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Research Article
Sero-Epidemiological Survey of the Main Respiratory Infections in Broiler Farms in the Peri-Urban Area of Dakar and Thies (Senegal)

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Abstract

Background and Objective: A seroepidemiological survey was carried out in Senegal in an area with a high concentration of poultry for Newcastle disease, infectious bronchitis (IB), LPAI subtype H9 and *Mycoplasma gallisepticum* mycoplasmosis in broilers. **Materials and Methods:** During the survey, 20 broiler farms with at least 1,000 clinically affected birds per flock aged 16-45 days with respiratory symptoms were visited. On each farm, a questionnaire was administered and blood samples were taken from 20 chickens in the most affected house. A serological investigation by indirect ELISA was carried out on 384 sera. Serological infection rates varied according to disease, age, vaccination program and husbandry system. **Results:** The infection rates were in order of importance 40% for LPAI subtype H9, 35% for infectious bronchitis and 5% for Newcastle disease. Serology was negative for *Mycoplasma gallisepticum*. Infection rates were higher in the department of Rufisque, in semi-intensive farms and older chickens. Co-infections were observed with higher importance for the IB-LPAI association. The evaluation of vaccine efficacy revealed that 70% of cases of Newcastle disease, 42.86% of cases of infectious bronchitis and 26.31% of cases of LPAI subtype H9 were suspected of being ineffective. **Conclusion:** This work shows the need to have a database for the implementation of a medical prophylaxis programme specific to the epidemiological conditions of these farms. Nucleic acid sequencing to identify circulating serotypes is the next step.

Key words: Broiler chicken, respiratory infections, newcastle disease, infectious bronchitis, LPAI subtype H9, Mycoplasma gallisepticum, seroepidemiology

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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

Respiratory infections are the main cause of mortality in poultry farms. They are complex diseases, difficult to control because of their multifactorial character and the importance of co-infection[1]. In addition, respiratory viruses are highly contagious and some of them have a great evolutionary capacity[2]. Five main viruses are of dominant importance in respiratory diseases of poultry namely Newcastle disease, Infectious Bronchitis, Avian Influenza, Infectious Laryngotracheitis and Chicken "Big Head" Infectious Syndrome viruses. The bacteria associated with these diseases are mainly *Mycoplasma gallisepticum* and *Mycoplasma synoviae*, responsible for mycoplasmosis, and *Avibacterium paragallinarum*, responsible for infectious coryza[3,4].

Furthermore, risk factors related to biosecurity and husbandry practices favour the occurrence, expression and spread of these diseases and thus increase the severity and economic losses[5]. Respiratory diseases have become very common in poultry farms in Senegal.

The prevention of clinical infections is based on vaccination, which is widely practised in poultry farming. Despite the existence of control methods, their prevalence remains high in Senegalese broiler farms. Control of these diseases requires the identification of new viral variants circulating in these farms to strengthen vaccination programs for better efficiency. However, few studies have been carried out in Sub-Saharan Africa on the viral and bacterial strains associated with respiratory infections in poultry and the suitability of effective vaccines initially developed for the European and North American markets to African viral strains deserves an evaluation[5].

The objective of this study was to determine the seroprevalence of the main respiratory infections in broiler flocks in the peri-urban area of Dakar and Thiès (Senegal).

MATERIALS AND METHODS

Field survey

Study area and period: The study concerned the peri-urban region of Dakar and Thies (Fig. 1) in the Niayes area where there is a high concentration of industrial poultry production. A cross-sectional survey was carried out in twenty modern broiler farms of the Cobb 500 strain, suspected of respiratory infections on the referral of poultry veterinary practitioners in four departments (Rufisque, Thies, Mbour and Guediawaye). After a suspected case was reported, the case was visited at the farm level within 1-2 days. The collection of data and blood samples was carried out for 4 months, from January-April, 2020.

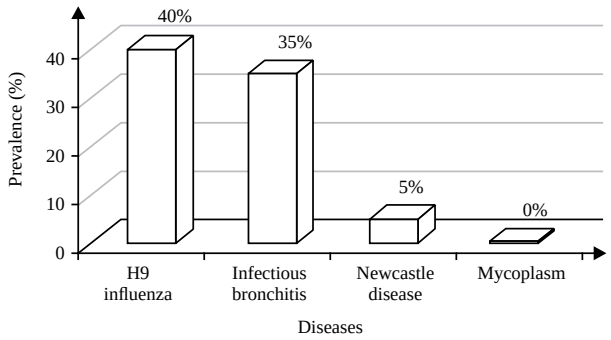

Fig. 1: Prevalence of respiratory infections on the broiler farms investigated

Questionnaire: The visit to each farm was recorded on an information sheet in the KoBoToolbox software. The information was collected from the farmers and based on observations made at the farm level. It concerned the characteristics of the farms, technical, sanitary and medical monitoring, breeding practices and biosecurity measures, the dominant diseases described, the factors influencing the appearance of these diseases, sanitary and medical prophylaxis, diagnosis and treatment of respiratory infections. Other information was collected from the veterinarians in charge of the follow-up and from the hatcheries of origin of the chicks, in particular on the various vaccines that the chicks had received. The KoBoToolbox software was used for statistical processing of the collected information and provided summary reports with graphs and tables, as well as geolocation of the surveyed area. The chi-2 test (implemented in R 4.03 software) was used to test the null hypothesis of no relationship between risk factors and the presence of respiratory infections.

Samples: A total of 384 blood samples were taken. The blood was taken from the venous sinus located under the base of the cerebellum (occipital sinus) of the chickens, directly at the farm. The samples were transported and stored in a cooler containing carbohydrate ice.

Laboratory tests

Serology: The blood samples sent to the EISMV serology unit were centrifuged the same day (3000 rpm for 15 min). The

collected sera 384 were kept in the freezer and were analysed by the indirect ELISA technique using kits from the company IDvet: ID Screen ®Newcastle Nucleoprotein indirect, ID Screen ®Influenza H9 Indirect, ID Screen

®Infectious bronchitis indirect and ID Screen ®Mycoplasma gallisepticum indirect. The serological analysis consisted of testing and quantifying antibodies of infectious, maternal or vaccine origin against Newcastle Disease Virus (NDV), Infectious Bronchitis Virus (IBV), IAFP (VIAFP) subtype H9 and *Mycoplasma gallisepticum*. The ELISA plate reader used (BIO-RAD) was used to obtain the optical densities (OD) transformed into antibody titers interpreted in the form of lists and graphs with the IDSoftTM software. The average titers obtained in each farm were compared with the general IDvet baselines to deduce infectious and vaccine titers. Probable seroprevalence were determined from the different infectious titers obtained.

RESULTS

Characteristics of the farms surveyed: About 75% of the farms surveyed were semi-intensive (1000-4000), 15% intensive (6000-40000) and 10% industrial (50000 and more). The age of the chickens sampled varied between 16 and 45 days. None of the farms had birds in the start-up phase (1-15 days), 40% had birds in the growth phase (16-28 days) and 60% had birds in the finishing phase (29 days and more).

Serology: Serological screening of flocks by indirect ELISA resulted in seroprevalence of 40% [CI: 18.53; 61.47] for LPAI subtype H9, 35% (CI: 14.10: 55.90) for infectious bronchitis and 5% (CI: 0.00: 14.55) for Newcastle disease. Serology remained negative for *Mycoplasma gallisepticum* (Fig. 1). Infection rates varied according to the areas surveyed, the farming system, the age of the chickens (production phase) and the vaccination protocol (Fig. 2-5). The highest prevalence were obtained in Rufisque (Fig. 2). Newcastle disease infection, as well as 25% of the infection rate for infectious bronchitis, was observed in the semi-intensive flocks that were the most infected. Chickens in the start-up phase as well as those from industrial farms were seronegative. Infected chickens were those in the growing and finishing stages. Indeed, the highest infection rates were obtained in the finishing flocks (100% for Newcastle disease, 86% for Infectious Bronchitis and 62.5% for Influenza H9).

Co-infections were also observed, notably one co-infection for infectious bronchitis and Newcastle disease and

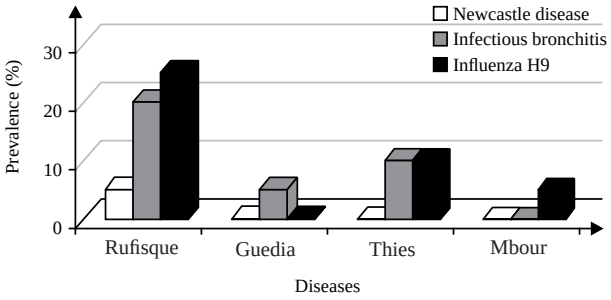

Fig. 2: Prevalence of respiratory infections by survey area

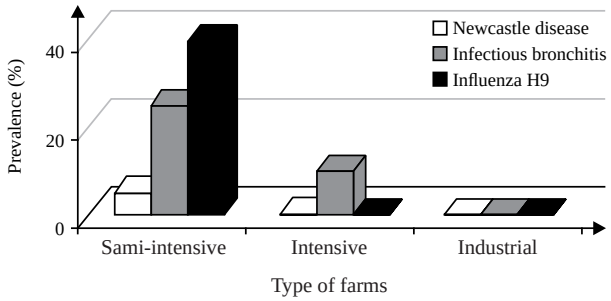

Fig. 3: Prevalence of respiratory infections according to the type of farm

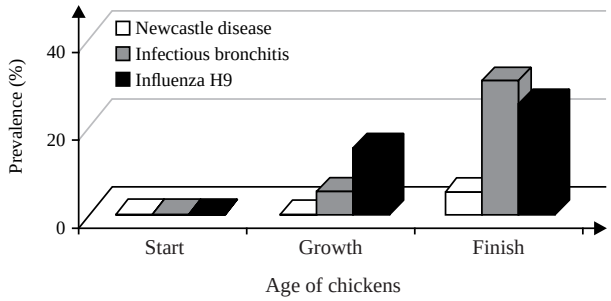

Fig. 4: Prevalence of respiratory infections according to age

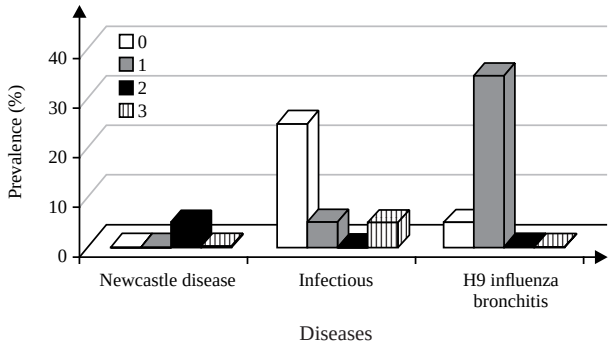

Fig. 5: Prevalence according to the vaccination protocols applied on the surveyed farms

Table 1: Risk factors for respiratory infections

Risk factors		Presence of respiratory infection (%) Yes	No	p-value
Irregularity of visits by the poultry advisor	Yes	60	25	0.0214
	No	0	15	
Manual watering system	Yes	60	25	0.0214
	No	0	15	
No second disinfection of the buildings after the stamping out	Yes	60	25	0.0214
	No	0	15	
Poor quality of bedding (wet in places)	Yes	45	5	0.00617
	No	15	35	
No foot bath	Yes	50	15	0.03527
	No	10	25	

Chi-square test: Significant at $p<0.05$

three co-infections for infectious bronchitis and LPAI subtype H9. According to the vaccination protocol, Newcastle disease was only observed in farms that had received primary vaccination with two boosters. For infectious bronchitis, among the 35% of infected farms, 25% did not vaccinate, 5% had a primary vaccination without a booster and 5% had a primary vaccination with two booster shots. None of the farms that received primary vaccination with a booster was infected. The seroprevalence of Influenza H9 (40%) was 35% in farms using a primary vaccination without a booster and 5% in those that did not vaccinate (Fig. 5).

Risk factors: There was a statistically significant association between certain variables such as irregular visits by the poultry advisor, manual watering system, lack of second disinfection of the buildings after sanitation, poor quality of bedding, lack of foot bath and the occurrence of respiratory infections (Table 1).

Medical prophylaxis: First of all, it appears that the vaccination protocols are very diverse. Each breeder has his protocol and seems to use it according to the time and money they spend on it (sometimes none). The vaccinations done are only directed against viral infections. Primary vaccinations are mostly done at the hatchery and all boosters, if done are done on the farm by drinking water (90%), dipping (30%) or spraying (15%). Vaccination against Gumboro disease and anti-coccidial use for prevention are also taken into account. No farms conducted serological tests to monitor vaccine uptake. The evaluation of vaccine efficacy based on the average titers obtained led to the suspicion of vaccine inefficacy in 70% of cases for Newcastle disease, 42.86% for infectious bronchitis and 26.31% for LPAI subtype H9.

DISCUSSION

This study assessed the infection rates of Newcastle disease, infectious bronchitis, VIAFP subtype H9 and *Mycoplasma gallisepticum* in the peri-urban area of Dakar and Thies. The high seroprevalence of 40% obtained for LPAI-H9 is much higher than the 7.78% found in Sonali chickens from Joypurhat district[6], but in both cases, the prevalence was higher in older subjects, which might be due to prolonged exposure to infections. The relatively high prevalence of infectious bronchitis infections could be explained by the low vaccination coverage in broiler farms but also by the presence of new variants that can appear by mutation (point mutations, deletions) or by recombination on the viral genome (if a cell is infected by two different strains of the same virus).

The low prevalence of Newcastle disease could be explained by the high vaccination pressure in farms.

Mycoplasma gallisepticum infection was not detected in any of the farms surveyed, probably due to the short production cycle of broilers. We noted in our study that respiratory diseases attack older chickens more easily[7].

One co-infection for infectious bronchitis and Newcastle disease and three co-infections for infectious bronchitis and LPAI subtype H9 were revealed, reflecting the importance of co-infection which makes these diseases complex and difficult to control. It is essential that good hygiene and biosecurity measures to prevent the introduction of viruses and bacteria be applied on poultry farms.

The serological results also suspected vaccine ineffectiveness in 70% of cases for Newcastle disease, 42.86% for Infectious Bronchitis and 26.31% for LPAI subtype H9. It, therefore, appears important to vaccinate chickens correctly with adequate vaccines[8,9]. always respecting the booster vaccination to optimize the protection of subjects. Indeed, chickens would be effectively protected only during the first 2 weeks of their life. Reliable knowledge of the circulating serotypes in the region is the prerequisite for successful vaccination. On the other hand, the immune status of breeding hens should be taken into account in chick vaccination programmes, which depend mainly on the neutralising power of maternal antibodies and their ability to

suppress active immunity. Progress could be made in the hatcheries by avoiding, for example, mixing hatching eggs of different origins for the same hatching. Indeed, the heterogeneity of batches is unfavourable to the effectiveness of vaccination because the titres pass below the inhibition threshold at very different times[10,11]. As the entry points for respiratory infectious agents are the ocular, nasal and oral routes, administration of vaccines by dipping or spraying develops local immunity in chickens, which would be much more effective than administration of vaccines by drinking water.

The limitations of the study lie mainly in the lack of data on the number of meat poultry farms in the study area and the interpretation of the serological results, which was rather complicated because only one blood sample was taken from the chickens.

CONCLUSION

In the 20 farms involved in this study, the presence of NDV, IBV, LPAI subtype H9 and *Mycoplasma gallisepticum* in the various meat poultry productions in Senegal was shown by serological analysis. The infection rate of the flocks visited was 40% for LPAI subtype H9, 35% for infectious bronchitis and 5% for Newcastle disease. This study did not reveal the presence of *Mycoplasma gallisepticum*. The serological results also showed a vaccine inefficiency of 70% for Newcastle disease, 42.86% for infectious bronchitis and 26.31% for LPAI subtype H9. This study should be extended to nucleic acid sequencing to identify the serotypes circulating in Senegalese farms and, at the same time, to evaluate the effectiveness of commercial vaccines and extended to other regions of the country to establish a map to serve as a basis for epidemiological surveillance.

SIGNIFICANCE STATEMENT

This study showed the strong involvement of NDV, IBV, LPAI subtype H9 and *Mycoplasma gallisepticum* in the respiratory diseases described in the Dakar and Thiès Regions. This study may be beneficial to farmers, poultry industry stakeholders in Senegal, researchers, and the Senegalese government. This study will help researchers to identify the different microbial agents in the region, to know their prevalence to better understand the degree of involvement of these agents in respiratory diseases.

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