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The Effect of Infectious Bronchitis Virus In Embryonated Chicken Eggs

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ABSTRACT

Specimens were collected from different farms in Iraq, then collected samples from (Baghdad, Babylon, Diyala, Wasit and Sallahulldin) were prepared by mortar and pestle and were inoculated into chicken embryos at age (9-11) days by allantoic sac inoculation. The death of embryos after 24 hr. post inoculation was ignored. There was no death of embryos on the first passage of the virus into chicken embryos and the allantoic fluid was harvested after 96 hours post inoculation. On second passage, one embryo was died and the allantoic fluid was harvested after 96 hr. post inoculation. On third passage, two embryos were died post inoculation and the allantoic fluid was harvested after 96 hr. The infected allantoic fluid was examined by RT-PCR to detect IBV. The result of RT-PCR was positive to detect IBV in infected harvested allantoic fluid.

Keywords: *Infectious bronchitis virus, embryonated chicken eggs, RT-PCR, allantoic sac*

INTRODUCTION

The avian infectious bronchitis virus, also known as IBV, is one of the most dangerous viruses that can infect chickens. Because of this virus, the chicken business in many parts of the globe faces a challenge (1, 2). It can infect all species of chicken (3). Since 1931, when it was first seen in the United States, it has been widespread (4). Since 1989, cases of illness have been recorded in Iraq (5). IBV is pleomorphic in shape, and in accordance with the Gamma coronavirus species and the Coronaviridae family, this illness is classified as belonging to the group Nidovirales (6, 7). IBV's genome is made up of a single strand of positive-sense RNA that is about 27.6 kilobases long and codes for four different proteins. These proteins are known as the spike glycoprotein (S), the membrane glycoprotein (M), the envelope protein (E), and the nucleocapsid protein (N) (8, 9).This virus causes a very contagious, severe form of the illness. Death rates range between 20% and 30%, while morbidity may exceed 100%. Secondary infections may increase flock mortality (10). The virus enters via inhalation and mostly multiplies in the respiratory organs in the ciliated epithelium and mucus secretion cells, which leads to breathing signs such as tracheal rales and respiratory symptoms, including wheezing, coughing, sneezing, and nasal secretions (11). Infected laying hens have been discovered to be suffering from respiratory problems and a drop in egg production as result of the infection (12). Along with implementation of vaccination plan for IBV, a significant number of flock that was impact shows respiratory signs of late-onset respiratory disease and increase in the airsaculities condemnation that was induced by IBV (13). Despite the fact that collective vaccination against infectious bronchitis has been practiced throughout the whole of the commercial chicken sector

outbreaks of this illness have continued to occur up until the present day (14). Infectious bronchitis virus infections can also be identified by the identification of viral RNA by RT-PCR, as a result of which the diagnosis can be obtained quickly and reliably (15). The goal of this study is to detect any pathological changes in chicken embryos via allantoic sac inoculation and confirm with real-time RT-PCR.

METHODOLOGY

Materials

Devices

The device and apparatus that were used in the collection and preparation of samples and virus isolation are shown in Table 2-1.

TABLE 2-1; the devices that used in virus isolation

No.	Equipment	Brand	Origin
1	Disposable test tube 10 ml	/	Local Markets
2	Cooling centrifuge	KUBOTA	Japan
3	Refrigerator	Hitachi	Japan
4	Incubator	Biobase	China
5	Disposable sterile insulin syringe	/	Local Markets
6	Biosafety cabinet II	Biobase	China
7	Bunsen burner	Amal	Turkey
8	Eggs candling light	/	Local Markets
9	Autoclave	/	

Collection of Samples

Fifty samples were collected from different farms in Iraq (Baghdad, Babylon, Wasit, Salahalhuddin and Diyala).

Fertilizing Eggs

The eggs, which were between 9 and 11 days old, were obtained from the Al-Taji hatchery in Baghdad and from the Al-Anwar hatchery in Babylon and were used for isolation of IBV.

Antibiotics

Antibiotic mix: Streptomycin, Crystalline penicillin at 10 mg/ml and 10,000 U/ml concentration.

METHODS

Preparation of samples

Tissues specimens from each flock were ground in an autoclave pestle and mortar with sterile phosphate buffer saline, and the tissues were cut into small pieces after that crushing the samples.

Virus isolation from embryonated chicken eggs

Tissue samples from each flock were ground with sterile phosphate buffered saline pH (7-7.3) in an autoclaved pestle and mortar. The organ suspension was first centrifuged at 3500 rpm for (5-10) min. at 4°C, after which the floating was aggregated. Antibiotics (streptomycin and crystalline penicillin at 10 mg/ml and 10,000 u/ml concentrations, respectively) were added. The age of embryos was determined at 9–11 days old, and a good injection site was chosen on the egg shell where no essential blood vessels were present and immediately sterilized with 70% ethanol. In addition, an insulin syringe was utilized in order to inject 0.2 ul of the supernatant into the allantoic sac. Where a negative control was inoculated with 0.2 ml of PBS. The puncture hole in the egg was sealed with molten paraffin or colored nails, so the eggs were incubated at 37°C with examination by candling light every day to check viability. Nonspecific symptoms were ignored the first day after inoculation, but deaths observed between 48 and 96 hours were investigated. The allantoic fluid from the death embryo was extracted with a sterile syringe after 4 hours of chilling at 4 °C in a refrigerator,

centrifuged at 3500 rpm for (5-10) min. to exclude mixed blood and tissue debris, and then stored at 20°C till next use.

Real Time RT-PCR to detect isolated IBV into inoculated in chicken embryos

To confirm IBV RT-PCR was used, was performed using RT-PCR, the harvested allantoic fluid from infected chicken embryos was collected, reverse transcribed, and amplified of to detect IBV.

RESULTS

Result of virus isolation from embryonated chicken eggs

The virus was isolated from chicken embryos by inoculation into the allantoic sac. After three passages, it recorded the death of the embryo and pathological changes like dwarfism and congestion. As shown in Figure (3-1) and table (3-1):

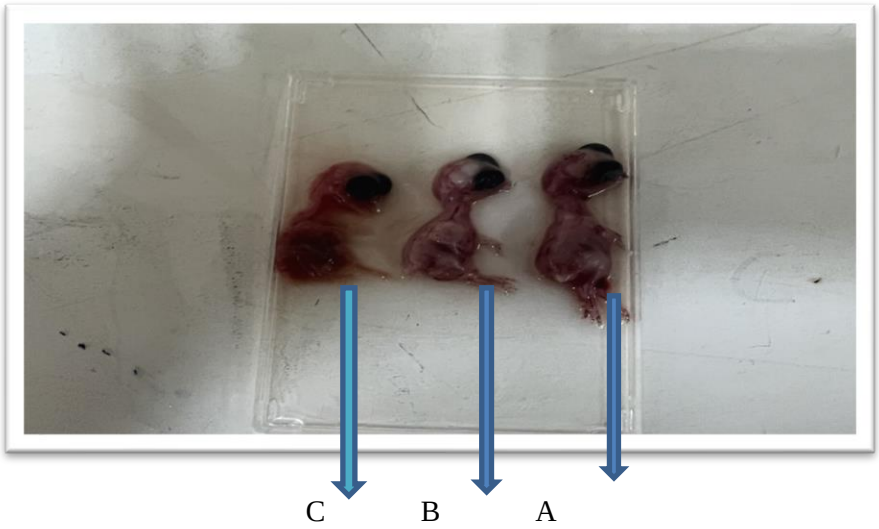

FIGURE 3-1: The result of eggs inoculation by IBV as the following:

(A) Is control negative (non- infected embryo).
(B) Infected embryo in second passage after 96 hr.
(C) Third passage after 96 hr. Dwarfism and
Congestion in compare with control.

TABLE 3-1: Number of passages, time of death during incubation period after virus inoculation into chicken embryos.

NO. of passages	Death after One day	Death after Two days	Death after Three days	Death after Four days	All
1	-	-	-	-	-
2	-	-	-	1	1
3	-	-	1	1	2
Total	-	-	1	2	3

Detection of an isolated virus in chicken embryos by using real-time RT-PCR

Allantoic fluid was extracted from virus-inoculated embryonated eggs, and viral RNA was extracted and amplified using real-time RT-PCR, with the results shown in table (3-2) and figure (3-2);

TABLE 3-2: Ct. value of detected isolated IB virus from chicken embryo by RT-PCR

NO. of isolated samples	Ct.
1	13.9
2	24.6

FIGURE 3-2: The plot of real-time RT-PCR amplification specific for IBV detection of isolated virus in chicken embryos.

DISCUSSION

The coronavirus causes avian infectious bronchitis (IB), a severe and highly contagious disease of poultry that can cause complex respiratory problems and is among the most wide spread poultry disease (16).The result is characterized by pathological lesions such as dwarfism and congestion in comparison with the control group. Positive RT-PCR samples were inoculated into chicken embryos for virus isolation; no embryos died in the first passage. In the second passage, one embryo died after 4 days post-inoculation. While, in the third passage, two embryos were checked for any pathological change in the embryo, positive specimens by RT-PCR were incubated with embryonated chicken eggs for IBV at 9–11 days old, and allantoic fluid was harvested five days post infection. Serial passage was used in embryonated chicken eggs to increase virus titer (17). The first and second passages showed no pathological change, but the third passage showed the macroscopic changes due to virus replication that caused congestion in the body and head with dwarfism. This result is in agreement with (18). Embryonated chicken eggs were initially used as a host system for the infectious bronchitis virus. The allantoic route is primarily used because these viruses replicate well in the epithelium lining the chorioallantoic membrane. Virus titers are high in these membranes associated with allantoic fluid; other research incubated a chicken embryo when it was 10–11 days old, and it showed the same pathological change in the third passage (19). In this step, the harvested allantoic fluid of the isolated virus from embryonated eggs were prepared and viral RNA was extracted and

amplified by real-time RT-PCR this result agreement with (20).

CONCLUSIONS

Serial passages were performed to obtain high titers of virus when injecting the virus into the allantoic cavity during 9-11 days, which causes pathological changes in the embryonated chicken eggs such as congestion.

CONFLICT OF INTEREST

The authors of this study say have no conflicts of interests.

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