ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2018.418.422

Research Article
Seroprevalence of Infectious Bronchitis Virus in Broiler Farms in Batna, East Algeria

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Abstract

Background and Objective: Avian infectious bronchitis is an infectious viral disease that induces major economic losses. It has a worldwide distribution and a high morbidity rate, that may reach 100%. Infectious bronchitis monitoring and typing are needed to determine its impact on the chicken industry and to choose adequate vaccine strains. The aim of this study was to determine the existence of the disease and to monitor its seroprevalence in unvaccinated broiler farms in the East of Algeria. **Methodology:** The study was conducted in the province of Batna (East of Algeria). One hundred and eighty-four blood samples were collected from different broiler farms and assayed by an indirect ELISA test. **Results:** Serological analysis showed a high rate of infection with high Infectious Bronchitis Virus (IBV) antibody titers. A total of 144 were positive (78.25%) with a significant average antibody titer (2080.277). **Conclusion:** The current study demonstrated a high seroprevalence of infectious bronchitis in unvaccinated broiler farms in the East of Algeria. Therefore, adequate preventive measures (vaccination, improvement of hygienic conditions) must be applied to reduce circulation of t the virus and the rate of infection.

Key words: Viral disease, chicken industry, broiler vaccination, infectious bronchitis virus, Algeria

Citation: Abdelheq Barberis, Nadir Alloui, Amine Boudaoud, Omar Bennoune and Ayachi Ammar, 2018. Seroprevalence of infectious bronchitis virus in broiler farms in Batna, East Algeria. Int. J. Poult. Sci., 17: 418-422.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Viral diseases such as Newcastle disease, avian influenza and Infectious Bronchitis (IB) are the most common respiratory diseases in the chicken farms. They cause significant economic losses, by the decline in production[1-3], mortality and high costs of treatment, mainly related to dealing with subsequent opportunistic sur-infections. One serological study showed that Infectious Bronchitis Virus (IBV) is more common (86%) than other respiratory pathogens such as *Mycoplasma gallisepticum*[4]. Also, IBV is considered as a primary disease in 59.2% of the cases[5]. Infectious bronchitis morbidity can reach 100% but mortality can vary from 18-84% depending on strain virulence, age of the chicken and secondary infections[6].

IBV is a gamma-coronavirus[7] that has a worldwide distribution and affects all countries practicing poultry breeding[8]. It causes upper-respiratory disease in both meat and layer-type birds. The IBV genome is organized as follow: UTR 5'-1a, (Polymerase genes)-S-3a, b, c (E)-M-4b, c-5a, b-N-6b-3'UTR 3' [9] and codes for four major structural proteins. The spike glycoprotein (S) consists of two subunits, S1 and S2. S1 contains three hypervariable regions (HVR1, HVR2 and HVR3) harboring antigenic epitopes and making the difference between IBV serotypes[10]. Differences in the spike proteins between different strains may promote a vaccine failure[11]. The nucleoprotein (N) and the membrane protein (M) are more stable than the spike protein, S1. Genetic modifications affecting the spike genes promote IBV evolution, co-circulation of different genotypes[12], variability in the pathogenicity[13] and differences in cell tropism[14]. More than 20 known serotypes are detected worldwide[15] and can simultaneously infect the same country[16]. Some types have a worldwide distribution[17] but others, remain localized in indigenous regions[18].

Control of IB is essentially based on the use of live attenuated and killed vaccines. However, the low level of cross-protection between vaccines of different serotypes and the coexistence of several genotypes in the same region are major obstacles to IB control[3]. In some countries, despite vaccination, outbreaks are regularly reported, indicating the ineffectiveness of vaccines against the field strains[8]. Therefore, monitoring and serotyping IBV field strains circulating in each area are required to update vaccination programs[2].

In Algeria, the only investigation that was interested in studying the IBV circulation was conducted by Sid *et al.*[19] in the center of country. Thus, no evidence of IBV circulation in the other provinces of the country was reported. The purpose of the present study was to determine the IBV circulation and prevalence among broiler farms in the eastern Algeria. The study focused on the province of Batna (eastern Algerian), a region characterized by a high density of poultry breeding.

MATERIALS AND METHODS

Sampling area: The study was conducted in four geographic areas (Ras-El-Aioun, Gouigba, Rahbat and Telkhemt) of province of Batna, where broiler farms are predominant. It was limited to the 7 un-vaccinated broiler farms of the mentioned areas. The size of broiler flocks included ranged from 1500-3500 birds. The geographical distribution of broiler flocks is shown in Table 1.

Blood sample collection: Blood samples were collected from seven unvaccinated broiler farms. Sampled chickens were 7-8 weeks old and showed no clinical signs of IB infection. Twenty to thirty-five blood samples per flock, were collected in blood collection glass tubes (Improve Medical®, Guangzhou, China) as described by Gharaibeh[2] , Roussan *et al.*[20] and Bourogaa *et al.*[21]. Collection tubes were kept upright for 24 h at room temperature to clot. After centrifugation (Sigma, Germany), sera were transferred into 1.5 mL tubes (Eppendorf®, Hamburg, Germany) and stored in a freezer (Liebherr™, Strasbourg, France) at -20°C until processed.

Serological tests: Serological tests are used in epidemiological studies for IBV detection, diagnosis[22] and the evaluation of vaccination programs efficiency[21]. The sera were analyzed by an indirect ELISA test (IDEXX infectious bronchitis virus antibody test, IDEXX, US). The test is designed to detect IBV specific antibodies conserved between different serotypes, which promotes the detection of all IBV types. The test consisted of five 96 well plates (precoated with a purified viral

Table 1: Geographical distribution of broiler flocks included in the serological study, as well as the number of bloods samples collected depending on the size of each flock

Town	Number of tested flock	number of chicken in each tested flock	Total samples collected by each flock
Ras El Aioun	2	1500-2000	20-25
Rahbat	2	3000-3500	30-35
Gouigba	1	1500-2000	20-25
Canda	1	1500-2000	20-25
Talkhamt	1	1500	20

antigen), positive and negative IBV antibody control sera (1.9 mL), conjugate (50 mL), sample diluent (235 mL), TMB substrate (60 mL) and stop solution (60 mL).

Serological analysis: The sera were diluted at 1/500 dilution and then vortexed before being used. Two positive and two negative controls were used systemically in each 96 well plate (2 wells for each control). Elisa test was performed according to the manufacturer's recommendations. The microplates were analyzed by the ELISA microplate reader (Metertech 960, Taipei, Taiwan), at a wavelength of 650 nm. The test was valid if the difference between the average value of the positive controls (PC X) and the average value of the negative controls (NC x) is greater than 0.075 and the mean value of negative controls is less than 0.150. The interpretation of the results was determined by the ELISA sample-to-positive (S/P) ratio for each serum. The threshold of positivity was fixed at 0.20 (antibody titer = 396). The samples with E/P$\leq$0.20 (antibody titer less or equal to 396) were negative, whereas samples with E/P>0.20 (antibody titer higher to 396) were considered positive.

IBV prevalence was calculated as the percentage of positive sera among the total number of analyzed sera. The average titer of antibodies was also calculated and compared with the positivity threshold of the test according to Ayim-Akonor *et al.*[4]. IBV antibody titer was classified as low (titer level ranging from 397-1,000), medium (titer levels ranging from 1,001-5,000) or high (titers>5,000).

RESULTS AND DISCUSSION

IBV antibody detection: In the absence of official reports or published research studies, there is no evidence of circulation of the IBV in the East of Algeria. The results of the present study showed, for the first time the circulation of the IBV in broiler farms in the province of Batna (east of Algeria), with a significant mean antibody titer. However, the circulation of the virus was detected in the center of Algeria[19]. Moreover, IBV specific antibodies were detected in poultry farms in different countries. In North Africa, IBV was detected in Egypt[23], Tunisia[21,24], Morocco[25] and Libya[26]. In the west of Africa, it was detected in Niger[27] and Nigeria[28,29]. IBV antibodies were also detected in other African countries such as Ethiopia[30], Ghana[4] and Zimbabwe[31].

Seroprevalence of IBV: In the present study, one hundred and forty-four samples (78.25%) from unvaccinated broiler farm were positive to IBV specific antibodies. It was reported that IB infection and high seroprevalence in unvaccinated adult broiler farms are attributed to infection with wild viruses[30,32-35].

The seroprevalence observed in the present study is very close to that reported in Nigeria (84%)[33], Ghana (85.5%)[4], Sudan (71%)[36] and south west of Nigeria (84.98%)[28]. However, results of the current study remain relatively low (94.5%) compared to that reported in Ethiopia by Hutton *et al.*[30]. Moreover, the seroprevalence observed in our study is relatively higher compared to that found in Pakistan (67%)[37] and France (61%)[38]. The lowest seroprevalence (18.02%) of sera from unvaccinated broiler chickens was reported in Grenada, an isolated island in central America[39].

It is obvious that the high infectivity of the IBV promotes its rapid transmission and consequently increases its seroprevalence[3]. Also because of their systematic use in live vaccines, virus particles can also spread easily to unvaccinated chicken flocks and increase the IB seroprevalences[3]. In this respect, Ahmed *et al.*[37], reported, in Pakistan, that 88% of the sera were positive for M41 serotypes, when only, 40, 52 and 8% were positive for D-274, D-1466 and 4-91serotypes, respectively. The high prevalence of the M strain was explained by its systematic use in vaccinating poultry farms in this country. In this respect it was stated that, despite their wide use in the surveillance of IBV, serological tests have some limitations (origin of antibodies, titer of the humoral response and IBV type), especially in countries where live attenuated vaccines are used. This last facilitates the spread of vaccine strains in the field and influences the interpretation of the results. Therefore, molecular studies should be performed to identify IBV field strains.

The high seroprevalence observed in this study can also be explained by the fact that all blood samples were collected from chickens at the slaughter age (7-8 weeks old). This hypothesis is consistent with results observed by Javed *et al.*[40], who reported that the seroprevalence of IBV increased with age, because of the long period of exposure to field viruses. Also, vaccine antibodies, (vaccines are usually given in the early stages of age), do not persist for a long time after administration; infection with wild viruses occurs after the decline of maternal antibodies. Ducatez *et al.*[33] reported that in chickens, IBV seroprevalence related to vaccine antibodies is reduced to 0% between 2 and 7 weeks of age. In the same way, Cavanagh and Naqi[3] and Emikpe *et al.*[28] also reported that maternal antibodies decline rapidly and early between the 3rd and the 4th weeks of age. It means that after the decline of vaccine/maternal antibodies, a positive result to serological tests occurs following exposure to a wild virus. Consequently, antibodies

detected in unvaccinated adult chickens could be considered as an indicator of infection with wild-type viruses.

The mean titer of IBV specific antibodies: The mean titer of IBV observed in the present study was 2080, significantly (5 times) higher than the threshold (396) reported for negative samples. This titer is considered as medium titer, between low titers (1,001) and high titers (5,000), because it was observed in industrial chicken flocks. An antibody titer of 1427 was considered high, by Hadipour *et al.*[41], because their serological study was conducted on endogenous chicken flocks. Moreover, the unvaccinated status of flocks and the age of chickens (slaughter age) would indicate a natural infection with a low virulence strain of IBV.

CONCLUSION

The present study showed for the first time, the exposition of unvaccinated broiler chickens to IBV field strain in the east of Algeria, as indicated by its high seroprevalence. Given the high prevalence observed in the study, IBV should be considered as one of the major respiratory diseases found in poultry farming in eastern Algeria. Therefore, the vaccination coverage needs to be broader.

SIGNIFICANCE STATEMENT

This study confirms that unvaccinated broilers are highly exposed to IBV in the East of Algeria, a region where there is the highest density of commercial poultry. This should convince breeders, practitioners and public authorities to systematize vaccination against infectious bronchitis and thus reduce the circulation of wild viruses.

ACKNOWLEDGEMENT

There were no external funding sources for this study. Authors thank Dr. Hamza Rahab for his insights and cooperation.

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