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Uncovering the Spread of Newcastle Disease Virus in Burkina Faso's Village Poultry Systems: seroconversion rate and viral detection

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Uncovering the Spread of Newcastle Disease Virus in Burkina Faso's Village Poultry Systems: seroconversion rate and viral detection

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Abstract

Background: Newcastle disease (ND) is one of the most important viral diseases reducing poultry production throughout Africa. This is one of the reasons why this disease is subject to a large-scale annual vaccination program. In Burkina Faso, over the past decade, there has been an increase in unconfirmed cases of ND. Our study aimed to identify ND virus (NDV) circulation in traditional livestock farms in Burkina Faso.

Results: one hundred and ninety-five (195) poultry were sampled in 42 farms from four regions of Burkina Faso in 2022. NDV seroconversion was assessed using the ID Screen® Newcastle Disease Indirect kit. Birds from each farm were swabbed (nasopharyngeal and cloacal) to detect the presence of the virus, for a total of 102 swab pools. RNA extracted from the swab samples was tested by RT-qPCR using the OneStep RT-PCR Kit (Qiagen, Hilden). Antibodies to Newcastle disease virus were detected in 70.77 ± 6.38% (138/195) of the poultry tested (i.e. chicken, Guinea fowl, ducks, quails and turkey) and 100% of villages were positive for Newcastle disease antibodies. 23.81% of samples were RT-PCR positive. The seroconversion rate shows the

presence of antibodies indicate exposure to NDV or NDV vaccine with a significantly higher in vaccinated poultry than in unvaccinated poultry (p=0,0006). All study regions demonstrated virus circulation and poly-specific farms showed significantly less virus presence than mono-specific farms (p=0,004).

Conclusions: the present study provides important information on anti-NDV antibody titers and the circulation of this avian virus in Burkina Faso. It also emphasizes the importance of maintaining vaccination programs and surveillance of avian diseases in Burkina Faso.

Keywords: Newcastle Disease; Prevalence; seroconversion; Burkina Faso.

Background

Newcastle disease (ND) is a highly contagious viral infection affecting domestic and wild birds. It is found in over 236 avian species (1). The virulence of the pathogen and clinical signs depend on the avian species and viral strain (2). Newcastle disease is enzootic in many countries around the world, especially in Africa (3, 4). It can cause significant economic losses on farms, both in terms of direct losses linked to morbidity and mortality and indirect losses linked to people's livelihoods (3, 5, 6).

Newcastle disease is caused by avian Paramyxovirus serotype 1 (APMV-1), an enveloped virus of the genus *Avulavirus* and belonging to the family *Paramyxoviridae* (7, 8).

Bird viruses belong to categories known as APMV-1 through APMV-21. Various species of birds are infected by these viruses. Newcastle disease virus identified as APMV-1 is the main virus affecting birds. Chickens face significant issues with this particular strain responsible for Newcastle disease. In contrast the remaining types from APMV-2 to APMV-21 are primarily discovered in both wild and domesticated birds. Small or unnoticed infections are unlikely to result from strains not APMV-1 (9, 10).

Newcastle disease virus strains are separates into two main clades : class I and class II (11). Class I viruses mainly include strains with low virulence detected in wild birds (10). Class II largely includes strains circulating in domestic poultry. It brings together the most virulent isolates associated with epizootics (1, 12).

Different vaccines strains are used for vaccination, and the majority are lentogenic strains, namely LaSota, Hitchner B1, Clone 30, Ulster 2C, VG/GA, Phy LMV42, C131, Brescia, C2, V4, Queensland, I-2 and F (5, 13). The vaccine strain used in Burkina Faso is the LaSota strain. This vaccine strain confers high protection against genotype II of the virus andprotects against both the onset of clinical signs and disease-related mortality (14). In Burkina Faso, the numbers poultry vaccinated against ND were 29,861,800 in 2020 and 28,064,300 in 2021 (15). In Burkina Faso raising chickens can be done in three distinct methods: free-range partly free-range and completely confined. A significant number of farmers (64%) prefer the free-range method. Minimal help is provided to the chickens as they search for food independently. A small flock of chickens typically around 25 or fewer is commonly

maintained by farmers. The partly free-range method is utilized by approximately 36% of farmers (16). Chickens are not confined to a single space; additional food housing and basic health care are provided for them. Keeping chickens indoors at all times is relatively rare. Special kinds of chickens are raised in a confined space primarily by large companies with their food and health managed properly. Raising chickens in this manner does not occur frequently among smaller operations. These vaccinations are carried out on modern, semi-modern and traditional farms throughout the country.

Studies have shown that the virus circulates in Africa, with serological prevalences ranging from 17.6% to 56.4% (3, 17-20). Conducted researche in Burkina Faso examined regions affected by Avian influenza virus. Newcastle disease was discovered among local and wild birds. No other viruses were identified. A percentage of 13.8 (39/283 samples) in bird populations tested positive using RT-PCR. Evidence indicates that the spread of the disease to domestic birds may occur due to wild birds (21). No weak forms of the virus have been detected in tests conducted in West Africa particularly in Burkina Faso where new more potent strains were identified (22). Small farms were involved in outbreaks. The virus is also thought to be evolving and disseminating throughout West Africa. It cannot be stated that the situation is under control.

Other studies focusing on the risk associated with ND have demonstrated risk factors such as the traditional nature of livestock farming, poor application of biosecurity measures, non-compliance with the vaccination schedule. In 2020 and 2021, 3270 suspected NDV outbreaks were reported in the 13 regions of Burkina Faso (15). Unfortunately, these suspected outbreaks are rarely confirmed or investigated. Our study aimed to highlight the circulation of NDV in traditional poultry farms in Burkina Faso. The study took into account only traditional type farms, due to the irregularity of vaccination and the virtual absence of the implementation of biosecurity measures (4, 23).

Materials and Methods

Study area

Burkina Faso has 13 regions and 45 provinces. The study covered 4 regions (Figure 1) of the country. The climate found in Burkina Faso is of the Sudano-Sahelian type (24). Burkina Faso's fauna, both domestic and wild, is strongly influenced by its four agro-ecological zones: North-Sahelian, South-Sahelian, North-Sudanian, and South-Sudanian (25). These zones, characterized by differing vegetation and climatic conditions, shape the distribution, abundance, and adaptability of animals across the territory.

These factors contribute to a more or less significant growth in livestock farming. The study focused on chickens from farms in rural and urban areas of Burkina Faso.

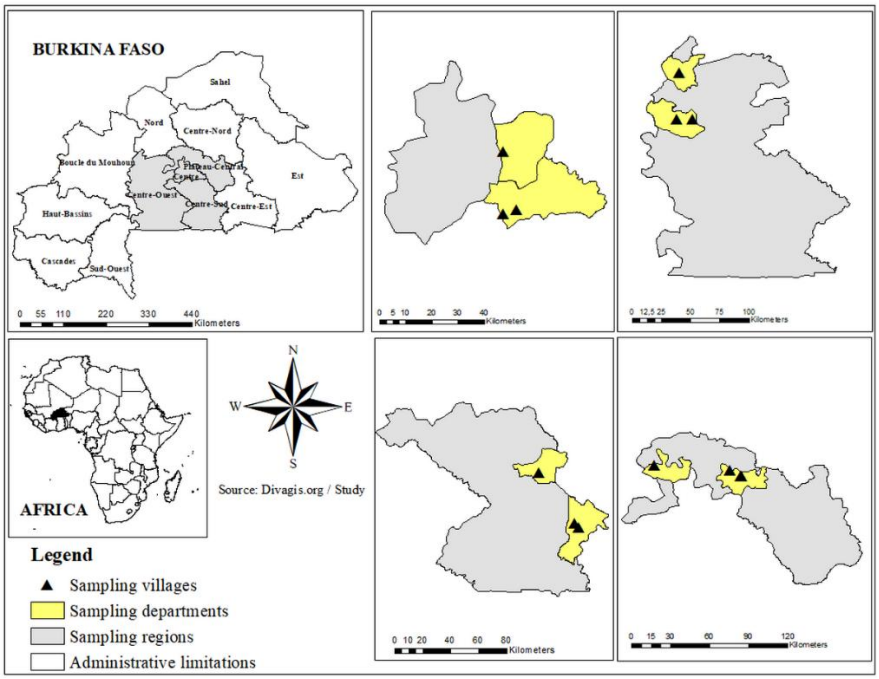


Figure 1. map of the study area

Sample size

The sample size was calculated with the epiTool (26) using the formula $n = (Z^2 \times P (1 / P)) / e^2$. Here Z = value of the standard normal distribution corresponding to the desired confidence level (Z = 1.96 for a 95% CI), P is the actual expected prevalence of disease and e is the desired precision with an expected seroconversion rate of 0.85, a precision of 0.05 and a confidence level of 0.95. The study was carried out in 4 regions of the country. In each region, three (03) villages were randomly selected. Four (04) poultry farms per village were sampled. A sampling plan was defined according to the number of poultry present on the farm (Table 1).

Table 1. Number of samples to be taken according to flock size

Number birds per farm	Number to be sampled	Number of samples expected by sample type		
		Sera	Tracheal swabs	Cloacal swabs
20 to 50 poultry	4	4	4	4
50 to 100 poultry	6	6	6	6
More than 100 poultry	8	8	8	8
Total	18	18	18	18

Sampling and data collection

The various samples were collected using a survey form developed with Sphinx 5.10 software. Blood samples on dry sampling tubes were taken for serum collection, and tracheal and cloacal swabs were also taken from apparently healthy poultry. In each village, a mean of 18 serum samples and 10 swabs were taken. After sampling, the swabs were placed in 15 ml tubes (Falcon) containing 2 to 4 ml of sterile Phosphate-buffered saline (PBS) at pH 7.4.

Primary data collection was carried out on farms using survey forms, which were used to gather information on the location of farms, poultry present, date of last vaccinations. Each farm had a data collection sheet highlighting all the information deemed useful about the farm.

Serological tests

Immune responses to Newcastle disease virus (NDV) were detected using a commercial indirect enzyme-linked immunosorbent assay kit ((IDvet's ID Screen® Newcastle Disease Indirect kit, France) as per the manufacturer's guidelines. This test demonstrated sensitivity and specificity of 100%. Poultry sera were collected on sterile collection tubes on 1.8ml, these sera were transported between 4 to 8°C in coolers and stored at -80°C freezers pending serological analyses. The sera were tested individually. This test does not distinguish between vaccine-type antibodies and those resulting from natural infection. The presence of antibodies was compared to a titer value of 993. When the concentration of antibodies surpassed the value 993, the sample was marked positive; if below, it was considered negative. A negative test result means that no antibodies are present or that the antibodies against Newcastle disease virus (NDV) in the analyzed serum sample are present at undetectable levels. A positive result means there are antibodies against NDV, which shows the person was either exposed to the virus before or has developed immunity after getting vaccinated.

RT-qPCR

Total RNA was extracted from the swabs with the Qiagen RNeasy kit according to the manufacturer's recommendations. The final elution volume was 30 µL. Viral RNA amplification was performed with the OneStep Real Time RT-PCR Kit (Qiagen, Hilden) as described by Wise et al., 2004. All swab samples were extracted using this method. The oligonucleotide sequences used were APMV 1 F 5'-AGTGATGTGCTCGGACCTTC-3' for the sense primer, APMV 1 R 5'-CCTGAGGAGAGGCATTTGCTA-3' for the antisense primer and APMV 1 - LNA 5'HEX-GGGACrGChTgCtatCc-BHQ3' for the probe for the detection of M gene of the virus. Samples were considered positive at a Cq of less than 36 for 40 cycles performed

Risk factors

The risk factors that are linked to ND presence in farms have been highlighted using an analytical approach that combines epidemiological and statistical data. The information was obtained by conducting as serological follow-up and gathering factors

that relate to livestock characteristics, flock composition, the presence of other bird species, and vaccination and health history. The seroconversion rates for each factor under study have been established and compared to each modality of these factors. To measure the strength of the observed relationships, coefficients (odds ratio) and probability values (*p-value*) were used.

Statistical analysis

Field survey data were collected and laboratory results compiled using Microsoft Office Excel. Statistical analyses were performed using R 4.3.2 software. These analyses consisted of a description of the data and a comparison of proportions using a generalized linear model (GLM), followed by the calculation of odds ratios. Variables were compared two by two using Tukey's test. Means were compared using the ANOVA test.

Results

Farm typology

The study took place in four (04) regions and eight (08) departments. A total of 42 farms were surveyed. These were traditional poultry farms (100%). These farms were mostly poly-specific (26/42), with a predominance of farms with two poultry species (72%). *Gallus gallus domesticus* (chickens) were present on all farms, followed by *Numida méléagris* (Guinea fowl: 52.38%), *Meleagris gallopavo* (turkey: 21.43%) and *Anas platyrhynchos* (duck: 9.52%), *Coturnix coturnix* (quails: 4.76%). The average flock size was 135 [Min= 17 - Max=519] birds. Pathologies were frequent in the flocks, with 50% of farmers mentioning the presence of diseases in the flocks over the period covering the last three (03) three months. Clinical signs included neurological signs (4/42), digestive signs (4/42), respiratory signs (7/42), locomotion problems (3/42) and sudden death (14/42). The majority of farmers (37/42) claimed to have encountered Newcastle disease in the last 12 months, and 90% of these farmers claimed to have vaccinated their poultry against the disease over a period ranging from 3 months to more than a year (Table 2).

Newcastle disease serology: seroconversion

Antibodies against Newcastle disease were detected in 70.77 ± 6.38% (138/195) of poultry with a mean antibody titer of 9498.34 [Min: 1109.65-Max:103526]. All of villages presents protectives poultry for Newcastle disease antibodies with variable levels [Min: 12.5% -Max:100%] (Table 3).

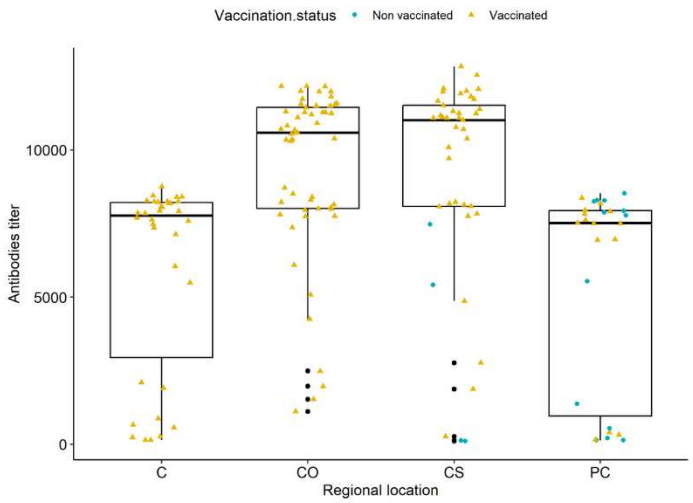
This seroconversion was highest in the Centre Ouest region (82.81% ± 9.24%) and lowest in the Plateau Central region (45.45% ± 14.71%). This seroconversion level differed significantly according to region ($p=0.0003$).

The seroconversion rate was significantly higher ($p<0.01$) on poly-specific farms (76.81% ± 7.04%) than on monospecific farms. The number of avian species present on the farm also had a significant effect ($p<0.05$) on the seroconversion rate. Specifically, the presence of guinea fowl ($p<0.05$) and duck ($p<0.05$) increased the seroconversion rate, with respective odds ratios of 2.82 [1.5 - 5.37] and 3.6 [1.18 - 15.63]. The presence of other species such as quail ($p>0.05$) and turkey ($p>0.05$) had no significant

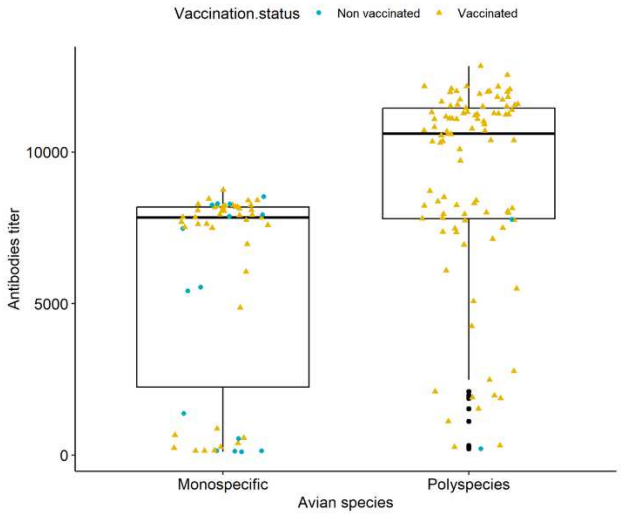
effect on the seroconversion rate. All farms reporting the presence of nervous-type clinical signs in the 3 months preceding the survey were seropositive ($p<0.05$), while the presence of other clinical signs had no significant effect.

The age of the birds sampled had no effect on the variation in seroconversion rate. On the other hand, vaccination status ($p<0.001$) and period of last vaccination ($p<0.001$) did have an impact on seroconversion rate. Vaccinated animals were 4.12 [1.77 - 9.89] times more likely to present antibodies than non-vaccinated animals. Similarly, animals vaccinated over a period of less than 3 months were 10.3 [3.73 - 30.55] times more likely to present antibodies than non-vaccinated poultry, with seroconversion rates decreasing over time (Table 4).

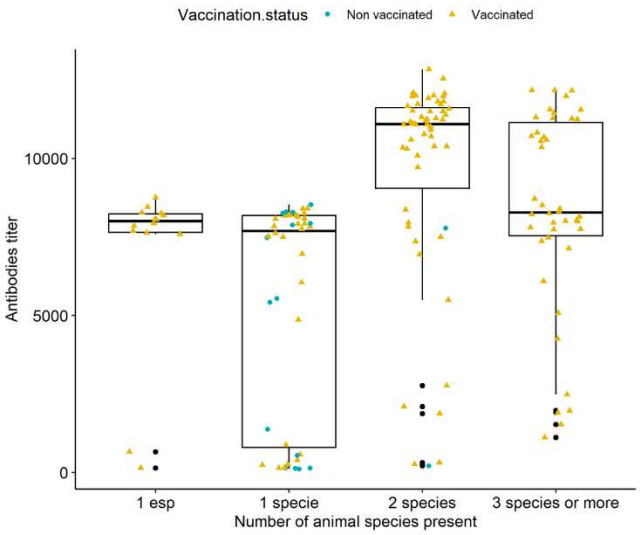
The mean antibody titer was 7889.4 [min=109.4-max=12834.1]. This rate was significantly ($p=5.76e-05$) higher in vaccinated poultry 8295.95 [min=138.34 - max=12834.09] than in unvaccinated poultry 4588.78 [min=109.43 - max=8528.19] (Figure 2).



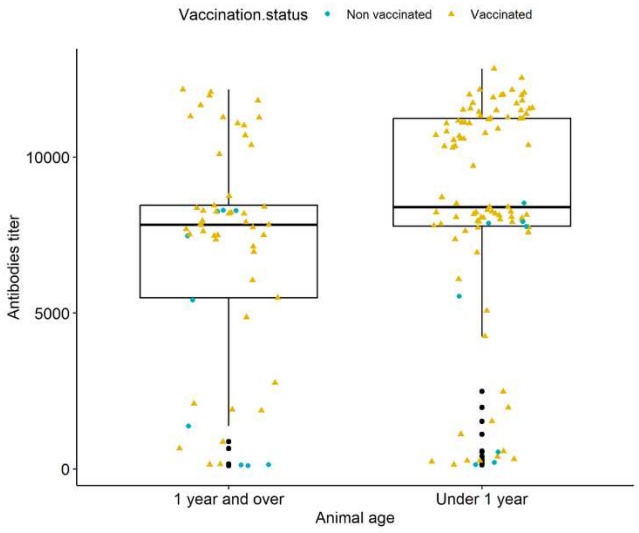
(A)



(B)

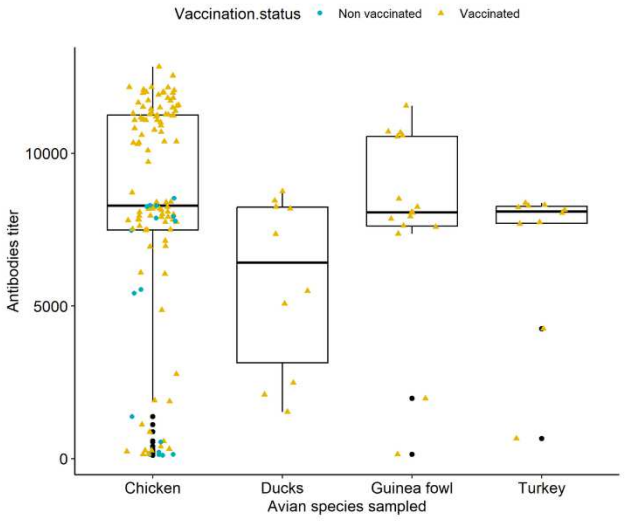


(C)

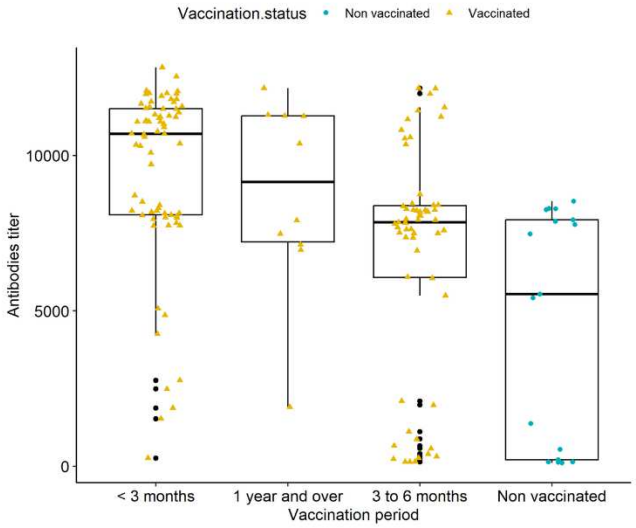


(D)

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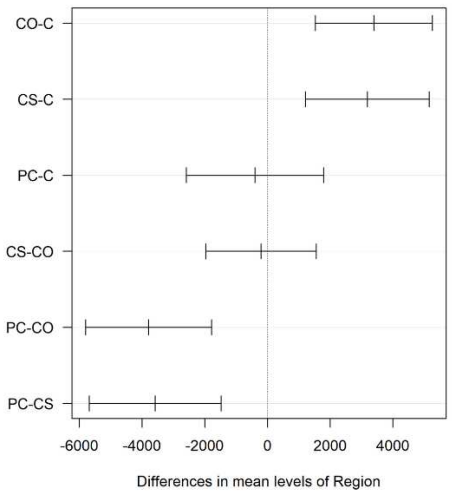
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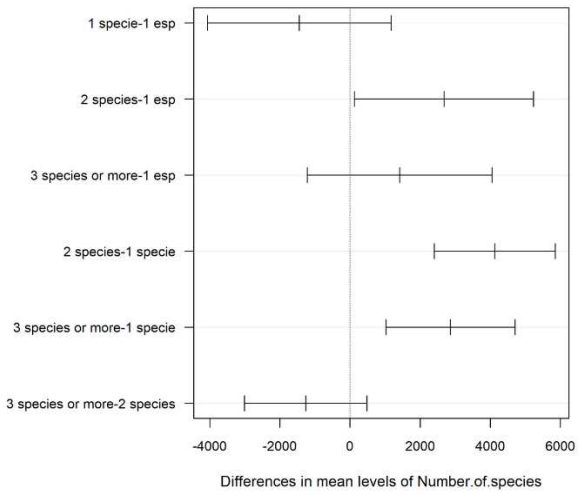
(F)

Figure 2. Comparison of the titers of antibody titers by vaccination status: (A) Differences in mean levels of region. (B) Differences in mean levels between mono and poly-specifics farms. (C) Differences in mean levels of Number of species presents. (D) Differences in mean levels according animal's age. (E) Differences in mean levels according to species. (F) Differences in mean levels of breeding period. 162 163 164 165

Pairwise comparison of mean antibody titers as a function of several explanatory variables yields significantly different values, except for the species variable (Figure 3). 166 167

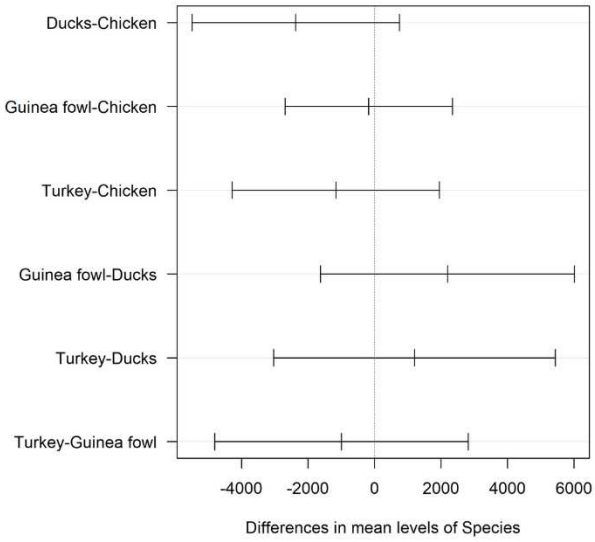


(A)

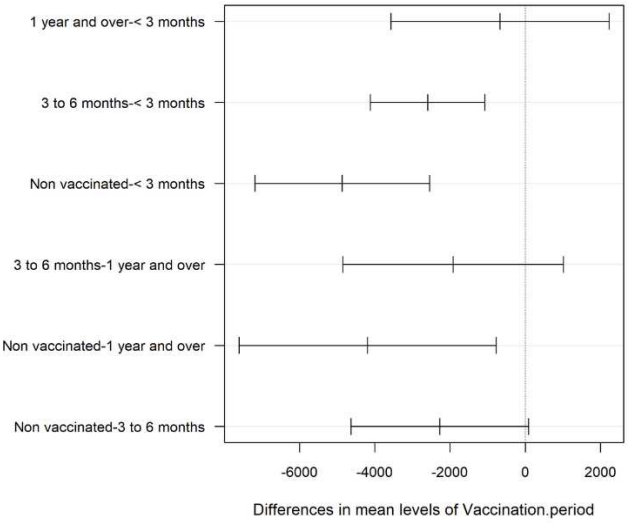


(B)

168



(C)



(D)

Figure 3. Pairwise comparison of antibody titers. (A) Differences in mean levels of region (B) Differences in mean levels of Number of species present, (C) Differences in mean levels of species sampled, (D) Differences in mean levels of vaccination period. 169 170

Molecular detection of the Newcastle disease virus genome 171

RT-PCR was positive in 16.66% of samples (17/102) and 23.81% (10/42) of farms (Table 5). Positive individuals were obtained in all regions and 50% (6/12) of villages. Monospecific farms had more positives (37.5%) than poly-specific farms (15.38%). However, within the poly-specific farms, those with two avian species had a higher positivity rate than those with 3 or more species. 172 173 174 175

Among the clinical signs mentioned, only the presence of respiratory signs (57.14%) had a significant effect on the molecular prevalence of the disease with p= 0.0435.

Discussion

Our study confirms the circulation of NDV in100% villages investigated. The regions chosen for the study are well known for their large local poultry populations (15) but also for their reserves and forest areas (27). These areas are part of the Sudanian and Sudano-Sahelian agroecological zones that are home to species receptive to NDV, highlighting the significant role of avifauna in maintaining and transmitting the virus (28).

As a result, information on the vaccination status of each subject was collected. This yielded an average seroconversion rate of 70.77% ± 6.38. This rate was significantly higher in vaccinated poultry than in unvaccinated poultry. The seroconversion rate among unvaccinated poultry remained higher than those obtained in Ivory Coast (22%) (3) in Nigeria (17) and Egypt (31%), in Libya (53%) (19) in Sierra Leone (56.4%) (18) in Ethiopia (17.06%) (20) which can attest to the circulation of the wild strain of the virus.

The LaSota vaccine strain used in Burkina Faso confers high antibody levels (6). Animals vaccinated less than 3 months ago showed the highest level of present antibodies, and were significantly different from those vaccinated between 3 and 6 months ago, thus demonstrating the drop in antibody levels and the need for a booster vaccination (29). The decline in antibody levels in the absence of a boost dose exposes poultry to new infection (20, 29, 30). Vaccination also induces a cell-type immunity, enabling vaccinated poultry to be protected despite low antibody titers (30).

The presence of more than one animal species on the farm revealed a significant effect on the poultry' seroconversion rate. Poly-specific farms had the highest seroconversion rate (77% ± 7%), with high antibody levels (31-33). This could be due to the presence on the farms of avian species such as guinea fowl, ducks, quail and turkeys, which are less susceptible to the virus than chickens, and could favor reinfection of the latter (34, 35). Vaccination may result in post-vaccination viral shedding that can affect unvaccinated poultry (36). High antibody levels following repeated vaccination could be a factor limiting viral excretion (36). In addition, traditional animal husbandry, where animals roam the village and come into contact with wild birds at watering holes or in the forest, could encourage infection (3, 4).

Among the different clinical signs found, only the presence of nervous signs was found to be significant with regard to seroconversion. Clinical symptoms such as digestive issues, motor dysfunction, and respiratory signs do not closely correlate with Newcastle disease. In fact, they have no specificity to Newcastle disease because these symptoms can easily also be present in other poultry diseases and are a reflection of the systemic response to generalized disease rather than an indication of specific viral pathology (10). Conversely, neurological symptoms, such as torticollis, paralysis and tremors, are much more strongly

associated with neurotropic pathogenic NDV infections and provide stronger evidence for clinical diagnosis and associated dynamic immune responses (37, 38).

Geographically, the Centre Ouest region had the highest seroconversion rate, and was significantly different from other three regions. This region is characterized not only by the important practice of local poultry farming (15) but also the presence of numerous protected areas and forests (39) unlike the plateau region central. The central plateau region also has a more arid climate, with high temperatures that can affect virus survival (40).

Real-time PCR demonstrated the presence of the virus in 23.8% of farms. All study regions demonstrated virus circulation. Poly-specific farms showed significantly less virus presence than poly-specific farms. This could be explained by the high seroconversion rate in these farms, which does not allow prolonged excretion of the virus, as avian species such as ducks, pigeons and other species can passively excrete the virus, thus favoring infection of local chickens (41, 42). This molecular prevalence is higher than that obtained in Ivory Coast (14.7%) (3), this higher prevalence could be explained by the larger sample size in Ivory Coast with 4562 samples tested from 3 regions of country, which yielded more refined results.

Traditional poultry farms, where management isn't always very strict, are a major factor in spreading Newcastle disease virus. In these systems, when many a lot of birds packed together, like ducks, pigeons, and similar species, it helps the virus spread around more easily (43). Maintaining diverse species separate and also reducing interaction to wild birds constitute critical biosecurity practices to reduce disease transmission. In Burkina Faso local poultry farms, is confronted to many challenges like vaccines disponibility, verification of poultry immunization statut, and implementation of biosecurity measures. Thes challenges complicate the disease prevention. The improvement of preventive measures might contribute reduce virus concentrations and also reduce the duration of virus spread, particularly in farms with multiple avian species where the virus circulates more frequently (44).

Newcastle disease virus strains found in Africa demonstrates significant genomic variation, including multiple harmful genotypes found in various parts of Africa. In West Africa, genotypes XIV, XVII, and XVIII commonly circulate simultaneously, demonstrating the complexity of the spread of the infection is in that area (12, 44). This genetic diversity might affect infection control since the majority of vaccines administered in poultry, including LaSota, are derived from genotype II viral strains.

Heterogenius vaccination generally help in preventing mortality, but they could be less effective at cutting down viral shedding and spreading (45). So, identifying the genome of NDV strains found in Burkina Faso, mainly focusing on the F gene by sequencing methods, is really important. This would help show if administering vaccines matched to the specific strains could improve control of Newcastle disease in Burkina Faso (46).

Conclusions

This study shows that Newcastle disease virus is circulating in traditional poultry farms in Burkina Faso. The lack of rigor in the implementation of vaccination and the virtual absence of biosecurity measures on poultry farms are crucial points which, if controlled, could break the chain of transmission of the disease. Better implementation of vaccination campaigns with close monitoring of livestock farms would provide better protection for animals, thereby breaking the chain of virus transmission.

Declarations

Ethics approval: This study has obtained approval from the animal health ethics committee of Burkina Faso under reference CE-UJKZ/2025-02.

Consent for publication: all authors have read the document and approve its publication.

Consent to participate: Not Applicable.

Availability of data and materials: The data and materials presented in this study are available on request from the corresponding author.

Competing interests: The authors declare no conflicts of interest.

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Authors' contributions: L.B.O., L.H.Z. and J.B.O.: Conceptualization, Methodology, Software, Validation, Formal analysis, Data curation, Writing-original draft, Visualization, Writing-review and editing. S.H.O., C.A.Z.L., D.G., M.G., K.A.T., M.Z.: Investigation, Resources, Writing-review and editing. P.R., A.K., N.B.: Writing-review, Project administration, Funding acquisition, and Supervision. All authors have read and agreed to the published version of the manuscript.

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Table 2. Farm typology

	Centre	Centre-ouest	Centre-sud	Plateau central	Percentage
Type of breeding					
Mono-specific	5	-	3	8	38.1 % (16/42)
Poly-specific	4	10	9	3	61.9 % (26/42)
Species presents					
Chicken	9	10	12	11	100 % (42/42)
Guinea fowl	2	10	8	2	52.38 % (22/42)
Ducks	1	2	-	1	9.52 % (4/42)
Turkey	2	5	2	-	21.43 % (9/42)
Quails	2	-	-	-	4.76 % (2/42)
Presence of suspected clinical signs over the last 3 months					
Nervous signs	3	-	1	-	9.52 % (4/42)
Digestive signs	-	-	4	-	9.52 % (4/42)
Respiratory signs	3	1	1	2	16.67 % (7/42)
Locomotion problems	2	1	-	-	7.14 % (3/42)
Sudden death	1	10	3	-	33.33 % (14/42)
Total	9	10	12	11	100 % (42/42)

Table 3. Seroconversion rates by village and livestock type

Region / village	Seroconversion		Total
	Mono-specific (positive / subtotal)	Poly-specific (positive / subtotal)	
Centre (C)			
Nabitenga	44. 44% (4/9)	70% (7/10)	57.89% (11/19)
Wedbila 1 and 2	100% (4/4)	100% (12/12)	100% (16/16)
Centre-Ouest (CO)			
Didyr	-	82.05% (32/39)	82.05% (32/39)
Koukouldi	-	87.5% (7/8)	87.5% (7/8)
Tio	-	82.35% (14/17)	82.3% (14/17)
Centre Sud (CS)			
Boussougou	-	56.25% (9/16)	56.25% (9/16)
Goulagon	-	80% (16/20)	80% (16/20)
Kaibo	83.33% (10/12)	75% (3/4)	81.25% (13/16)
Plateau Central (PC)			
Daguilma	100% (4/4)	25% (2/8)	50% (6/12)
Kekelma	66.67% (8/12)	100% (4/4)	75% (12/16)
Kolguessé	12.5% (2/16)	-	12.5% (2/16)
Total	56.14% (32/57)	76.81% (106/138)	70.77% (138/195)

Table 4. Antibodies presence (seroconversion) rates associated to statistical parameters

Variable	seroconversion (%) ± 2σ	Positive / Total (%)	OR	P-value
Region				
C a	77.14% ± 14.19%	(27/35)	4.05 [1.56 – 11.39]	0.0003
CO a	82.81% ± 9.43%	(53/64)	5.78 [2.45 – 14.4]	
CS a	73.08% ± 12.3%	(38/52)	3.25 [1.41 – 4.8]	
PC	45.45% ± 15.01%	(20/44)	Ref [-]	
Type of breeding				
Mono-specific	56.14% ± 13.14%	(32/57)	Ref [-]	0.004
Poly-specific	76.81% ± 7.18%	(106/138)	2,59 [1.34 – 5]	
Number of bird species present				
1	61.11% ± 11.49%	(44/72)	Ref [-]	0.03
2	72.22% ± 10.55%	(52/72)	1,65 [0.82 – 3.36]	
3	82.35% ± 10.67%	(42/51)	2,97 [1.29 – 7.35]	
Presence of guinea fowl				
No	56.94% ± 11.67%	(41/72)	Ref [-]	0.001
Yes	78.86% ± 7.36%	(97/123)	2,82 [1.5 – 5.37]	
Presence of ducks				
No	68.04% ± 7.17%	(115/169)	Ref [-]	0.04
Yes	88.46% ± 12.53%	(23/26)	3.6 [1.18 – 15.63]	
Presence of quail				
No	71.20% ± 6.55%	(136/191)	2.47 [0.29 – 21.02]	0.58
Yes	50% ± 50%	(2/4)	Ref [-]	
Turkey presence				
No	69.08% ± 7.49%	(105/152)	Ref [-]	0.33
Yes	76.74% ± 12.88%	(33/43)	1.48 [0.69 – 3.38]	
Presence of clinical signs over the last 3 months				

No	69.31% ± 9.83%	(61/88)	Ref [-]	0.69
Yes	71.96% ± 8.68%	(77/107)	1.13 [0.61 – 2.11]	
Nervous signs				
No	68.33% ± 6.93%	(123/180)	Ref [-]	0.009
Yes	100% ± 0%	(15/15)	NA [NA - NA]	
Presence of digestive signs				
No	70.94% ± 6.78%	(127/179)	1.11 [0.34 – 3.21]	1
Yes	68.75% ± 23.17%	(11/16)	Ref [-]	
Presence of respiratory signs				
No	71.34% ± 7.21%	(112/157)	1.15 [0.52 – 2.43]	0.072
Yes	68.42% ± 15.08%	(26/38)	Ref [-]	
Presence of locomotion problems				
No	70.87% ± 6.73%	(129/182)	1.08 [0.28 – 3.48]	1
Yes	69.23% ± 25.6%	(9/13)	Ref [-]	
Presence of sudden death				
No	66.38% ± 8.77%	(77/116)	Ref [-]	0.1
Yes	77.21% ± 9.44%	(61/79)	1.72 [0.9 – 3.34]	
Age of animal 2 categories				
1 year and more	72.46% ± 10.75%	(50/69)	1.14 [0.6 – 2.08]	
Less than_1 year	69.84% ± 8.18%	(88/126)	Ref [-]	0.7
Period of last vaccination				
inf 3 months b	88.31% ± 7.32%	(68/77)	Ref [-]	2.953e-05
3_6 months a	63.63% ± 10.96%	(49/77)	4,32 [1.93 – 10.45]	
1 years and over a	66.67% ± 24.34%	(10/15)	3,77 [1 – 13.48]	
No vaccinated a	42.31% ± 19.38%	(11/26)	10,3 [3.73 – 30.55]	
Vaccination status				
No vaccinated	42.31% ± 19.38%	(11/26)	Ref [-]	0.0006
Vaccinated	75.15% ± 6.65%	(127/169)	4,12 [1.77 – 9.89]	
Total	70.77% ± 6.51%	(138/195)		

Table 5: molecular prevalence of Newcastle disease

Variable	Positive / total	Percentage
Region		
C	4/9	44.44%
CO	2/10	20%
CS	2/12	16.67%
PC	2/11	18.18%
Number of bird species present		
1	6/16	37.5%
2	2/17	11.76%
3	2/9	22.22%
Species presents		
Chicken	10/42	23.81%
Guinea fowl	3/22	13.64%
Ducks	1/4	25%
Turkey	2/9	22.22%
Quails	1/2	50%
Presence of clinical signs over the last 3 months		
Neurological signs	1/4	25%
Digestive signs	1/4	25%
Respiratory signs	4/7	57.14%
Locomotion problems	0/3	0%
Sudden death	2/14	14.29%
Total	5/21	23.81%