Seroprevalence and associated risk factors of influenza D virus infection in cattle in the peri-urban areas of Dakar and Thiès, Senegal

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Abstract

Introduction

Influenza D virus (IDV) is an emerging pathogen playing a role in bovine respiratory disease complex, contributing to significant economic losses in the livestock industry. Despite its global spread, the epidemiology of IDV in Senegal remains largely unexplored. This study aims to assess the seroprevalence of IDV and identify key factors associated with its infection in cattle within the peri-urban areas of Dakar and Thiès, Senegal.

Methods

A cross-sectional study was conducted, with 168 cattle sampled from intensive, semi-intensive, and extensive farming systems. Blood samples were tested using hemagglutination inhibition assays to determine antibody titers against IDV. Logistic regression was employed to identify potential factors associated with the seropositivity of cattle in the study area.

Results

The results showed an overall seroprevalence of 31.5%. Larger herds (≥50 cattle) had a significantly higher seroprevalence (47.1%) compared to smaller herds (27.6%, $p<0.05$), while cattle from Senegal had a higher seroprevalence (53.3%) than imported animals (24.2%, $p<0.05$). In multivariate analysis, smaller herds and mixed-breed cattle were found to have lower risks of infection, while Senegalese-origin cattle exhibited a much higher risk of infection.

Conclusion

This study provided the first evidence of IDV circulation in cattle in Senegal, with herd size and animal origin being key factors influencing infection risk. Strengthened biosecurity measures and further research into genetic resistance are recommended to mitigate the spread of IDV in the region.

Keywords: Influenza D virus (IDV), bovine respiratory disease, Seroprevalence, peri-urban cattle farming, Senegal

Introduction

In Senegal, livestock is a very important part of the agricultural ranking as the second-largest activity in the primary sector after agriculture (Diouf, 2019). The livestock production sector contributes to the employment and income generation of approximately one-third of households in Senegal and helps in building resilience against exogenous shocks (Sow et al., 2021). Under the Emerging Senegal Plan, it is a sector of priority interest since it contributes 21% to added value and 3.4% to national GDP (Assocle, 2024). This development option includes that of increasing food security and reducing dependence on imports of food products. Livestock farming contributes to improving agricultural productivity through animal traction and organic manure in rural development poles.

The cattle population in Senegal encompassed 4.1 million heads in 2023 (MEPA, 2023)(DSV, 2024). This production system is one of the core activities within the economy that boost food self-sufficiency, job opportunities, and social integration (Moussa, 2023; Ferrari et al., 2024). With the increased demand for most dairy products, Senegal imports cattle from Western countries and integrates them into the production of dairy products.

However, cattle production in Senegal encounters numerous challenges combining technical, socio-economic, political, health-related issues (Kanh et al., 2019).

Among health issues, viral diseases play a big role and zzonotic concerns have been raised for influenza D virus infection (Nissly et al., 2020). The pathogen was first detected in the United States in 2011, and quickly linked to the bovine respiratory disease complex (Hause et al., 2014).

In Africa, the virus circulates in countries in East, North, and West Africa (Murakami et al., 2019; Salem, 2018; Sanogo et al., 2021). Although no studies have confirmed its presence in Senegal, the country's practice of importing cattle from Western regions where influenza D is prevalent raises concerns about its possible introduction. Hence the need to investigate the presence of this virus in local herds to ensure effective livestock health management and to implement appropriate control measures.

Therefore, this study investigates the seroprevalence of influenza D and aim at identifying factors associated with its infection in cattle within the peri-urban areas of Dakar and Thiès. Understanding the epidemiology of influenza D in Senegal is essential for developing effective control strategies if needed, thus protecting the livestock sector, and safeguarding public health.

Methods

Study Area

The study was carried out in the urban centers of Dakar and Thiès, and more precisely on the Niayes area. Niayes is a coastal zone in northwest Senegal, extending from Dakar to Saint-Louis along the Atlantic coast over approximately 200 km long and from 5 to 30 km inland. This area is well recognized by its high concentration of intensive and semi-intensive farming systems of livestock. The Niayes area enjoys modal temperatures and relatively larger rainfall compared to other regions of Senegal, therefore allowing the growth of lush vegetation with a wide range of ecological diversity (Fare, 2018). According to MEPA (2024), although this area accounts for just 1% of the national cattle herd, it plays an important role in the country's livestock production.

The Niayes region offers several benefits for cattle farming, including shallow aquifers and natural water sources that provide year-round hydration for livestock. Its fertile soils and favorable climate support the growth of high-quality pastures throughout the year, ensuring a consistent and nutritious food supply for cattle. Additionally, its close proximity to major urban centers like Dakar and Saint-Louis makes it easier to market and sell livestock products. These factors contribute to a higher economic return for local farmers compared to other crops, making Niayes one of Senegal's leading regions for cattle production (Njong, 2006).

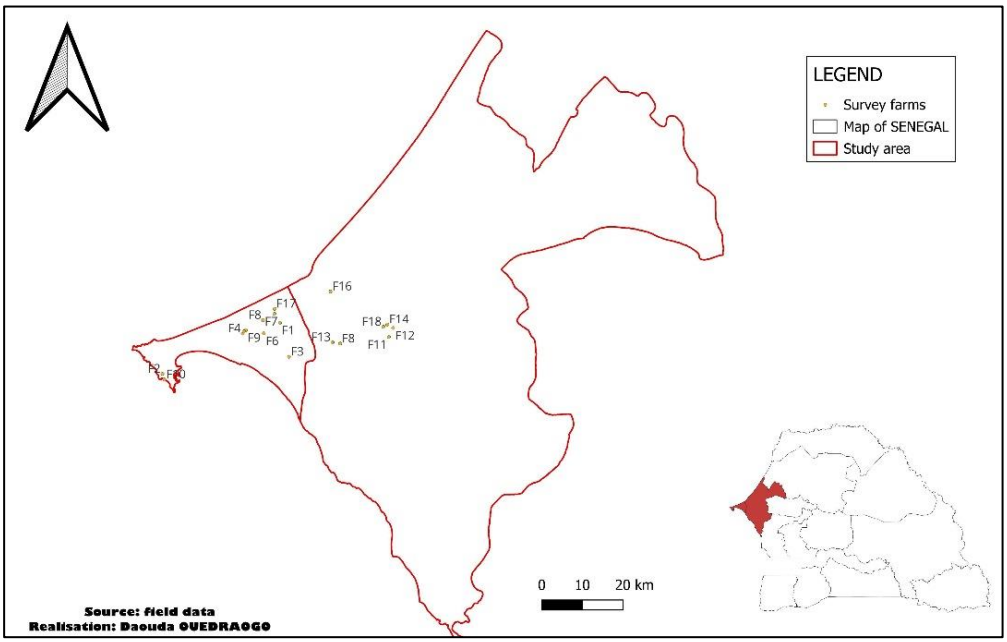

Figure 1: Location of the study area in the country

Study Design

This cross-sectional study was undertaken to determine the seroprevalence of influenza D virus in the Dakar and Thiès regions. Eight communities with high cattle populations: Médina, Bambilor, Niaga, Yen, Diender Gedji, Keur Moussa, Northern Thiès and Western Thiès were purposely selected to ensure maximum representation of cattle farming in said regions. Farms

in each region were selected based on their accessibility and the consent of the farmers. Blood samples were collected from animals older than 8 months to avoid interference from maternal antibodies, without consideration of breed or general health condition. The number of animals randomly chosen from each herd ranged from 5 to 10, depending on the size of the farm.

Questionnaire administration

Additionally, a comprehensive questionnaire was administered to the farm owners or managers, which included questions on the location of the farm, type of enterprise, type of livestock production, and herd size. Information specific to each animal sampled was also collected including species, sex, breed, age, and origin of each sampled animal and vaccinations administered was also collected.

Sample Size Determination

The Cochran formula (Bolarinwa, 2020), which considers the desired confidence level expressed as Z, expected proportion (p), and precision as d, was used to find the number of samples required in this research study as follows:

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

Assumptions made for this study included the following:

- Confidence level: 95% where Z= 1.96
- precision: 5% where d = 0.05
- An expected prevalence of 10% was guided by the findings of (Sanogo *et al.*, 2021) on influenza D virus in neighbouring West African countries with a similar ecological and farming context to Senegal.

Assuming these values, the initial estimated sample size was 138 cattle. However, a total of 168 cattle from local, hybrid, and exotic breeds were sampled using simple random sampling.

Blood Sample Collection

The blood samples were collected aseptically by a 16-gauge needle with a Vacutainer® system from the jugular vein in volumes of 10 mL. Blood was drawn into plain tubes without anticoagulant and allowed to clot at room temperature. After clotting, the samples were transported in a cooler containing ice packs to keep the samples at 2-8°C to the laboratory. Upon arrival in the laboratory, the samples were centrifuged at 700 × g for 10 minutes to clearly separate the serum. The clear serum obtained was pipetted into labelled 2-mL cryovial tubes with care and stored at −20°C until further testing.

Hemagglutination HA and Hemagglutination Inhibition HI Tests

To begin the laboratories analysis, blood was drawn from the wing vein of a healthy chicken into Alsever's solution to prevent clotting. The collected blood was then washed three times in PBS, centrifuging at 1,500 × g for 5 minutes each. Following each centrifugation, the supernatant was removed and RBCs resuspended in PBS. After the final wash, the RBCs were resuspended to make a 1% RBC suspension and used in both hemagglutination and hemagglutination inhibition assays.

Further, the hemagglutination (HA) assays were performed using antigen from the Influenza D virus (D/bovine/France/5920/2014) obtained from National Veterinary School of Toulouse. Accordingly, the HA titter of the antigen was calculated at 64 according to FAO protocols (FAO, 2023). To standardize the antigen to 4 HA units, the titter 64 was divided by 4 and yielded a dilution factor of 16. Therefore, the antigen was diluted in appropriate volumes in PBS, preparing it up to 25 mL of the diluted antigen for testing across 10 microwell plates.

This was followed by the hemagglutination inhibition (HI) test in the identification of antibodies against Influenza D virus. The HI test followed FAO guidelines, as described by Onidje et al., (2024). In each well of a V-bottom microtitterplate, 25 µL of PBS was first added, followed by 25 µL of serum in the first well, with serial two-fold dilutions carried out across the plate. Next, 25 µL of the standardized antigen (4 HA units) and 25 µL of the 1% RBC suspension were added to each well. The plate was incubated for 30 minutes at room temperature, followed by 45 minutes at 4°C after the RBC suspension was added.

Hemagglutination inhibition was read after incubation by tilting of the plate to observe the movement of RBCs. Comparison of the sample wells with the control wells was done for reading. The highest dilution of serum that had inhibited hemagglutination, giving a clear RBC dropping pellet at the bottom of the well, was recorded as the antibody titter. Samples with titters ≥1:16 were considered positive for antibodies against the Influenza D virus as recommended in Sagerman et al (2021).

Data Management and Analysis

Data from the field surveys and serological tests were entered and recorded in Microsoft Excel (Office 2016). A farm was considered positive if at least one animal tested positive for the virus. Before conducting the analysis, variables like "age" and "herd size" were recoded into binary qualitative categories. All statistical analyses were performed using R Studio (version 2023.12.1) for descriptive statistics and association tests. Chi-square tests were employed to assess associations, while Fisher’s exact test was used when Chi-square test conditions were not met.

Odds ratios (ORs) were also calculated to evaluate the strength of associations. In this analysis, the serological status of the animals was treated as the dependent variable, while the other field-collected variables were considered independent. A univariate logistic regression was performed to determine unadjusted odds ratios for potential factors associated with infection. Variables with a p-value ≤ 0.20 in the univariate analysis were subsequently included in a multivariate logistic regression model. Factors associated with Influenza D virus infection were identified by analysing the odds ratios, their confidence intervals, and p-values. For all analyses, a significance threshold of 0.05 was applied.

Ethical Considerations

The sample collection process for this study on the seroprevalence of influenza D in cattle adhered to strict ethical guidelines. Informed consent had been acquired from farm owners before any blood samples were collected from the cattle. Participants were fully informed about the study's objectives and procedures, and their involvement was entirely voluntary. The privacy of the respondents was protected, and all collected data and biological samples were treated confidentially. Blood sampling was performed humanely to reduce the animals' stress as much as possible, in line with the Ethical Guidelines for Animal Welfare (Marr, 2015).

Result:

The herds and animals observed in this study were primarily part of an intensive farming system, with 67.3% of farms practicing intensive farming, 30.4% practicing semi-intensive, and only 2.4% operating extensively as shown in **table 1.** Most of the herds, 79.8%, consisted of less than 50 cattle, while 20.2% had more than 50 cattle. Dairy production was the dominant farming activity, making up 91.1% of the operations, followed by beef fattening at 4.2%, and traction at 4.8%. In terms of breed, 86.9% of the animals were exotic, 8.3% were mixed breeds, and 4.8% were local breeds. The herds were predominantly female, with 92.3% being female cattle, while males accounted for only 7.7%. In terms of age, 70.8% of the cattle were over two years old, and 29.2% were under two years. The origin of the cattle showed that 71.4% were imported, 26.8% were from Senegal, and 1.8% had unidentified origins. Lastly, vaccination was common, with 70.8% of the animals vaccinated, leaving 29.2% unvaccinated.

Table 1: Characteristics of the surveyed Herds and Animals

	Variables	Count	Proportion (%)
Farming System	Intensive	113	67.3
	Semi-intensive	51	30.4
	Extensive	4	2.4
Herd Size	Less than 50 cattle	134	79.8
	More than 50 cattle	34	20.2
Farming Activity	Dairy production	153	91.1

	Beef fattening	7	4.2
	Traction	8	4.8
	Exotic	146	86.9
Breed	Mixed	14	8.3
	Local	8	4.8
Sex	Female	155	92.3
	Male	13	7.7
Age	Over 2 years	119	70.8
	Under 2 years	49	29.2
	Imported	120	71.4
Origin	Senegal	45	26.8
	Unidentified	3	1.8
Vaccination	Yes	119	70.8
	No	49	29.2

The serological results are presented in **Table 2**, detailing the distribution of antibody titters among the cattle tested. Overall, 31.5% (n=53) of the animals were seropositive. Among the seropositive animals, antibody titters varied: 9.4% (n=5) had a titter of 1:16, 3.8% (n=2) exhibited a titter of 1:32, and 26.4% (n=14) showed a titter of 1:64. Higher titters were observed in 3.8% (n=2) of animals with a titter of 1:128, 9.4% (n=5) with a titter of 1:256, 11.3% (n=6) with a titter of 1:512, and 26.4% (n=14) with a titter of 1:1024. The highest titers recorded were 1:2048 in 5.7% (n=3) and 1:5096 in 3.8% (n=2) of the animals.

Table 2: Distribution of Antibody Tittrs and Seroprevalence of Influenza D in Cattle

Titters	<16	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:5096
Count	115	5	2	14	2	5	6	14	3	2
Percentage	68.5 %	9.4 %	3.8 %	26.4 %	3.8%	9.4%	11.3 %	26.4%	5.7%	3.8%
Overall Prevalence			**Positive**				31.5%			
			Negative				68.5%			

The results of the association tests between seroprevalence and various factors are summarized in **Table 3**. The statistical analysis revealed significant differences in herd size and animal origin. Herds with more than 50 cattle had a higher seroprevalence of 47.1%, compared to 27.6% in herds with fewer than 50 cattle ($p<0.05$). Additionally, local breed exhibited a significantly higher seroprevalence of 53.3% compared to imported animals, which had a seroprevalence of 24.2% ($p<0.05$). Other factors, including region, farming system, farming activity, breed, sex, age, and vaccination status, did not show statistically significant differences

in seroprevalence (p>0.05), though trends were observed, such as higher seroprevalence in females and older animals.

Table 3: Statistical Analysis of Factors Associated with Influenza D Seroprevalence in Cattle

Variables	Category	Total Tested	Positive	Seroprevalence (%)	p-value
Regions	***Dakar***	90	29	32.2	0.9715
	Thiès	78	24	30.8	
Farming System	***Intensive***	113	38	33.6	0.784
	Semi-intensive	51	14	27.5	
	Extensive	4	1	25.0	
Herd Size	***< 50 cattle***	134	37	27.6	**0.03346**
	>50 cattle	34	16	47.1	
Farming Activity	***Dairy production***	153	50	32.7	0.5548
	Beef fattening	7	2	28.6	
	Traction	8	1	12.5	
Breed	***Exotic***	146	49	33.6	0.1077
	Crossbred	14	1	7.1	
	Local	8	3	37.5	
Sex	***Female***	155	52	33.5	0.06466
	Male	13	1	7.7	
Age	***Over 2 years***	119	40	33.6	0.546
	Up to 2 years	49	13	26.5	
Origin	***Imported***	120	29	24.2	**0.002131**
	Senegal	45	24	53.3	
Vaccination	***Yes***	119	39	32.8	0.3272
	No	49	14	28.6	

In this study, several factors were identified as significantly associated with the outcome of interest through univariate and multivariate regression analyses (**Table 4)**. Herd size was significantly associated with the outcome, as herds with more than 50 cattle had a higher likelihood of being seropositive compared to herds with fewer than 50 cattle (adjusted odds ratio (AOR) = 5.2, 95% confidence interval (CI): 2.1–12.9, $P < 0.001$). The breed of cattle was another significant factor, with exotic breeds showing a markedly increased risk compared to crossbred cattle (AOR = 28.8, 95% CI: 2.9–281.1, $P = 0.004$), while local breeds showed a marginal association (AOR = 11.8, 95% CI: 0.8–176.7, $P = 0.073$). Cattle origin was a strong predictor of the outcome, with Senegalese-origin cattle having a significantly higher risk compared to imported cattle (AOR = 20.7, 95% CI: 6.8–62.9, $P< 0.001$). While sex of the cattle showed an increased risk for females in the univariate analysis (AOR = 8.9, 95% CI: 0.7–104.1, $P = 0.079$), this association was not statistically significant.

Table 4: Univariate and Multivariate Logistic Regression of Influenza D Virus Infection in Relation to Various Variables

		Univariate regression analysis		Multivariate regression analysis	
Variables	**Category**	**Crude odds ratio (95% CI)**	**P value**	**Adjusted odds ratio (95% CI)**	**Pvalue**
Regions	***Dakar***	Ref.	-	-	
	Thies	1.1 (0.5 – 2.0)	0.840	-	
Farming System	***Intensive***	1.5 (0.15 – 15.1)	0.721	-	
	Semi-intensive	1.1 (0.1 – 11.8)	0.916	-	
	Extensive	Ref.	-	-	
Herd Size	***< 50 cattle***	Ref.	-	Ref.	
	>50 cattle	2.5 (1.1 – 5,4)	0.022	5.2 (2.1 – 12.9)	0.000
Farming Activity	***Dairy production***	3.4 (0.4 – 28.4)	0.259	-	
	Beef fattening	2.8 (0.2 – 40.0)	0.448	-	
	Traction	Ref.	-	-	
Breed	***Exotic***	6.6 (0.8 – 51.7)	0.074	28.8 (2.9 – 281.1)	0.004
	Crossbred	Ref	-	Ref.	
	Local	7.8 (0.6 – 93.8)	0.106	11.8 (0.8 – 176.7)	0.073
Sex	***Female***	6.6 (0.8 – 47.9)	0.088	8.9 (0.7 – 104.1)	0.079
	Male	Ref.	-	Ref.	
Age	***Over 2 years***	1.3 (0.6 – 2.8)	0.432	-	
	Up to 2 years	Ref.	-	-	
Origin	***Imported***	Ref.	-	Ref.	
	Senegal	3.1 (1.5 – 6.3)	0.001	20.7 (6.8 – 62.9)	0.000

Discussion

The present study aimed to assess the seroprevalence of IDV and to identify factors associated with its infection in cattle within the peri-urban areas of Dakar and Thiès. The serological findings revealed a 31.5% seroprevalence, indicating active viral circulation in the cattle population. This figure is notably higher than previous reports from West Africa, which documented seroprevalence rates between 1.9% and 10.4% (Sanogo et al., 2021). These differences could be due to technical issues. The cattle sera were indeed not pre-treated in the present study due to unavailability of receptor destroying enzyme. This may have led to false positives. The differences could also be attributed to regional variations in exposure, management practices, or viral persistence. Previous studies, such as those by Sreenivasan et al., (2015) and Gaudino et al., (2022), similarly underscore the widespread occurrence of IDV, particularly in cattle, which are considered the primary host.

A notable finding in the present study is the significant relationship between herd size and infection risk. Larger herds (≥50 cattle) were more likely to be infected, as confirmed by both univariate and multivariate analyses (OR=0.40, p=0.02 in univariate; OR=0.19, p=0.0003 in

multivariate). This observation aligns with the work of Salem, (2018), who found that larger herd sizes and higher animal densities facilitate viral transmission, especially for respiratory pathogens. Moreover, Sreenivasan et al., (2015) and Chiapponi et al., (2020) demonstrated that IDV spreads efficiently through direct contact, a factor that is exacerbated in densely populated herds.

In addition to herd size, animal origin emerged as another significant factor influencing IDV infection. The study found that Senegalese cattle had a higher likelihood of infection compared to imported animals (OR=3.13, p=0.00144). This finding is in line with reports by Sanogo et al., (2021), which highlighted the role of regional factors, such as differences in biosecurity protocols and environmental exposure, in shaping IDV seroprevalence rates. Imported cattle, often subjected to more stringent health checks and quarantine procedures, are likely to have reduced exposure to the virus, thus explaining their lower infection rates.

Another intriguing finding is the significantly lower infection risk observed in Crossbred cattle (OR=0.034, p=0.00384). This suggests that genetic diversity may confer some resistance to IDV. Similar patterns have been reported by Ferguson et al., (2015), who found that mixed-breed cattle displayed greater resilience to respiratory diseases. This trend may also extend to IDV, further emphasizing the importance of genetic diversity in infection resistance. Gaudino et al., (2022) also noted increasing IDV genetic diversity, especially in Europe, suggesting that genetic factors may play a critical role in infection dynamics.

Although sex was not identified as a significant predictor of infection risk in this study, a trend towards lower infection rates in male cattle (p=0.079) was noted. While earlier studies, such as that of Salem, (2018), have not reported significant sex-based differences in IDV infection rates, this trend hints at the possibility that behavioral or physiological factors could influence susceptibility.

Limit of the study

The study is limited by its focus on the peri-urban areas of Dakar and Thiès, making the findings less applicable to other regions in Senegal. Additionally, the use of serological tests, which detect past exposure rather than active infections, limits the assessment of current influenza D virus circulation. More advanced methods, like PCR, and broader geographic coverage would improve future research.

Conclusion

In conclusion, this study reveals active circulation of influenza D virus (IDV) in cattle within the peri-urban areas of Dakar and Thiès, with herd size, cattle origin, and genetic diversity emerging as key factors influencing infection risk. Larger herds and cattle of Senegalese origin

were found to have a higher susceptibility, while mixed-breed cattle demonstrated potential genetic resistance.

To effectively manage the spread of IDV, it is recommended to strengthen biosecurity measures, particularly in larger herds, and employ molecular diagnostics like PCR for more accurate detection of active infections. Additionally, research into the genetic resistance of mixed-breed cattle should be pursued to inform breeding programs aimed at enhancing herd resilience. Expanding surveillance to include more regions across Senegal will further support the development of comprehensive control strategies and improve our understanding of the virus's prevalence and impact.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' contributions

The research concept was developed by Mireille Catherine Kadja, Laibané Dieudonné Dahourou, Mariette Ducatez and Souhaibou Sourokou Sabi. Daouda Ouedraogo was responsible for sample collection and statistical analysis. Laboratory analyses were conducted by Daouda Ouedraogo and Rianatou Bada-Alambedji. The manuscript draft was prepared by Edmond Onidje and Benjamin Obukowho Emikpe. All authors reviewed and approved the final version of the manuscript and made equal contributions to its content.

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