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Molecular Epidemiology and Maternal Humoral Immunity of Avian Infectious Bronchitis (AIB) in Sulaymaniyah Governorate/Kurdistan Region-Iraq

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ABSTRACT

Avian infectious bronchitis, caused by the avian infectious bronchitis virus (IBV), is endemic in Sulaymaniyah, the Kurdistan Region of Iraq, causing significant economic losses. Fertilized broiler eggs are imported from different countries and used by the poultry farms in the region. However, a systematic comparative study about the rate of IBV infection and maternal antibodies has not been conducted in the poultry farms of this area. Accordingly, this study was designed to compare the levels of maternal antibodies in relation to infection rates between broilers imported from different sources. From the first day until the birds reached marketing ages, eighty-five broiler farms were supervised in the Sulaymaniyah Governorate from January to December 2019. The sources of chicks were firstly recorded, and maternal antibody titers against IBV in 1–4 days old chicks were measured in 10 chicks randomly selected from each of the 85 farms. Later, the birds were observed for clinical signs relevant to infection with avian infectious bronchitis, and infections were confirmed by polymerase chain reaction (PCR). The results indicated that fertilized eggs were imported from five sources: Belgium, Bulgaria, Iran, Turkey, and the Netherlands. All chicks had maternal antibodies against IBV, with no statistically significant difference between the antibody titers ($p > 0.05$). However, infections occurred in 51.8% (44/85) of the farms at an average age of 22.8 days. We conclude that maternal antibodies cannot protect >10-day-old broilers, and suitable vaccination programs using local variants of the virus are necessary to eradicate IBV in Sulaymaniyah governorate, Kurdistan Region.

Keywords: Avian infectious bronchitis virus, IBV, maternal immunization, antibody titer.

1. Introduction

Infectious bronchitis (IB) is an acute, highly contagious disease responsible for remarkably significant economic losses in the poultry industry worldwide[1]. The disease was first documented in 1931 in the USA and is caused by the avian infectious bronchitis virus (IBV)[2], a single-stranded RNA virus belonging to the Coronaviridae family of order Nidovirales[3]. IBV primarily causes respiratory infection and can also cause infection according to its strain in the digestive, urinary, and reproductive systems, leading to reduced egg production[1]. The continuous emergence of new antigenic and genetic variants of IBV is due to its high substitution and recombination rate characterizations with heterogeneous biological and immunological properties[4]. Vaccination in poultry, especially live attenuated and inactivated virus vaccines, is considered a novel approach in preventing and controlling IBV worldwide. However, sometimes vaccine failure occurs when high maternal antibodies occur due to the neutralization of the live vaccine[5].

Maternal antibodies are transferred naturally through the placenta, colostrum, milk, or egg and are known as passive immunity[6]. Therefore, pathogen-specific maternal antibodies play an essential role in the modern-day broiler chicken industry due to their short life span[7]. In chicken, the transfer of maternal

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antibodies requires two main steps. First, antibodies are transferred from the hen and deposited in the egg yolk (IgG or IgY) and albumen (IgA and IgM). Second, IgY is transferred to offspring through egg yolk through embryonic circulation. This process starts from day 7 of embryonic development until a few days before hatching. IgA and IgM are transferred to the embryo by absorption of albumen by the embryonic gut, which functions as a protein source and protective immunoglobulin in young chicks.

Due to a lack of immune system development in young chicks and hatching in a sterilized egg environment, they are vulnerable to many pathogens. Therefore, maternal antibodies have a significant role in chicks' protection, mainly covering the first 10–14 days of their lives. A proper and effective vaccination program should be conducted since maternal antibody reduces and sometimes disappears after that period[8]. The maternal antibody level transferred to the chick is entirely proportional to the blood antibody titer and hens' age. Before production, revaccination of chicken is required to increase the antibody titer[6].

Despite a good vaccination program and technique, IBV outbreaks still occur in poultry farms, depending on the type of vaccine used[9]. There is a chance of infection in using a live vaccine, which necessitates using these vaccines with precaution. Also, the quality of vaccine, storage, and method of administration is another factor[10]. Hence, it is crucial to determine the duration of protection provided by different titers of maternal antibodies against IB in broilers.

The poultry industry in Sulaymaniyah Governorate, Kurdistan Region, comprises an important sector in agriculture and economy[11]. Unfortunately, IBV outbreaks represent a severe threat to that sector, accounting for substantial economic losses in the affected farms. For incubation, fertilized chicken eggs are imported from different countries such as Iran, Turkey, and Belgium. A few day-old chicks have high titers of maternal antibodies to provide protection. However, outbreaks still occur in the region. Accordingly, this study aims at determining the prevalence rate of IBV and the best source of imported fertilized eggs in which the maternal antibodies can provide sufficient protection against the virus in Sulaymaniyah.

2. Material and Methods

2.1 Study area and farms

This study was conducted in the Sulaymaniyah Governorate, Kurdistan Region, from January to December 2019. About 85 broiler farms were supervised from the first day until the chickens were brought to the abattoir. Details on the farm capacity, source of chicks, ventilation system, and disease outbreaks were recorded. The owners of these farms provided verbal consent to participate in the study. Ten chicks (1–4 days old) were taken randomly from each batch to determine the maternal IBV antibody titers using blood taken after the bird was slaughtered. The farms were then supervised until the broilers reached marketing weight.

When IB infection was suspected based on clinical signs on a farm, ten birds were selected and euthanized. During necropsy, lungs, tracheal mucus, and kidneys were pulled and homogenized well for RNA extraction.

2.2 Detection of maternal IBV antibodies

Blood samples were taken from the first batch of chicks and put into plain tubes. The serum was separated by centrifugation at 3,500 revolutions per minute (RPM) and used to detect IBV antibodies using ELISA.

Serum IBV antibody titers were determined using an ELISA-based IBV antibody detection kit from IDEXX Inc., USA. Serum samples were diluted with the sample diluent (provided within the kit) by a 1:500 ratio before assay. One hundred microliters of the diluted serum were taken and put into a 96-well microtiter plate containing IBV antigen. The plate was then covered with parafilm and incubated at 25°C for 30 minutes. Then, the plate's content was aspirated and washed automatically by Microplate Strip Washer. Washing was done five times by adding 300 µl distilled water to each well. The microplate was decanted on an absorbent tissue paper to absorb distilled water remains after each washing. A multichannel micropipette was used to transfer 100 µl of the conjugate to each well. The plate was covered with parafilm and incubated at 25°C for 30 minutes. 100 µl of the substrate was transferred into each well by a multichannel micropipette. The plate was incubated at room temperature for 15 minutes, and 100 µl of the stop solution was added to each well. Finally, the absorbance was read using an ELx800TM Absorbance Microplate Reader at 650 nm. The result interpretation was based on the manufacturer's software XCheck plus infectious bronchitis test kit by IDEXX. Negative and positive control wells were also included in each plate. Each test was run in triplicate.

2.3 RNA extraction and polymerase chain reaction

RNA extraction was conducted using the Total RNA Mini Kit (blood/cultured cell) developed by Geneaid Biotech Ltd. Primer sets of the S1 gene of IBV were produced by Macrogen® (Korea). The forward primer sequence was 5`-GTTTACTACTACCAAAGTGCCTT-3`, and the reverse primer's nucleotide sequence was 5`-GTGTAAACAAGGTCACCATTTA-3`. These primers were initially designed by Rauf[12]. The 20 µl PCR reaction master mix was prepared according to the manufacturer's protocol. The thermocycler was initiated with a five minute-denaturation at 95°C, followed by 40 cycles, each constituting a denaturation at 95°C annealing at 52°C, and extension at 72°C. A final extension step at 72°C was also included.

2.4 Interpretation of results and statistical analysis

Serum antibody titers were measured using ELISA at different ages. These were used to predict flock protection from IB. The PCR results were used to determine whether the flock was infected by a natural outbreak of IBV or not. Differences in the maternal antibody titers between the farms infected with IB and farms which did not report the infection were compared statistically using an independent variable t-test. The differences between the antibody titers of chicks imported from different countries were compared using a one-way analysis of variance

(ANOVA), followed by Duncan's post hoc. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1 Sources of fertilized eggs and antibody titers

Eighty-five broiler farms located in the districts of Chamchamal (25), Darbandikhan (2), Dukan (32), Kalar (2), Koya (1), Mawat (7), Qaladiza (1), Qaradagh (9), and Slemani (6) were included. Our survey showed that fertilized broiler eggs were imported from Belgium, Bulgaria, Iran, Netherlands, and Turkey (Table 1). About 41.2% (35/85) of the farms included in this study used imported fertilized eggs from Belgium, and 22.4% (19/85) used fertilized eggs imported from Turkey. About 15.3% of the farms (13/85) used eggs imported from Bulgaria. The remaining 18 farms used imported eggs from Iran and Netherlands (nine farms each). None of the farms vaccinated the birds against IBV.

All farms included in the study did not vaccinate the chicks, and they relied on the presence of maternal antibodies and following a strict quarantine measure to protect against IB. The results showed that chicks from all sources contained maternal antibodies against IBV to protect against natural infection. No statistically significant differences were observed between the antibody titers of chicks from different sources (Figure 1A). Also, no statistically significant difference in the IBV antibody titers was observed between the farms infected later with the virus and farms that did not report the occurrence of IB infection, even though the average titer was slightly higher in the infected farms (Figure 1B). Taking the country sources of the chicks into account, we also compared the antibody titers between infected farms and farms that did not report any IBV infection (Figure 1C). The results revealed no statistically significant difference in the titers of antibodies between the groups. These results revealed that none of the sources of fertilized eggs was superior at protecting against the natural infection of infectious bronchitis.

3.2 Infectious bronchitis rates and age of infection

The PCR tests confirmed the natural infections with IBV, and it was shown that 44 of the 85 farms (51.8%) were infected with the virus, with infections occurring from four days to seven weeks (Figure 2). The average age of infection in the 44 farms was 22.8 ± 1.6 days.

We compared the average ages of chicks at the time of the first infection between the different sources (Figure 1D), and the results showed that infections occurred at 30.0 days in farms raising chicks imported from Bulgaria. Infections occurred after 23.1 days and 22.5 days in farms raising imported chicks from Turkey and Belgium. However, infections occurred as early as 12.8 days and 15.0 days in farms raising imported chicks from Iran and Netherlands. These results indicated that IBV infections occurred in imported chicks from all sources, even though infections occurred later in farms raising chicks from Bulgaria. Each antibody titer represents the mean of 10 samples per farm. * = The farms are categorized into not-infected (-) and infected (+).

Table 1: Antibody titer, source of chicks, and infection status of broilers in 85 farms.

Farm no.	Chicks' age (days)	Source	IBV titer	Infection with IBV*	Age of infection (days)
1.	1	Belgium	1604	+	4
2.	1	Belgium	4169	+	19
3.	1	Belgium	3291	+	31
4.	2	Belgium	2618	+	13
5.	1	Belgium	2789	+	14
6.	1	Belgium	5037	-	-
7.	2	Belgium	1668	+	41
8.	1	Belgium	6306	-	-
9.	2	Belgium	6000	-	20
10.	1	Belgium	8136	+	34
11.	1	Belgium	2282	-	-
12.	1	Belgium	3986	-	-
13.	4	Belgium	943	-	-
14.	1	Belgium	3563	+	24
15.	2	Belgium	8134	+	15
16.	1	Belgium	4462	-	-
17.	1	Belgium	2992	-	-
18.	1	Belgium	5857	+	38
19.	1	Belgium	10202	+	11
20.	3	Belgium	6133	-	-
21.	1	Belgium	7353	-	-
22.	1	Belgium	3178	-	-
23.	1	Belgium	5117	+	28
24.	1	Belgium	3246	+	27
25.	1	Belgium	4134	+	12
26.	2	Belgium	4145	-	-

Farm no.	Chicks' age (days)	Source	IBV titer	Infection with IBV*	Age of infection (days)
27.	1	Belgium	2523	+	20
28.	5	Belgium	498	+	25
29.	1	Belgium	4586	+	18
30.	1	Belgium	9008	-	-
31.	1	Belgium	4663	-	-
32.	1	Belgium	2682	+	19
33.	1	Belgium	3991	-	-
34.	1	Belgium	3867	-	13
35.	1	Belgium	3086	+	35
36.	2	Bulgaria	3920	+	41
37.	1	Bulgaria	5922	-	-
38.	1	Bulgaria	2538	-	-
39.	1	Bulgaria	5775	+	26
40.	4	Bulgaria	5692	-	20
41.	1	Bulgaria	5510	+	39
42.	1	Bulgaria	2191	+	41
43.	2	Bulgaria	8279	+	25
44.	1	Bulgaria	6081	+	17
45.	4	Bulgaria	3844	+	30
46.	1	Bulgaria	6384	+	15
47.	1	Bulgaria	11282	+	30
48.	2	Bulgaria	2305	-	29
49.	2	Iran	2194	-	-
50.	1	Iran	8237	-	-
51.	1	Iran	13619	+	15
52.	1	Iran	4282	-	-
53.	3	Iran	3389	-	-
54.	1	Iran	6343	+	10
55.	2	Iran	4501	-	-
56.	2	Iran	2116	+	7
57.	4	Iran	1531	+	19
58.	2	Netherlands	1896	-	-
59.	2	Netherlands	4721	-	-
60.	3	Netherlands	3432	-	-
61.	4	Netherlands	3392	-	-
62.	2	Netherlands	6680	+	12
63.	1	Netherlands	5083	+	18
64.	4	Netherlands	2191	+	15
65.	3	Netherlands	2612	-	-
66.	1	Netherlands	1735	-	-
67.	3	Turkey	2575	-	44
68.	3	Turkey	2688	+	45
69.	1	Turkey	5026	+	15
70.	3	Turkey	6184	-	-
71.	1	Turkey	4410	+	31
72.	1	Turkey	4507	-	-
73.	2	Turkey	1926	-	-
74.	4	Turkey	5896	-	-
75.	1	Turkey	5077	+	25
76.	1	Turkey	5492	-	32
77.	1	Turkey	8571	+	25
78.	3	Turkey	6707	-	-
79.	2	Turkey	1844	-	-
80.	1	Turkey	3984	+	15
81.	1	Turkey	2888	+	19
82.	1	Turkey	2982	+	21
83.	1	Turkey	5609	-	-
84.	1	Turkey	3295	+	12
85.	1	Turkey	1425	-	-

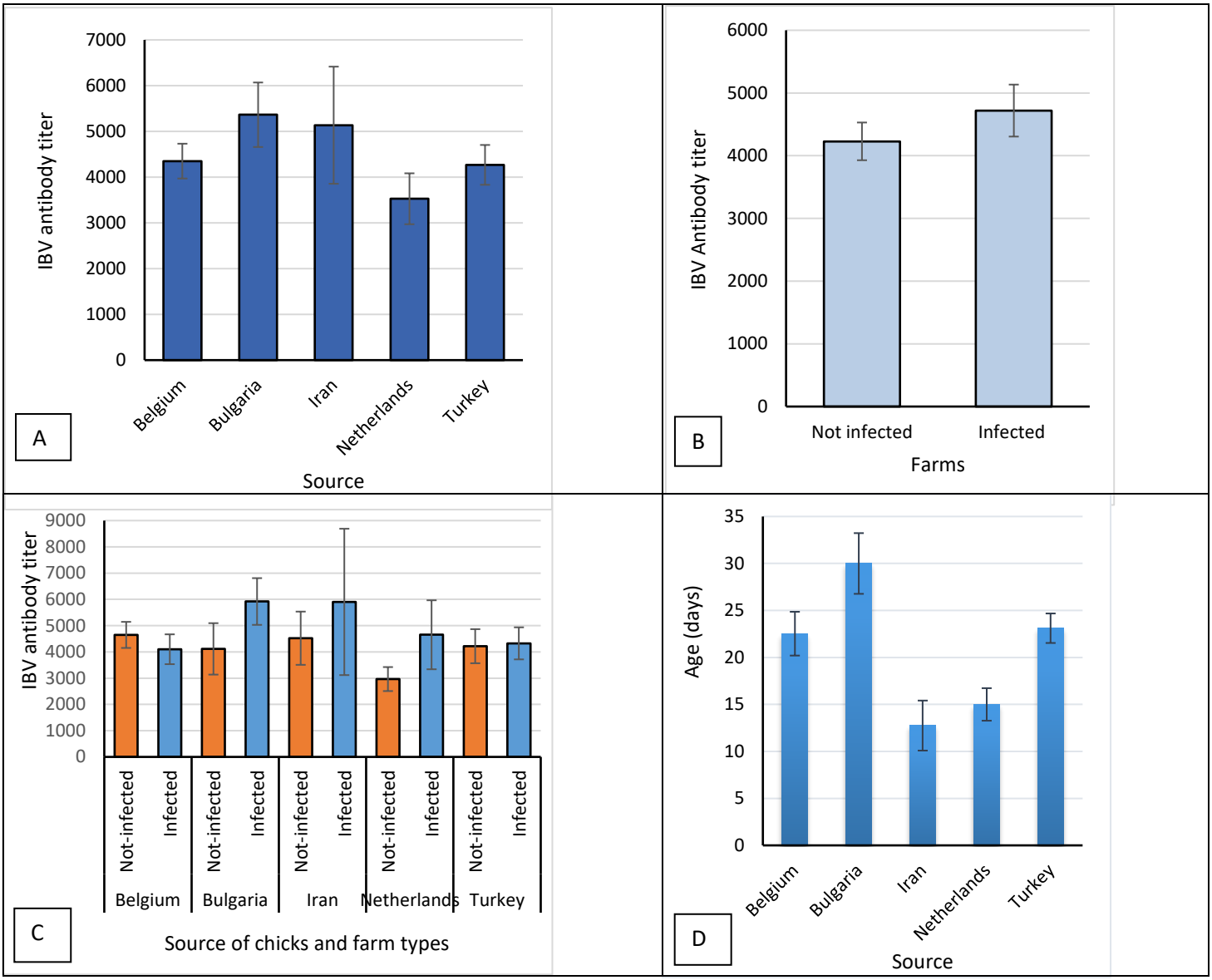

Figure 1: A) Antibody titers in chicks imported from different countries. No statistical difference was present between the different sources. B) IBV antibody titers of broiler chicks from 41 non-infected farms and 44 farms infected later with the virus. Columns represent the mean antibody titers, and error bars represent the standard errors of the mean (SEM). No statistical difference ($P > 0.05$) was present between the two groups ($p = 0.341$). Test: independent sample t-test. C) Antibody titers of chicks imported from different sources. Values represent mean ± SEM. There was no statistical difference between the groups ($p > 0.05$), using a one-way analysis of variance followed by Duncan's post hoc. D) Age of infection with IBV in farms raising chicks from different sources. Infections occurred at an average of 30.0 days in farms raising chicks imported from Bulgaria, which was statistically significant ($p \leq 0.05$) between farms raising imported chicks from Iran (12.8 days) and the Netherlands (15.0 days).

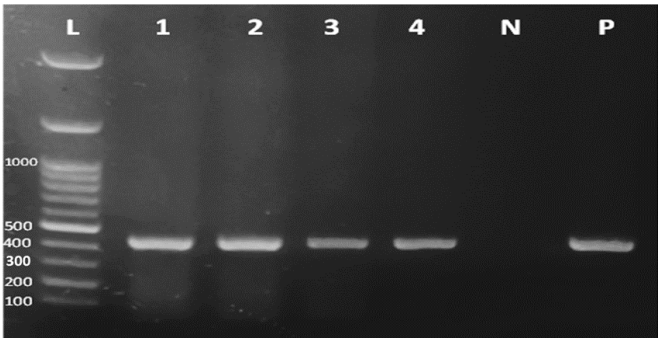

Figure 2: Agarose gel electrophoresis of amplified S1 gene fragments (448 base pairs) stained with ethidium bromide. Lane L revealed a 100-bp DNA ladder. Lanes 1–4 were the IBV-positive samples, and lane N was the negative control.

4. Discussion

Avian infectious bronchitis still represents a significant threat to the poultry industry in the Kurdistan region. Our survey indicated that about 51.8% of the farms in the area are infected with IBV annually, causing 10–15% mortality rates in the affected farms. This study compared the rates of IBV infection in broilers imported from different countries with maternal antibodies against the virus. The aims were to determine which source chicks harbor the highest titer of maternal antibodies with the least infection rates. The results revealed no statistical differences in the levels of maternal antibodies and infection rates. A study by Gharaibeh and Mahmoud[13] showed that the half-life of antibodies against IBV is about 3.9 days. The researchers showed that maternal antibodies against IBV can last for up to nine days

in their study. The average antibody titers in our study were 4719.4 and 4228.3 in the infected and not infected farms. The average age of infection in the farms was 22.8 days, meaning that the birds were protected in the first ten days by maternal antibodies. However, farms number 1 and 56 were infected with IBV as early as 4 and 7 days, probably due to the low antibody titers against the virus, rendering the birds susceptible to infection after four days.

Farms using fertilized eggs imported from Bulgaria became infected with IBV after 30 days. However, the antibody titers in the blood of the chicks were not higher than in chicks from other sources. Moreover, the antibody titers in these birds could protect for no later than ten days. Hence, the late infection in these farms is not related to maternal antibodies and is due to management factors[9,14].

No differences between the mean antibody titers of the infected farms and those that did not report any infection of IBV were present in farms that used fertilized eggs from the same country of origin, indicating that maternal antibodies can protect the birds for a limited time. After that, the birds should be vaccinated with a suitable vaccine.

Typically, day-old chicks should be vaccinated in the hatchery, and broilers raised for longer than 49 days are vaccinated again when they are 16 to 18 days old[10,15]. Broilers are kept for longer than 45 days in the farms of Sulaymaniyah due to consumer preferences, making them more vulnerable to infection. Hence, to control IBV outbreaks in Sulaymaniyah, broiler farms should follow a vaccination program using viral strains endemic to the region.

5. Conclusion

Avian infectious bronchitis caused by IBV is endemic to the Kurdistan Region in the north of Iraq, causing significant economic losses in the broiler farms, as the infection accounts for 10–15% mortality rates. Imported fertilized chicken eggs from Belgium, Bulgaria, Iran, the Netherlands, and Turkey are endowed with sufficient maternal antibodies to protect them for up to ten days. However, vaccination with local strain viruses seems to be the only way to control the infection in the region.

Conflict of interests

None.

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