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Abstract

Purpose: This study aimed to identify associated factors and the prevalence of major avian respiratory diseases, including Mycoplasma gallisepticum, Influenza H9, Infectious Bronchitis (IB), and Newcastle disease in poultry farms in the Dakar and Thiès regions of Senegal.

Methodology: A cross-sectional observational study was conducted on purposively selected farms with over 1000 birds exhibiting respiratory symptoms. Blood samples (400) were collected for serological analysis, and post-mortem examinations were performed to assess disease prevalence. Additionally, data on biosecurity, vaccination, medication practices, general management, and farm infrastructure were obtained through direct observation. Student t-tests were used to analyze the association between different farming practices and respiratory infections.

Findings: The results indicated that proximity to neighboring farms, non-compliant buildings, mixed-age groups, and lack of farmer training were associated with respiratory diseases in the surveyed farms. Serological analysis revealed a high prevalence of Mycoplasma gallisepticum and Influenza H9, each detected in 50% of the farms. Infectious Bronchitis was found in 40% of the farms, while Newcastle disease was detected in 35%. Co-infections were common, with the combination of H9 influenza and Mycoplasma gallisepticum being the most prevalent (33%).

Unique Contribution to Theory, Practice and Policy: The study emphasizes the need for targeted interventions, including improved biosecurity measures, vaccination strategies, and farmer training, to control respiratory infections. Strengthening biosecurity protocols and enhancing farmer education will help reduce disease.

Keywords: *Respiratory Disease, Senegal, Biosecurity, Layer Hens*

INTRODUCTION

Respiratory infections in poultry have been considered as one of the major concerns for the poultry industry due to their negative impacts on both bird health and productivity and, thus, on economic outcomes(Ali, 2020; Yehia et *al*., 2023). Actually, such infections usually have a synergistic consequence of viral and bacterial pathogens, which may worsen the disease severity and increase the mortality rate (Umar et *al.,* 2019). Notable viral respiratory infections include Newcastle disease (ND), infectious bronchitis (IB), Avian influenza (AI), and infectious laryngotracheitis (LTI).

Newcastle disease and avian influenza are the two most important viral respiratory infections in poultry that exist in West Africa, specifically in Senegal (Kone et *al*., 2013; Molia et *al.*, 2017). The ND is endemic to this region, with high seroprevalence rates while AI, especially the H5N1 subtype of highly pathogenic avian influenza, has been a concern since its outbreaks in 2006. In 2017, outbreaks of Low Pathogenic Avian Influenza subtype H9N2 and ND were reported in Dakar and Thiès (Ministry of Livestock and Animal Productions, 2018). More recently, in 2021, outbreaks of High Pathogenic Avian Influenza H5N1 were recorded in poultry farms in Ndiakhirate, Pout, and Thiès (Tancrede, 2021; Ndiaye, 2023).

Besides viral pathogens, bacterial infections especially by Mycoplasma species are of great importance in the respiratory disease complexes in poultry. Such infections often occur together with viral infection and sometimes increase their clinical expression, leading to mortality. For example, Abdelaziz et *al*. (2019), concluded that mixed infections of infectious bronchitis virus, low pathogenic avian influenza virus H9N2, and *Mycoplasma gallisepticum* have resulted in severe economic loss in both backyard and commercial broiler flocks. According to Kadja et *al.,* (2021), respiratory diseases are more prevalent in layer and broiler farms especially in peri-urban areas of Dakar and Thies.

The interplay of several respiratory pathogens and their interaction which has severe consequences on poultry health argues for a complex approach to disease management. This would involve improved biosecurity, effective vaccination, and health monitoring for the reduction of risks of respiratory infections (Al-Natour et *al*., 2024). Knowledge of the epidemiology and risk factors of these infections, therefore, provides a foundation for the design of focused interventions meant for the control and prevention of outbreaks in poultry populations.

Despite significant research on respiratory diseases in poultry, little attention has been given to the peri-urban areas of Senegal, particularly in medium-sized layer farms, which are subject to unique disease transmission risks due to their proximity to urban centers and neighboring farms. Previous studies, such as the one conducted by Kadja et *al*., (2021), have primarily focused on peri-urban and large-scale broiler farms, leaving a gap in understanding the prevalence and co-infections of viral and bacterial pathogens, like Mycoplasma gallisepticum and Influenza H9, in layer farms in these densely populated regions. The present study, therefore, aims to identify associated factors for respiratory diseases in laying hens and establish the prevalence of major avian respiratory diseases and co-infections using serological tests (ELISA). Supported by the Epidemiologic Triad Theory, this research offers critical insights for farmers, veterinarians, and policymakers, helping improve disease prevention strategies in Senegal.

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METHODOLOGY

Study Areas

The present study was conducted in Senegal, principally in the Dakar and Thiès regions (Figure 1). These areas were purposively selected considering their very significant contribution to the Senegalese poultry industry. Dakar is a 550 km² large city located about 14.6928° N latitude and -17.4467° W longitude. At the time of the 2019 census, its population was 3,732,282. On an administrative basis, Dakar is further divided into four departments, including Dakar, Pikine, Guédiawaye, and Rufisque. This region was purposely selected because of its fine climate, the extensive facilities needed in poultry farming, and its being the large urban center.

Thiès, covering an area of 6,882 km², was located at about 14.7901° N latitude and 16.9241° W longitude, with a population of 2,105,711 in 2019. It comprises the Niayes area, which is very suitable for poultry farming. These two regions have 80% of Senegal's modern poultry farms; knowledge of their dynamics and health issues is therefore of paramount importance for the industry.

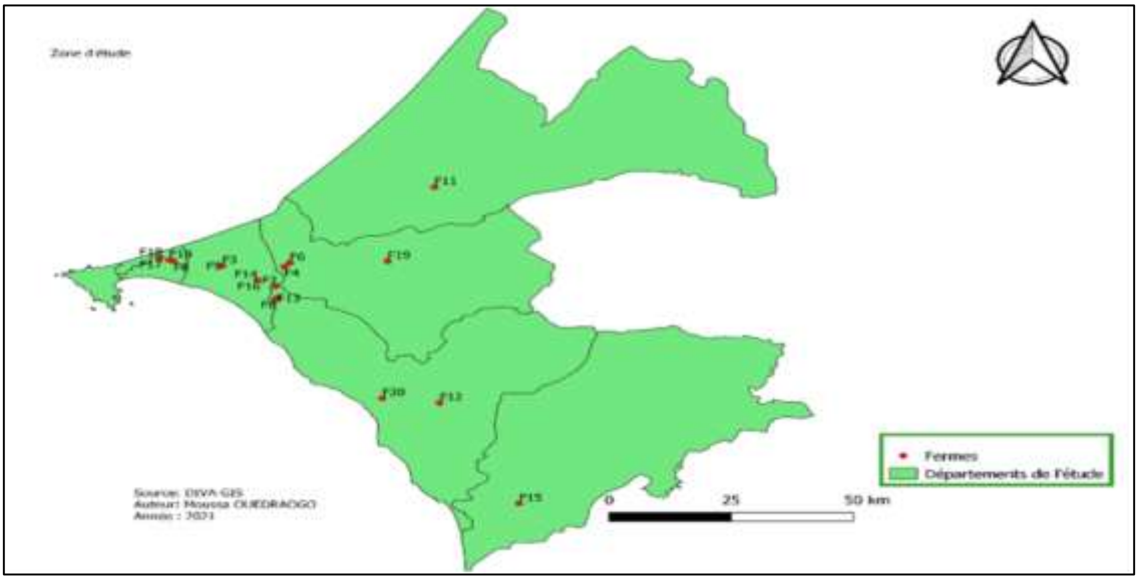

Figure 1: Map of the Study Area

Study Design

In this study, a cross-sectional observational design was adopted. Farms were stratified into two regions: Dakar and Thiès. From these strata, purposive sampling was done in order to get a sample size of 20 farms with more than 1,000 birds experiencing respiratory symptoms.

Blood samples were then taken from 20 birds randomly selected in each farm to ascertain the serological prevalence of some of the important respiratory diseases, such as Newcastle Disease, Influenza H9, Infectious Bronchitis, and *Mycoplasma gallisepticum*.

Furthermore, on each farm, post-mortem was on 10 birds to further investigate the extent of respiratory diseases and pathological changes.

Information on management practices about biosecurity measures, vaccination and medication practices, general management practices, and farm infrastructure was obtained through direct observation of the research team in each of these farms. Georeferencing of every farm's location relative to other farms was also obtained to assess any geographical risk factors and dynamics of disease transmission.

Sample Size Determinations

The Cochran formula (Bolarinwa, 2020) was used to determine the sample size with a 95% confidence level, proportion, and margin of error.

$$\mathrm{n} = \frac{Z^2 . p . (1 - p)}{\mathrm{d}^2}$$

The following parameters were employed in this study: the proportion expected to be covered = 0.50, the margin of error, d = 0.05, and the confidence level, 95% = Z = 1.96. The estimated sample size was 384 based on these values.

Collection of Blood Samples

A total of 3 ml of aseptic blood samples were obtained from the brachial vessels of layer birds using disposable 22G needles and syringes. The blood was subsequently transferred to sample containers that were clearly labelled. After clotting, the samples were transported to the serology laboratory of the Inter-State School of Veterinary Science and Medicine for analysis on ice and kept cold. The serum was obtained by centrifuging the samples at 978g for 10 minutes upon their arrival at the laboratory. The serum was maintained at a temperature of -20°C in 2-mL cryovial containers until it was tested.

Serological Examination

The collected sera were analysed by the indirect ELISA technique with the diagnostic kits from IDvet. Such assays allow detection of antibodies against certain pathogens: Newcastle Disease, Influenza H9, Infectious Bronchitis, Mycoplasma gallisepticum. The optical density was directly converted by IDvet's IDSoft™ software into antibody titters. The program interpreted the titters, generating lists and graphs of the distribution of the antibody titters within the farms. These graphs provided information about protection and inhibition thresholds of the maternal antibodies with the maximum and minimum values and coefficients of variation.

Data Management and Analysis

Data were recorded in Microsoft Excel® 2019 and analyzed in STATA software. Descriptive statistics summarized the findings in the form of tables, graphs, and charts. Among the statistical tests conducted were student t-tests to probe the association between different farming practices and the presence of respiratory infections in surveyed poultry farms. Contingency tables were put forth to show associations between certain variables and respiratory diseases.

RESULTS

Factors Associated with Respiratory Infections in Surveyed Farms

The analysis of risk factors associated with respiratory infections in surveyed farms reveals significant associations with specific farm practices (Table1). Respiratory infections were significantly associated with proximity to neighbouring farms (p-value < 0.0001), non-compliant buildings (p-value < 0.0001), and buildings that were inadequately oriented in relation to the prevailing winds (p-value < 0.0001)Furthermore, the presence of distinct age groups (p-value = 0.00021), a sanitary gap of less than 15 days (p-value < 0.0001) and a lack of training among poultry farmers were all associated with higher infection rates (p-value =

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0.00007). Strong associations were also observed with non-daily cleansing of drinkers, poor litter quality, and the absence of litter treatment prior to use (p-value < 0.0001).

Intriguingly, the absence of water analysis and treatment, the use of manual irrigation systems, and the proximity to residences did not demonstrate significant associations with respiratory infections (p-values: 0.3299 for all). Nevertheless, the need for rigors biosecurity measures was underscored by the strong association observed in farms that were accessible to outsiders (p-value < 0.0001).

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Tableau 1: Analysis of Risk Factors Associated with Respiratory Infections in Surveyed Farms

Variables		Presence of Respiratory Infections in the farm		p-value
		No	Yes	
Proximity to neighboring farms less than 500m	No	0	1	0,0001
	Yes	14	5	
Proximity to residences	No	0	1	0,3299
	Yes	3	17	
Non-compliant buildings	No	0	1	0,0000
	Yes	1	19	
Poorly oriented buildings with respect to prevailing winds	No	0	1	0,0001
	Yes	14	5	
Irregular visits by the poultry advisor	No	0	1	0,1864
	Yes	4	15	
Untrained poultry farmers	No	0	1	0,0007
	Yes	12	7	
Presence of different age groups	No	0	1	0,0021
	Yes	8	11	
Manual watering system	No	0	1	0,3299
	Yes	1	19	
Duration of sanitary gap (less than 15 days)	No	0	1	0,0000
	Yes	1	19	
No second disinfection of buildings after the sanitary gap	No	0	1	1,000
	Yes	1	18	
No prophylaxis program against pests	No	0	1	0,1036
	Yes	5	14	
No analysis of drinking water for chickens	No	0	1	0,3299
	Yes	1	19	
No treatment of drinking water for chickens	No	0	1	0,3299
	Yes	1	19	
Non-daily cleaning of drinkers	No	0	1	0,0000
	Yes	17	2	
No treatment of litter before use	No	0	1	0,0000
	Yes	18	1	
Poor litter quality (wet in places)	No	0	1	0,0000
	Yes	16	3	
No footbath	No	0	1	0,3299
	Yes	3	16	
Accessibility of the farm to outsiders	No	0	1	0,0000
	Yes	13	6	
No dusting of nets during the batch	No	0	1	0,1864
	Yes	4	15	
No measurement of temperature and humidity in breeding buildings	No	0	1	0,5770
	Yes	2	17	

Post Mortem Examination

The comprehensive postmortem examination performed on 200 birds from all surveyed farms indicated a variety of lesions that were classified according to the systems that were affected (Figure 2). Actually, respiratory lesions were the most prevalent, affecting 39.75% of the birds. The second most frequent were the digestive lesions, occurring in 18.67% of the birds.

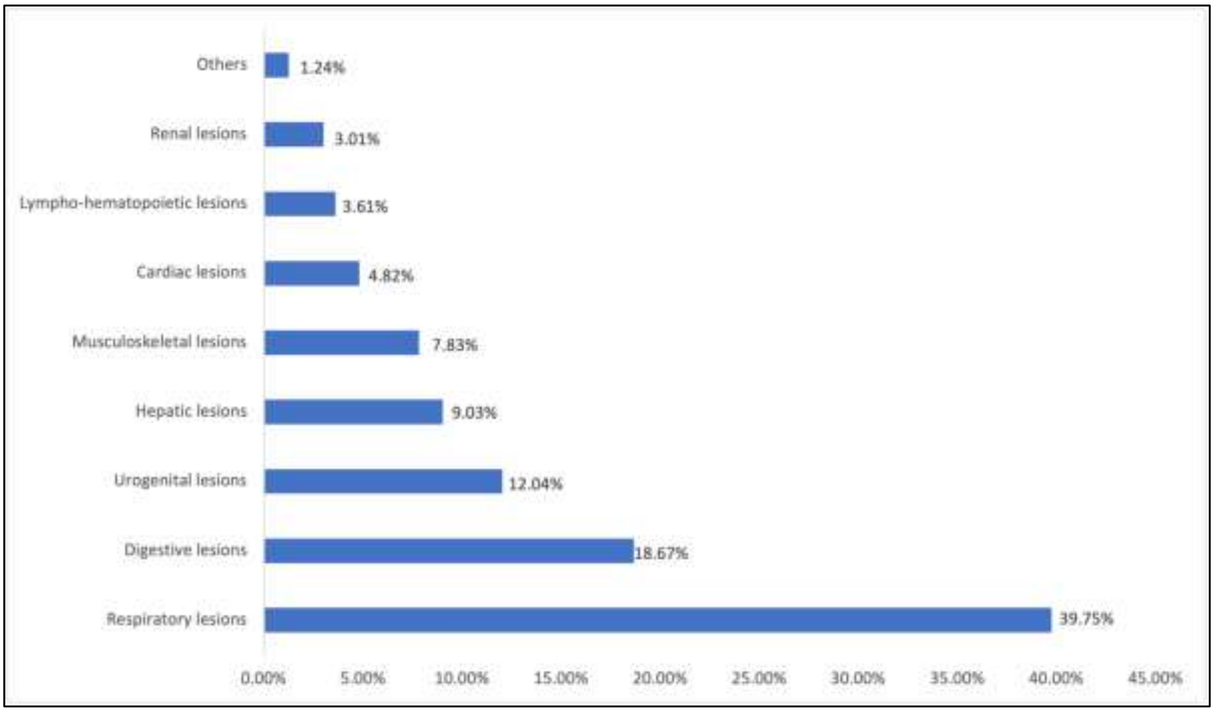

Figure 2 : Distribution of Lesions Identified in Postmortem Examination

Digestive lesions were followed in frequency by urogenital lesions, which occurred in 12.04% of the birds.

Moreover, 9.03% of the birds had hepatic lesions. Another 7.83% of the birds had musculoskeletal lesions. Cardiac lesions were recorded in 4.82% of the birds. Besides, 3.61% of the birds showed lesions in the lymphatic and hematopoietic systems; 3.01% showed renal lesions. Lastly, 1.24% of the birds had lesions categorized as "others."

Serological Test

Farm Prevalence

The serological result (Table 2) indicated *Mycoplasma gallisepticum* and Influenza H9 to be the most prevalent respiratory disease, each being isolated in 10 of the 20 farms tested. Infectious Bronchitis was detected in 8 out of the 20 farms with a prevalence rate of 40%. The least common of the major respiratory infections was Newcastle disease, which was found in 7 of the 20 farms to give a prevalence rate of 35%.

Table 2: Prevalence of Major Respiratory Infections Across Surveyed Farms

Disease	Farms Tested	Farms Positive	Prevalence	95% Confidence Interval
Mycoplasma gallisepticum	20	10	50%	[28.087; 71.913]
Influenza H9	20	10	50%	[28.087; 71.913]
Infectious Bronchitis	20	8	40%	[18.529; 61.471]
Newcastle Disease	20	7	35%	[14.096; 55.904]

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Regional prevalence

Further, the study delineated the distribution of these infections across the different regions (Figures 3). Rufisque exhibited the greatest prevalence rates for the majority of diseases. Rufisque had the maximum prevalence of infectious bronchitis at 25%, while Pikine, Thiès, and Mbour each had a prevalence of 5%, and Tivaoune had none. Newcastle Disease was most prevalent in Rufisque at 20%, while Thiès and Mbour presented prevalence of 10% and 5%, respectively, with no cases in Pikine and Tivaoune. Influenza H9 had a prevalence of 20% in Rufisque and in both Thiès and Mbour 15%, but none in Pikine and Tivaoune. Mycoplasmosis revealed a different trend, with the highest prevalence being observed in Thiès at 20%. Whereas 15% was observed in Rufisque and Mbour, it was only as low as 5% in Pikine and Tivaoune.

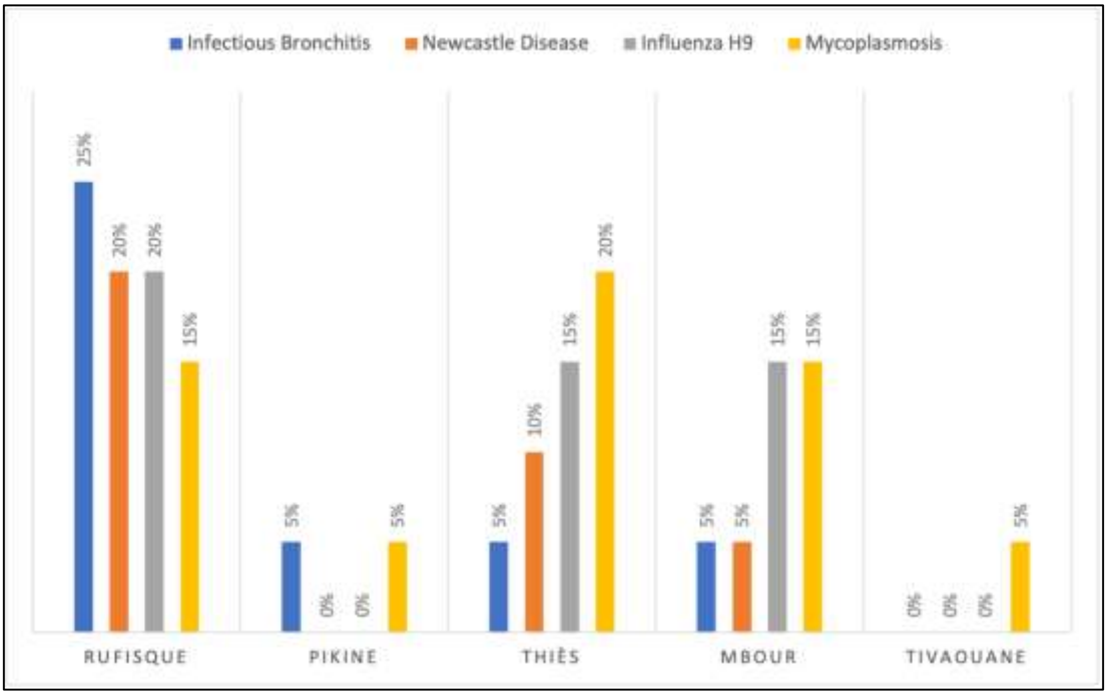

Figure 3: Seroprevalence of Major Respiratory Diseases in Poultry Farms across Dakar & Thies Departments

Prevalence according to Vaccination Programs

The seroprevalence of Newcastle disease, infectious bronchitis, and IAFP varied significantly according to the vaccination programs used (Figure 4). For Newcastle disease, the highest seroprevalence (42.85%) was observed with program P5 (3x live vaccine, 1x Vectormune HTV NDV), followed by program P4 (3x live vaccine, 1x inactivated vaccine) at 28.57%, and programs P1 (2x live vaccine, 2x inactivated vaccine) and P2 (3x inactivated vaccine, 1x live vaccine) each at 14.29%, while no seroprevalence was detected in programs P3 (4x live vaccine) and P6 (2x inactivated vaccine, 1x live vaccine). For infectious bronchitis, programs P1, P3, and P5 each had a seroprevalence of 25%, while programs P2 (4x live vaccine (Mass)) and P6 (1x live vaccine (Mass)) showed 12.5%, and no seroprevalence was found in program P4 (2x live vaccine (Mass + 793/B)). IAFP seroprevalence was highest in program P2 (2x inactivated vaccine) at 80%, followed by program P1 (1x inactivated vaccine) at 20%, with no seroprevalence observed in program P3 (3x inactivated vaccine). No farm vaccinates against avian mycoplasmas infection.

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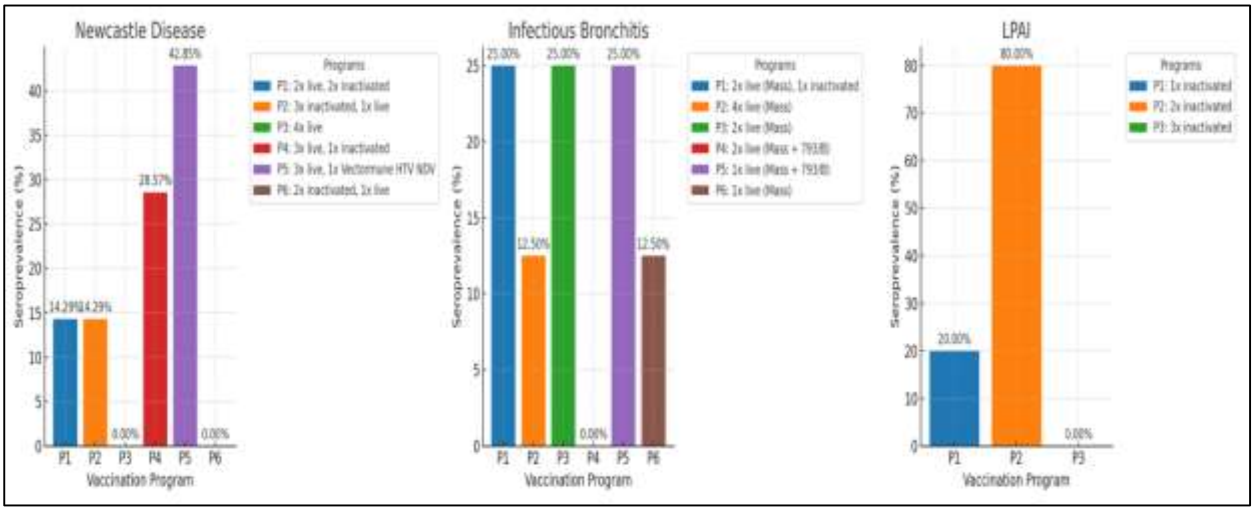

Figure 4: Sero-prevalence according to Vaccination Programs

Co-infection Prevalence

In this study, co-infections were identified in certain farms (Table 3). The combination of H9 influenza and mycoplasma gallisepticum infection was the most prevalent, accounting for 33% of cases. Newcastle disease coupled with infectious bronchitis and Newcastle disease in conjunction with mycoplasma gallisepticum infection were the second most prevalent, both representing 17% of the cases. The combinations of infectious bronchitis with H9 influenza, infectious bronchitis with mycoplasma gallisepticum infection, and Newcastle disease with H9 influenza were the least prevalent, both at 11%.

Table 3: Prevalence of Co-infection across Surveyed Farms

Co-infection	Prevalence (%)
H9 influenza + Mycoplasmosis	33
Newcastle disease + Infectious bronchitis	17
Newcastle disease + Mycoplasmosis	17
Infectious bronchitis + H9 influenza	11
Infectious bronchitis + Mycoplasmosis	11
Newcastle disease + H9 influenza	11

Discussion

The current study was conducted to identify risk factors and the prevalence of avian respiratory diseases and co-infections in poultry in order to inform effective prevention and control strategies for the poultry sector in Senegal. The result revealed several key risk factors associated to respiratory disease in the surveyed farms. Proximity to neighboring poultry farm significantly increased the incidence of respiratory diseases (p-value < 0.0001). This can be linked to the fact that farms close to each other might facilitate pathogen spread through shared airspace, personnel, or equipment. This finding aligns with Wells et *al.* (2017) and Ssematimba et *al.* (2019) who highlighted the importance of geographic location and inter-farm distance in disease spread.

Additionally, non-compliant and poorly oriented buildings emerged as significant risk factors (p-value < 0.0001). Proper infrastructure and ventilation are crucial for controlling respiratory diseases. Kadja et *al.* (2021) and Wang et *al.* (2023) concluded that poorly designed buildings facilitate pathogen accumulation and spread. Furthermore, the presence of mixed-age groups within the same farm was associated with higher respiratory infection rates (p-value = 0.00021). Mixed-age groups increase the risk of younger birds being exposed to pathogens carried by older birds, thereby supporting all-in/all-out systems. In line with this, East (2007) highlighted the benefits of single-age groups in reducing infection rates.

Moreover, lack of training among poultry farmers (p-value = 0.00007) emerged as a significant risk factor. This aligns with Kadja et *al*. (2021) and Meher et *al*. (2020) who found that farmer education significantly improves biosecurity practices. Non-daily cleaning of drinkers, poor litter quality, and the absence of litter treatment prior to use were significantly associated with higher infection rates (p-value < 0.0001 for all). Effective cleaning methods can decrease respiratory pathogens (Burbarelli et *al*., 2020).

In contrast, the absence of water analysis and treatment and manual systems did not demonstrate significant associations with respiratory infections (p-values: 0.3299 for all). Although these factors may be relevant in certain situations, they were not the primary driving force behind this investigation. However, the health of poultry can be influenced by a variety of water sources, with filtered water demonstrating some advantages ELSaidy et *al*. (2015).

On the other hands, the presence of respiratory infections was significantly correlated with the accessibility of farms to outsiders (p-value < 0.0001). This emphasises the necessity of stringent biosecurity protocols to mitigate pathogen risk and restrict external access. Studies conducted by Guinat et *al*. (2020) and Kadja et *al*. (2021) have demonstrated that the implementation of rigorous access controls can substantially reduce the incidence of disease.

Postmortem examinations revealed respiratory lesions as the most prevalent (39.75%), followed by digestive (18.67%) and urogenital lesions (12.04%), underscoring the need for targeted respiratory health interventions. Serological testing identified Mycoplasma gallisepticum (MG) and Influenza H9 as the most common respiratory diseases, each found in 50% of farms. Additionally, Infectious Bronchitis and Newcastle Disease were significant, found in 40% and 35% of farms, respectively. These findings align with studies by Habte et *al*. (2022), Aquino-Sagastume et *al*., (2016), and Callaby et *al*., (2016), which reported high seroprevalence rates for these respiratory diseases.

Co-infection analysis revealed significant prevalence rates for combinations such as H9 Influenza with Mycoplasmosis (33%) and Newcastle disease with Infectious Bronchitis (17%), highlighting the complexity of diagnosing and treating multiple infections and the need for comprehensive diagnostic approaches to detect multiple pathogens simultaneously (Abdelaziz et *al*., 2019).

Study Limitations

The study was conducted in only two locations, Dakar and Thiès, which may not provide a comprehensive understanding of the diverse poultry farming practices and disease prevalence throughout Senegal, thus restricting the generalizability of the results even though the locations are hubs of poultry production in Senegal. Additionally, reliance on farm owners and managers for reporting biosecurity measures and management practices could result in reporting bias, as adherence to recommended policies might be exaggerated.

CONCLUSION AND RECOMMENDATIONS

In conclusion, this study identified significant risk factors for respiratory diseases in laying hens in the peri-urban regions of Dakar and Thiès, Senegal. Key factors such as proximity to neighboring farms, non-compliant buildings, mixed-age groups, and inadequate farmer training were strongly associated with disease prevalence. The detection of Mycoplasma gallisepticum and Influenza H9 in 50% of farms, along with common co-infections, highlights the urgent need for improved disease management. Strengthening biosecurity measures, enhancing management practices, and providing continuous farmer education are essential to mitigate the impact of these diseases. Policymakers should prioritize investment in infrastructure, training, and disease surveillance to ensure the long-term resilience of the poultry sector.

This study extends the Epidemiologic Triad Theory by showing how farm management practices and urban proximity contribute to disease spread in peri-urban settings. It also offers practical insights for improving biosecurity, vaccination, and training, which are crucial for reducing disease prevalence. Finally, the findings provide a strong basis for policy recommendations aimed at supporting medium-sized poultry farms with enhanced biosecurity regulations and educational initiatives.

Conflicts of Interest

The authors declare no conflict of interest.

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Authors' Contributions

The research concept was developed by Mireille Catherine Kadja and Souhaibou Sourokou Sabi. Sample collection and laboratory analysis were carried out by, Moussa OUEDRAOGO. Statistical analysis was done by Edmond Onidje and Derrick Adu Asare. The draft of the manuscript was prepared by Edmond Onidje and Benjamin Obukowho Emikpe. All authors read and approved the final version of the manuscript and contributed equally to its content.

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