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Research Paper

Infectious Bronchitis Virus in Broiler Chickens: Seroprevalence and Associated Risk Factors

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ABSTRACT

Introduction: Infectious bronchitis virus (IBV) is one of the most important viral diseases, causing considerable economic losses in the poultry industry worldwide. The present study was conducted in Algeria to investigate the seroprevalence, associated risk factors, clinical signs, and lesions of IBV disease in broiler chicken.

Materials & Methods: The study was conducted in 63 chicken farms, where three IBV vaccination protocols were used in the first protocol: A single H120 dose was administered to the chicks at 7 days of age, in the second protocol: H120 at 3 days with an MA5 booster at 14 days, and in the third protocol: H120 at 3 days with MA5 and 4/91 boosters at 14 and 35 days, respectively. A total of 2142 sera were collected and analyzed using the enzyme-linked immunosorbent assay (ELISA) test.

Results: IBV was detected in 77.77% of broiler farms. The most common clinical signs observed were wheezing, sneezing, coughing, nasal discharge in the respiratory form, aqueous diarrhea, and dehydration in the nephropathogenic form. Lesions included tracheitis, fibrin deposition in the respiratory form, hemorrhagic nephritis, uric acid deposition at the renal, visceral, and articular level in the nephropathogenic form. Antibody titers were higher in winter compared with autumn and spring ($P<0.01$). Antibody titers were also higher in chickens aged >30 days, in farms with a high density, poor hygiene level, and under the first vaccination protocol, compared with chickens aged ≤30 days ($P<0.01$), farms with a low density ($P<0.001$), farms with good hygiene level ($P<0.01$) and those under the third vaccination protocol ($P<0.01$).

Conclusion: These findings showed a high seroprevalence of IBV in Algeria and highlight the importance of continuous surveillance and molecular characterization of persistent strains to optimize vaccination strategies against emerging variants.

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1. Introduction

Infectious bronchitis virus (IBV), belonging to the genus gamma Coronavirus, is an important viral disease in chickens industry worldwide, causing several economic losses [1]. The virus is characterized by high genetic and pathogenic variability, and new strains continue to emerge [2]. Transmission occurs via inhalationor contact with contaminated objects [1].

In broiler chickens, the disease is characterized by respiratory signs (tracheal rales, coughing, and sneezing), weight losses, reducing in feed efficiency, and bacterial super-infections [1, 3]. IBV infections may result in reduced egg production of up to 70%, declines in eggshell quality in layer and breeders, and low mortality rate [1]. Nephropathogenic IBV strains,however, can cause high mortality due to kidney failure [1].

Different diagnostic methods are routinely used to detect antibody responses in sera samples, such as enzyme-linked immunosorbent assay (ELISA) [4]. Moreover, many molecular methods have been developed to detected IBV genotypes [5].

Reported seroprevalences of avian IBV varied between countries; ranging from 43% in South Africa [6] and 84% in Nigeria [7].

IBV has become a serious problem, especially in north Africa, due to intensive poultry farming practices [8]. In Algeria, it was first detected by Sid et al. [9] in Medea regions. The present study was conducted to investigate the antibody titers, the seroprevalence, clinical signs, and lesions of IBV in broiler chickens, and to assess the associated risk factors.

2. Material and Methods

2.1. Study area and design

The study was carried out in north- central, eastern and western Algeria from September 2022 to May 2023. It was conducted in sixty- three (63) broiler chicken farms, each containing 3,000 to 15,000 chickens with different strains (Arbor acres, Cobb 500, Big fast), aged between four and seven weeks.

In all farms, broiler chickens were vaccinated against IBV with live vaccines using three different protocols:

Protocol 1: Chicks were vaccinated against IBV at 7 days of age with a live IB H120 strain vaccine.

Protocol 2: Chicks were vaccinated against IBV at 3 days of age with a live H120 strain vaccine, followed by a booster at 14 days of age with a live MA5 strain vaccine.

Protocol 3: Chicks were vaccinated against IBV at 3 days of age with a live H120 strain vaccine, followed by a booster at 14 days of age with a live MA5 strain vaccine and a second booster at 35 days of age with a live 4/91 strain vaccine.

2.2. Clinical and lesional diagnosis

Clinical diagnosis of IBV disease was based on symptoms accompanied by lesion examination.

2.3. Blood samples

From the 63 farms, a total of 2142 chickens were randomly selected for two blood samples. The first sample was taken as soon as the first clinical signs of IBV appeared, and the second sample was taken 2 to 3 weeks later.

Blood samples were collected aseptically from the wing vein of each chicken. About 3-5 mL were collected in dry tubes and centrifuged (5000 rpm for 10 min) immediately to recover sera, which were stored at -20 °C until analysis.

2.4. Serological analysis

Serological analysis was conducted to measure antibody titer level against IBV and performed using the indirect ELISA method. The test was performed with a commercial kit (ID Screen® IBVS Indirect) of IDvet Innovative Diagnostics kits (Montpellier, France).

The protocol was standardized according to the manufacturer's instructions. Sera were diluted 1:500, then loaded to ELISA plates to start immunosorbent reaction. ELISA plates were read using ELx800 spectrophotometer (BioTek™, USA) equipped with a 405 nm filter; where the measured optical density (OD) was transformed into titrated antibody. The averages of the antibody titers and the coefficient of variation (CV) were automatically calculated by band and by series of samples using the software provided by the laboratory (ID Soft™ 5.05, Montpellier, France).

2.5. Interpretation of the ELISA results

According to the manufacturer's instructions, the average antibody titers expected 3-5 weeks after using one live IBV vaccine range from 500 to 1000. For two live vaccines+Variant, the antibody titers vary from 1000 to 2000 after 3-5 weeks after vaccination. The CV should be between 40% and 60% for effective vaccination.

Antibody titers below 500 indicate poor or no vaccination or presence of an immunosuppressive disease and above 1000 for a single live vaccine and 2000 for two live vaccines+variant, indicate IBV disease.

2.6. Statistical analysis

Descriptive statistics were used to characterize flocks according the different factors. Statistical analyses were performed with SAS (Version 9.1.3; SAS Institute Inc., Cary, NC). Antibody titers over the time were analyzed by fitting the fixed effects of day, group and the interaction of day×group in a repeated measures variance analysis using PROC MIXED models, with the random effect of herd (SAS Inst. Inc. 9.1). Covariance structure used (compound symmetry or autoregressive [AR1]) was chosen based on the Akaike information criterion. The layout of our model can be summarized as follows: $Yijk = \mu + Gi + Tk + GTik + \varepsilon ijk$. Where Yijk=Antibody titer, μ=overall mean, Gi = effect of group, Tk = effect of time of sampling (k=1 and 2), GTik=effect of group × timeand εijk= random residual error. A stacked line plots of Antibody titer changes were generated using Prism 5.01 (GraphPad Software, Inc. La Jolla, CA USA).

3. Results

Out of 63 broiler chicken farms, 49(77.77%) were seropositive for IBV. A low CV (8-35%) was observed and the global means of antibody titer in the second time of sampling (4676) was significantly higher compared with the first time of sampling (1973) (SE=326.53) ($P<0.0001$).

At the second time of samples, antibody titers varied significantly according to the season, chicken age, chicken density in the farms, hygiene level in the farms and vaccination protocol (Table 1, Figure 1). The an-

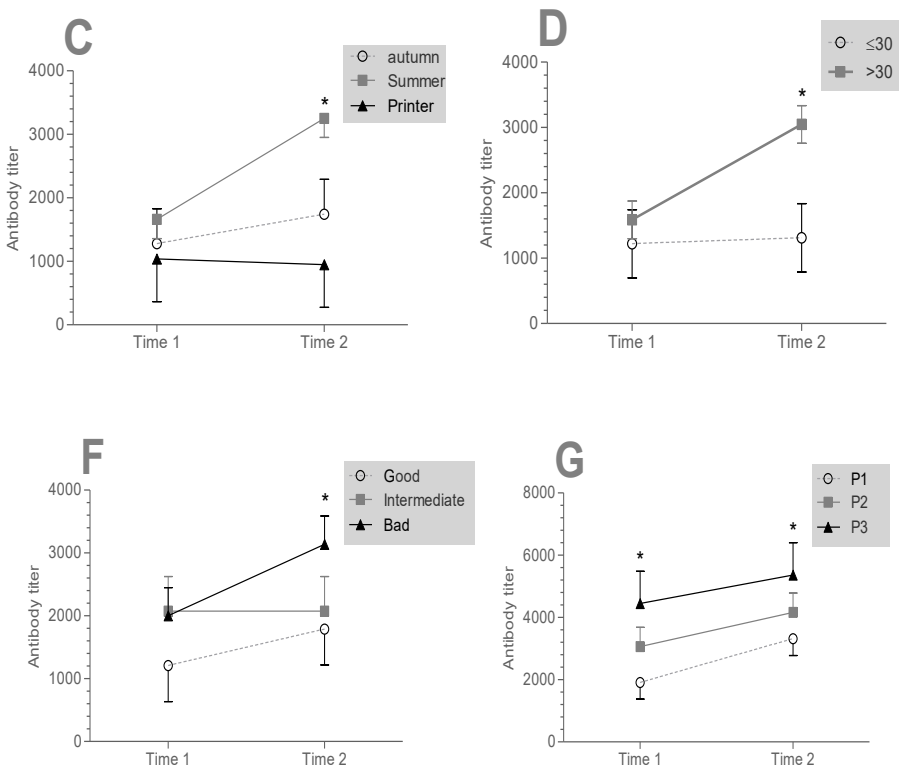

Figure 1. Risk factors affecting antibody titers of IBV

Note: Time 1: First sample; Time 2: Second sample; C: Season, D: Age, F: Hygiene, G: Protocol of vaccination.

Table 1. Antibody titers of IBV in the first and second time of sampling (TS1 and TS2) according to the different factors

Factors		TS1	TS2	SE	P
Area	East	4448.16	5366.03	1038.28	0.62
	Center	1912.21	2314.65	537.54	
	West	3065.21	4162.75	618.19	
Climate	Dry	1715.66	2624.74	535.39	0.89
	Wet	1577.13	2872.91	382.58	
Season	Autumn	1278.5	1740.5[a]	550.78	<0.01
	Winter	1661.25	3252.4[b]	301.67	
	Spring	1038.5	948.00[a]	674.56	
Age (d)	≤30	1220.14	1312[a]	522.13	<0.01
	>30	1587.35	3047.78[b]	288.05	
Density (birds/m^2)	>10	1772.33	3658.6[b]	332.69	<0.001
	≤10	1277.73	1771.55[a]	388.5	
Mortality	<10	1812.72	3048.55	705	0.71
	≥10	1687.62	2805.03	371.14	
Hygiene	Good	1206.86	1788.71[a]	571.71	<0.01
	Intermediate	2076.32	2076.32[b]	549.96	
	Bad	2001.62	3139.7[b]	447.8	
Strain	Arbor acres	2108.08	3274.8	564.63	0.48
	Cobb 500	1227.18	1227.18	1227.18	
	Big fast	2304.99	3231.72	610.57	
Protocol of vaccination	3	1912.21[a]	3314.65[a]	537.54	<0.01
	2	3065.21[ab]	4162.75[ab]	618.19	
	1	4448.16[b]	5366.03[b]	1038.28	

SE: Standard error.

Note: Values with different letters in the same column were different significantly.

tibody titers were higher in winter season compared with autumn and spring season (P<0.01). Moreover, the antibody titers were higher in chickens aged >30 days, in farms with a high density (>10 birds/m^2), in farms with poor hygiene level and under the first vaccination protocol., In contrast, titers were lower chickens aged ≤30 days (P<0.01), in farms with a low density (≤10 birds/m^2) (P<0.001), in farms with good hygiene level (P<0.01) and under the third vaccination protocol (P<0.01) (Table 1, Figure 1).

However, there was no significant effect of area, climate, and strain groups on the antibody titers.

All chickens clinically suspected of IBV were confirmed seropositive. The most common clinical signs observed were: Wheezing, sneezing, coughing, and nasal discharge for respiratory form, and aqueous diarrhea, dehydration, and high rate of mortality for nephropathogenic form.

The most frequent lesions observed were: Tracheitis (congestion of the tracheal mucosa) and fibrin deposition for respiratory form, and hemorrhagic nephritis, uric acid deposition at the renal and visceral level (visceral gut), and uric acid deposition at the joint level (articular gout) for nephropathogenic form.

4. Discussion

The IBV is characterized by the continuous emergence of new serotypes. Therefore, identification of the genotype of strains is necessary to reduce the low degree of cross-protection of commercial vaccines among the different serotypes [10]. It is well known that the occurrence of IBV disease in vaccinated flocks is quite frequent, possibly due to inappropriate vaccination or the emergence of new strains of the virus [11]. In many African countries, mass-produced IBV serotypes cause sporadic outbreaks in the commercial poultry industry [12]. Vaccination success also depends on the choice of vaccine strain and vaccination protocol [13].

The incidence of IBV in vaccinated flocks may be influenced also by inadequate vaccination practices. Messaï et al. [14] reported that the following factors influence the success of vaccination: Poor water quality, water that may contain disinfectants neutralizing the live vaccine, insufficient number of troughs on farms, and failure to maintain the vaccine storage cooling chain.

Effectively, all these factors can explain the presence of IBV, in this study, in broiler chicken farms despite vaccination.

The seroprevalence of IBV observed in this survey (77.77%) aligns with the reports from Nigeria 82.7% [15]. This high prevalence of IBV could be attributed to several factors including the presence of carriers of the virus in the environment, its ability to spread over significant distances via aerosol, and the highly transmissible nature of the IBV [16].

The overall seroprevalence of IBV revealed in this survey is higher than other studies conducted in Grenada (31.01%) [17] and Maiduguri (26.6%) [18], likely due to differences in agro-climatic conditions, sample size, management systems and increased IBV activity in the study area [19].

Protected poultry flocks should have an average antibody titer above the protection threshold without being very high compared to the titer resulting from vaccination, and without specific clinical signs [20]. In this study, the sampled chickens were suspected of being infected with IBV and showed typical clinical signs and necropsy lesions despite being vaccinated. Two blood samples were taken; the first sample was taken at the onset of clinical signs of the disease and the second sample was taken two to three weeks later. The antibody titer increased significantly between the 0.2 sets of sera taken indicating a stimulation of the immune system due to recent infection, reported by Lopez [21], Salhi et al. [22], Messaï et al. [14].

In this investigation, the most common clinical signs observed were: Rales, sneezing, coughing, nasal discharge for the respiratory form, and aqueous diarrhea and dehydration, for the nephropathogenic suspected form. These findings are in agreement with Abdel-Moneim et al. [3].

Bing et al. [23] signaled that nephropathogenic IBV was prevalent in the vaccinated chickens and resulting in a high mortality for the 20 to 50-day-old chickens. This is in line with our finding herein.

The observed lesions were: Tracheitis, fibrin deposition and colibacillosis complication for the respiratory form. Interstitial nephritis, uric acid deposition at the renal level and viscera, uric acid deposition at the articulation level were observed in the nephropathogenic form. These observations are consistent with those reported by Abdel-Moneim et al. [3].

In this study, older hens (over 30 days old) were the most infected,likely due to continuous exposure to the disease, resulting in a higher prevalence in adult chickens [24]. This indicates that strengthening biosecurity measures on farms can help reduce virus transmission with adequate ventilation and reduction of overcrowding [24]. Overcrowding on farms is thought to be a factor in the introduction and implantation of the virus [1].

The IBV is observed mainly in winter [25], consistent with our results. The origin of this seasonality may be attributed to environmental changes, changes in host physiology or alterations in the virus [26].

The prevention of IBV disease is based on hygiene and medical prophylaxis, therefore, it is important to highlight that no vaccine can solve the problem of IBV if the necessary precautions are not taken, such as compliance with husbandry methods like cleaning and disinfection and sanitary vacuums [1]. In our case the dirty farms effectively presented the highest levels of antibody titers.

De Wit et al. [27] reported that vaccination with a single mass strain protects hens from 76.5% up to 100%, while using mass and a booster with a variant gives protection in the range of 99-100%, against wild variant strains circulating in the field, consistent with our results. Indeed, when we did boosters in the second and third vaccination protocols, the antibody titers were not high in the first and second sampling times, explaining why the herds were better protected compared to those herds using a single vaccine (first protocol).

The chicken strain had no effect on infection status in this study. However, in some studies, it has an effect on seroprevalence linked to a difference in the level of seroprotection of the disease or to a genetic difference in strains against the disease [27].

5. Conclusion

In conclusion, the IBV is a highly prevalent disease (77.77%) in broiler chicken farms in Algeria despite vaccination. The continuous appearance of new serotypes of the virus leads to the vaccine failure. The antibody titers were significantly higher in chickens sampled in winter, in chickens >30 days old, in high- density farms, and in farms with poor hygiene. The control of all these factors significantly certainly reduces the prevalence of IBV within farms. The continuous emergence of new variants of the virus underlines the importance of a continuous surveillance of IBV to optimize vaccination strategies. Therefore, the identification and molecular characterization of persistent serotypes of IBV circulating in the field are recommended.

Ethical Considerations

Compliance with ethical guidelines

Experimental procedures were approved by the Institutional Committee for the Protection of Animals of the National Administration of Higher Education and Scientific Research of Algeria (Code: 98-11, Act of 22 August 1998).

Data availability

Data are available upon request from the corresponding author.

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Authors' contributions

Conceptualization, study design, and experiments: Omar Salhi and Chafik Redha Messai; Data analysis and interpretation: Mustapha Nabi, Nassim Ouchene and Nadjet Amina Khelifi Touhami; Writing: Omar Salhi, Nassim Ouchene, and Nadjet Amina Khelifi Touhami.

Conflict of interest

The authors declared no conflict of interest.

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