's ineffectiveness against IBV. Due to ribavirin's broad-spectrum inhibition of RNA viruses, it was believed that it could be useful in treating human coronavirus infection. Ribavirin has been demonstrated in numerous studies to have beneficial activity against SARS-CoV *in vitro* (33). However, other research has shown that ribavirin did not inhibit the virus in vivo or aid in the recovery of patients with SARS-CoV infection (34).

Conclusion

According to experimental data, RT-PCR, clinical signs, postmortem, and histopathological examinations, this study concluded that ribavirin was not effective against IBV. However, it can reduce the clinical signs of some of the chickens in groups treated with different doses of this drug.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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تأثير الريبافيرين على التهاب الشعب الهوائية المعدي المحدث تجريبيا في فروج اللحم

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الخلاصة

يسبب فيروس التهاب الشعب الهوائية المعدي (IBV) التهاب الشعب الهوائية المعدي(IB) ، وهو مرض شديد العدوى يؤثر على الجهاز التنفسي والإنجابي والكلى في الدجاج. لا يوجد دواء معتمد، وغالبًا ما يفشل التطعيم في الحماية بسبب ارتفاع معدلات الطفرات وإعادة التركيب. قيمت هذه الدراسة تأثير الريبافيرين على IB التجريبي لفروج اللحم. تم التأكد من إصابة دجاج التسمين بالـ IB باستخدام تفاعل البوليميراز المتسلسل بالنسخ العكسي .(RT-PCR) تم تمرير فيروس IBV في بيض الدجاج المضغي ثلاث مرات لنشر الفيروس. تم استخدام السائل السقاء (AF) من التمرير الفيروسي الثالث لإصابة كتاكيت الدجاج اللاحم. تم استخدام أربعة وخمسين كتكوتًا غير محصنة، عمر كل منها 17 يومًا، لتقييم التأثير المضاد للفيروسات للريبافيرين. تم تقسيم الكتاكيت إلى مجموعتين مصابتين (36 كتكوت) وغير مصابة (18 كتكوت). تم تقسيم الكتاكيت الـ 36 إلى أربع مجموعات متساوية، بينما تم تقسيم الطيور الـ 18 المتبقية إلى مجموعتين وتم حفظها في منشأة منفصلة. عندما بلغ عمر الطيور 17 يومًا، أصيب 36 طائرًا بفيروس IBV عن طريق إعطاء 0.2 مل من AF عن طريق الأنف. بدأ العلاج بالريبافيرين في نفس اليوم الذي أصيبت فيه الكتاكيت بفيروس IBV وتكرر مرتين يوميًا لمدة سبعة أيام. وتم أخذ عينات مسحة من القصبة الهوائية من الطيور في اليوم الخامس من الإصابة للتأكد من وجود فيروس IBV باستخدام تقنية PCR، وكانت النتيجة إيجابية لجميع الطيور، ولكن لم يتم ملاحظة أي نفوق. أظهرت بياناتنا التجريبية لنتائج اختبارPCR ، ووزن جسم الكتاكيت، ودرجة الحرارة، والتغذية، واستهلاك الماء، والعلامات السريرية، وفحوصات ما بعد الوفاة، والفحوصات النسيجية أن الريبافيرين غير فعال على فيروس.IBV .

الكلمات الدالة: مرض، فايروس، PCR، الريبافيرين.