Infectious Bronchitis Virus in broiler chickens: Seroprevalence and Associated Risk Factors

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ABSTRACT

Infectious Bronchitis Virus (IBV) is one of the most important viral diseases that causing considerable economic losses in the poultry industry worldwide. The present study was conducted, in Algeria, to investigate the seroprevalence, associated risk factors, clinical sings and lesions of infectious bronchitis viral (IBV) disease in broiler chicken. The study was conducted in 63 chicken farms of which 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 realized and analysed using ELISA test. The IBV was detected in 77.77% of broiler farms. The most common clinical signs observed were: wheezing, sneezing, coughing, nasal discharge for respiratory form and aqueous diarrhea, dehydration for nephropathogenic form. For lesions, there were: tracheitis, fibrin deposition for respiratory form, hemorrhagic

nephritis, uric acid deposition at the renal, visceral and articular level for nephropathogenic form. The antibody titers were higher in winter season compared with autumn and spring season ($p<0.01$). Also, the antibody titers were higher, respectively, in chickens aged >30 days, in farms with a high density, in farms with bad hygiene level and in the first protocol of vaccination, in comparison 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 with the third protocol of vaccination ($p<0.01$). 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.

Keywords: Broiler chicken; Infectious bronchitis virus; risk factors; Seroprevalence.

1. Introduction

Infectious Bronchitis Virus (IBV), belongs to the genus *gamma Coronavirus*, is an important viral disease in chickens industry worldwide that causes several economic losses (1). The virus is characterized by high genetic and pathogenic variability, and new strains continue to emerge (2). Transmission of the virus is made by inhalation, or by 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 qualityin layer and breeders and low mortality rate (1). Nephropathogenic IBV strains can cause high mortality due to kidney failure (1).

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

The seroprevalences of avian IBV varied between countries; 43% in South Africa (6) and 84% in Nigeria (7).

IBV became a serious problem, especially in north Africa, due to intensive poultry farming practices (8). In Algeria, it was detected for the first time 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 center, east and west Algeria, from September2022to Mai 2023.It wasconducted insixty three (63) broiler chicken farms and each farm 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 againstIBV with live vaccines through 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

The clinical diagnosis of IBV disease was made on the basis of the 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 3-5ml were collected in dry tubes and centrifuged (5000 rpm for 10 min) immediately to recover the sera that were stored at -20°C until analysis.

2.4. Serological analysis

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

The protocol was standardized according to the manufacturer instructions. Prior, sera were diluted to 1:500, and then loaded to ELISA plates to start immunosorbent reaction. ELISA plates were read by ELx800 spectrophotometer (BioTek™, USA) equipped with the 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 with the software provided by the laboratory (ID Soft™ 5.05, Montpellier, France).

2.5. Interpretation of the ELISA results

According to the manufacturer instructions, the average antibody titers expected after 3-5 weeks after using one live IBV vaccine range from 500 to 1000. For the use of two live vaccines + Variant, the antibody titers vary from 1000 to 2000 after 3-5 weeks after vaccination. The coefficient of variation (CV) should be between 40% and 60% for a good vaccination.

Antibody titers below the threshold of 500, means that there is a 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 means that there is an IBV disease.

2.6. Statistical analysis

Firstly, descriptive statistics were used to characterize flocks according the different factors. Thus, statistical analyses were performed with SAS (Version 9.1.3; SAS Institute Inc., Cary, NC).Antibody titer of disease through 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 a 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:$Y_{ijk} = \mu + G_i + T_k + GT_{ik} + \varepsilon_{ijk}$. Where Y_{ijk}=Antibody titer, μ=overall mean, G_i = effect of group, T_k = effect of time of sampling (k=1 and 2), GT_{ik}=effect of group × timeand ε_{ijk}= 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

A total number of 49/63 (77.77%) broiler chicken farms were seropositive for IBV. A low coefficient of variations (CV=8-35%) was observed and the global means of antibody titer in

the second time of sampling (4676) was significantly higher in comparison with the first time of sampling (1973) (SE=326.53) ($p < 0.0001$).

In the second time of samples, the antibody titers were varied significantly according to the season, chicken age, chicken density in the farms, hygiene level in the farms and protocol of vaccination (Table 1, Figure 1). The antibody 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 bad hygiene level and in the first protocol of vaccination, in comparison, respectively, with chickens aged ≤30 days ($p<0.01$), farms with a low density (≤ 10 birds/m^2) ($p<0.001$), farms with good hygiene level ($p<0.01$) and with the third protocol of vaccination ($p<0.01$) (Table 1, Figure 1).

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

All chickens suspected clinically of having IBV were revealed seropositive. The most common clinical signs observed were: wheezing, sneezing, coughing and nasal discharge for respiratory form andaqueous diarrhea, dehydrationand 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 in order to reduce the low degree of cross-protection of commercial vaccines between 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 which neutralizes the live vaccine, insufficient number of troughs on farms, and the 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%) is in agreement with the report of 82.7% (15) in Nigeria. 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).

The difference in seroprevalence could be due to variations 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 samplewas taken two to three weeks later. The antibody titer increased significantly between the 02 sets of sera taken indicating a stimulation of the immune system due to recent infection as reported by Lopez (21), Salhi et al. (22), Messaï et al. (14).

In this investigation, the most common clinical signsobserved were: rales, sneezing, coughing, nasal discharge for the respiratory form, and aqueous diarrhea and dehydration, for the nephropathogenic suspected form.These findingsare 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 lesionswere: 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 inthe 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. This could be 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). This is in accord 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). Effectively, in our case, the dirty farms 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, which correlates 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, which may explain why the herds were better protected compared to those where we used 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 that has been linked to a difference in the level of seroprotection of the disease or to a genetic difference in strains against the disease (27).

In conclusion, the IBV is a highly prevalent disease (77.77%) in broiler chicken farms in Algeria and this, in spite of vaccination. The continuous appearance of new serotypes of the virus leads to the failure of the vaccine. 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 in order to optimize vaccination strategies. Therefore, the identification and molecular characterization of persistent serotypes of IBV circulating in the field are recommended.

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Authors' Contribution

Study concept and design: O.S. and CR.M.

Conducting the experiment: O.S. and CR.M.

Analysis and interpretation of data: M.N., N.O. and N.A.K.T.

Drafting of the manuscript: O.S., N.O. and N.A.K.T.

Critical revision of the manuscript: O.S., N.O. and N.A.K.T.

Ethical approval

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

Conflict of interests

The authors declare that they have no known conflict of interest in the conduction of the current study.

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Data Availability

Data are available upon request from the corresponding author.

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