

Molecular Epidemiology of Rabies in Burkina Faso: A Case Study of Samples Analyzed at the National Livestock Laboratory

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Abstract

Rabies remains a major public health problem in Burkina Faso, where epidemiological and molecular data are still limited. This study aimed to assess the prevalence of infection in biting animals and to compare the performance of direct immunofluorescence (DFA) and conventional RT-PCR. A total of 97 brain tissue samples from biting animals collected between 2018 and 2019 at the National Livestock Laboratory were analyzed. Direct immunofluorescence (DFA) was performed according to the standard reference method, and conventional RT-PCR was carried out using primers targeting a conserved region of the Lyssavirus N gene. Epidemiological data (species, age, vaccination status, number of people bitten, and origin) were associated with the diagnostic results. DFA revealed an overall infection rate of 88.6%, which was higher in dogs (90.2%) and in unvaccinated animals (92.6%). Infection was more frequent in animals aged three months or older and was systematic in those that had bitten at least twice. RT-PCR showed an overall infection rate of 36.1%, with no positivity detected in vaccinated animals. The agreement between the two methods was low (Kappa =

0.13), probably due to prolonged preservation and suboptimal storage conditions of the samples. This study confirms the central role of dogs in the transmission of rabies and highlights the need to strengthen the vaccination of domestic animals. DFA remains a reference method well suited to the local context, while the optimization of molecular techniques and the improvement of sample preservation conditions represent key perspectives for strengthening epidemiological surveillance and moving toward the elimination of human rabies by 2030.

Keywords

Rabies, Fluorescent-Antibody Test (FAT), RT-Conventional PCR, Burkina Faso

1. Introduction

Rabies is an acute zoonotic viral disease that is almost always fatal once clinical symptoms appear [1]. Caused by a virus of the Lyssavirus genus and belonging to the Rhabdoviridae family, it exhibits a marked tropism for nerve cells [2] [3]. Rabies is mainly transmitted through the bites or scratches of infected animals, particularly dogs, which account for about 99% of human cases and represent the main reservoir of the virus [4] [5]. Although rabies can be controlled in some European countries through the vaccination of foxes, it remains endemic in many regions of the world, particularly in Africa and Asia, where it continues to pose a major public health problem [5] [6].

The rabies virus consists of a single-stranded, negative-sense RNA genome [5]. This 12 kb genome includes five main genes: N (nucleoprotein), P (phosphoprotein), M (matrix protein), G (glycoprotein), and L (large protein, involved in replication) [3]. The G protein plays a crucial role in infection by allowing the virus to bind to cellular receptors and is often targeted during vaccine development [7]. The genetic variability of the rabies virus is relatively low; however, it exists in the form of several Lyssavirus subtypes, which mainly correspond to different animal species that serve as reservoirs for the virus [8]. This genetic variability is an important factor for epidemiological surveillance, as it can influence both transmission dynamics and the effectiveness of vaccination strategies [8].

Each year, rabies kills approximately 60,000 people worldwide, with a particularly high number of victims in Africa, where the disease accounts for about 44% of global deaths [9]. Burkina Faso, like other West African countries, faces an endemic situation in which thousands of cases of attacks by infected animals are reported each year, mainly in the capital city, Ouagadougou [10] [11]. The vaccination of domestic animals, particularly dogs, is the main method of prevention, as it breaks the cycle of virus transmission to humans [12] [13].

Despite the progress made in vaccination, many challenges remain, particularly regarding access to post-exposure treatments and the implementation of reliable diagnostic methods such as Direct Immunofluorescence (DFA) and RT-PCR [13].

In recent years, research efforts have intensified; however, many areas—particularly rural and remote ones—still lack the infrastructure and resources needed to effectively combat this disease. The present study focuses on the molecular epidemiology of rabies, particularly the use of RT-PCR as a diagnostic method, in order to better understand the spread of the virus and to propose strategies adapted to local contexts, especially in Burkina Faso.

2. Methodology

2.1. Study Setting

The study was conducted in Ouagadougou, the capital of Burkina Faso, within the General Directorate of Veterinary Services, which houses the National Livestock Laboratory (LNE). The LNE is the largest diagnostic and analytical laboratory dedicated to the livestock sector in Burkina Faso. It plays a central role in both animal health protection and public health. In terms of animal health, the laboratory enables the accurate and early detection of animal diseases, the establishment of their epidemiological mapping, the improvement of diagnostic techniques, and the development of treatment methods and prophylactic measures. Regarding public health, the LNE contributes to the protection of human health through two main components: on one hand, the control of the quality of food products of animal origin, and on the other, the diagnosis and control of zoonotic diseases such as rabies. Finally, the LNE is equipped with a modern technical platform that allows it to conduct fundamental research in molecular biology and genetics.

2.2. Type and Period of Study

This is a cross-sectional study conducted from January 2018 to December 2019 at the National Livestock Laboratory (LNE) in Ouagadougou.

2.3. Sampling

A total of 277 heads of animals suspected of rabies were received at the LNE during the study period. Systematic sampling by selecting every k -th specimen from the list of 277 (with $k = 3$) allowed the selection of 100 samples, of which 97 were retained for analysis (3 were excluded due to lack of identification). Brain tissues (hippocampus) were collected from the animal heads using sterile equipment after opening the skulls. The samples were placed in sterile containers and stored for further analysis.

2.4. Study Population

The samples were mainly obtained from dogs, most of which originated from Kadiogo Province. Only a very small proportion of the examined animals had been vaccinated against rabies. Most of the animals had bitten at least one person before being culled.

2.5. Diagnosis of Rabies

2.5.1. Direct Immunofluorescence (DFA)

The diagnosis by direct immunofluorescence was based on the detection of the

viral nucleoprotein under a fluorescence microscope after fixation and staining of brain smears with a fluorescent anti-nucleocapsid conjugate. Positive controls (infected mouse brain) and negative controls (healthy mouse brain) were used to validate the procedure.

2.5.2. Conventional RT-PCR

Viral RNA was extracted from brain tissues using the RNA Extracol® kit (Eurx, Gdańsk, Poland) according to the manufacturer's instructions. The amplification reaction was carried out with the Access RT-PCR System kit (Promega Corporation, Madison, WI, USA) and specific primers described by De Benedictis *et al.* [14]. Each reaction was performed in a final volume of 25 µL, containing 5 µL of RNA extract, 5 µL of RT buffer (5X), 3 µL of MgSO₄ (3 mM), 0.5 µL of dNTPs (0.2 mM), 1 µL of forward primer and 1 µL of reverse primer (0.2 mM each), 0.2 µL of Tfl DNA polymerase (0.04 U/µL), 0.2 µL of AMV/Tfl reverse transcriptase (0.04 U/µL), and 9.1 µL of PCR-grade water.

The amplification program consisted of an initial reverse transcription step at 50 °C for 30 minutes, followed by polymerase activation at 95 °C for 15 minutes. This was followed by 45 successive cycles comprising denaturation at 94 °C for 30 seconds, annealing at 52 °C for 30 seconds, and extension at 72 °C for 40 seconds, with a final extension step at 72 °C for 5 minutes.

2.5.3. Agarose Gel Electrophoresis

The PCR products (expected size: 600 bp) were separated by electrophoresis on a 1.5% agarose gel. A volume of 7 µL of amplicons mixed with 2 µL of 6X loading buffer (Sigma) was loaded into each well, and electrophoresis was carried out at 144 V for 45 minutes in the presence of a Smart Ladder molecular weight marker (Invitrogen, Life Technologies). The amplified fragments were then visualized under UV light (312 nm).

2.6. Statistical Analysis

Data were entered using Excel 2010 and analyzed using Fisher's exact test ($p < 0.05$). Fisher's exact test was used to compare proportions between groups, specifically to assess the association between diagnostic outcomes (DFA and RT-PCR results) and variables such as vaccination status and species. This test was selected because some contingency table cells contained small, expected counts, making it more appropriate than the chi-square test. The agreement between DFA and RT-PCR results was evaluated using Cohen's Kappa coefficient.

3. Results

3.1. Characteristics of the Analyzed Samples

A total of 97 samples were included in the study. All were obtained from biting animals that had been culled, and their heads were submitted to the laboratory for analysis. Most of the samples (77.3%) originated from Kadiogo Province. Regarding the number of victims, 83.5% of the animals had bitten one person, while

16.4% had bitten two or more people. The distribution by animal species showed a clear predominance of dogs (94.8%). In contrast, only 4% of the samples came from animals that had received anti-rabies vaccination (**Table 1**).

Table 1. Characteristics of the analyzed samples.

Characteristics	Number (n)	Percentage (%)
Origin of the samples		
Kadiogo	75	77.3
Others origin	6	6.1
Unknown	16	16.4
Species		
Canine	92	94.8
Feline	4	4.1
Others	1	1.03
Vaccination status of the animals		
Vaccinated	7	7.2
Non vaccinated	55	55.6
Unknown	36	37.1
Age of the animals		
< 3 months	4	4.1
≥ 3 months	50	51.5
Unknown	43	44.33

3.2. Rabies Infection Rate by the DFA Method

The overall rabies virus infection rate determined by the DFA method was 88.6%. This rate varied according to the characteristics of the analyzed samples. The specimens were mainly obtained from canine species, along with a few feline samples and one cervid (doe). Diagnosis revealed an infection rate of 90.2% in dogs and 50% in cats, while the single cervid sample also tested positive.

Regarding vaccination status, the infection rate was significantly higher in unvaccinated animals (92.6%) and those with unknown vaccination status (91.7%) compared to vaccinated animals (42.8%; $p = 0.010$). Similarly, infection was more frequent in animals aged at least three months (88%) and in those of unknown age (93.0%; $p = 0.06$).

Furthermore, the results indicated that infection was systematic among dogs that had bitten at least two people (100%), compared to 86.4% among those that had bitten only one person ($p = 0.20$) (**Table 2**).

Table 2. Parameters influencing the infection DFA method.

Settings	Total	Positive samples (n)	Infection rate (%)	p-value
Species				
Canine	92	84	90.2	0.2
Feline	4	2	50	
Doe	1	1	100	
Vaccination status				
Vaccinated	7	3	42.8	0.01
Non vaccinated	54	50	92.5	
Unknown	36	33	91.6	
Animal ages				
< 3 months	4	2	50.0	0.06
≥ 3 months	50	44	88.0	
Unknown	43	40	93.0	
People bitten				
1 person	81	70	86.4	0.20
≥ 2 people	16	16	100	

3.3. Rabies Infection Rate by the PCR Diagnostic Method

The 97 samples tested by DFA were also subjected to RT-PCR diagnosis. Agarose gel electrophoresis (1.5%) of the RT-PCR products revealed, for several samples, a specific band of approximately 603 base pairs, corresponding to a positive result (Figure 1).

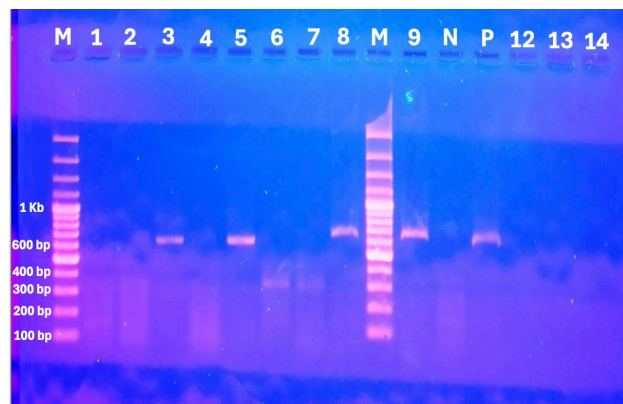


Figure 1. Agarose gel electrophoresis (1.5%) of PCR products. Samples 3, 5, 8, and 9 tested positive, while the remaining samples were negative. M = molecular weight marker; N = negative control; P = positive control; bp = base pairs.

The overall infection rate detected by RT-PCR was 36.1%. This rate varied according to the characteristics of the analyzed samples. Among dogs, the infection rate was 36.9%, compared with 25% in cats, while the sample from the cervid (doe)

tested negative. With respect to vaccination status, the infection rate was higher among animals with unknown vaccination status (44.4%) and unvaccinated animals (35.1%), whereas no vaccinated animal tested positive ($p = 0.15$). According to age, the infection rate was 32% in animals aged three months or older, 41.8% in those of unknown age, and 25% in animals younger than three months ($p = 0.65$). Finally, a trend toward a higher infection rate was observed with increasing number of people bitten: 34.5% among animals that had bitten one person, compared to 43.7% among those that had bitten two or more people ($p = 0.20$) (Table 3).

Table 3. Parameters influencing the infection rate by RT-PCR.

Parameters	Total	Positifs	Taux d'infection (%)	P value
Animal species				
Canine	92	58	36 > 9	
Feline	4	3	25	1
Doe	1	0	0	
Vaccination status				
Vaccinated	7	0	0	
Non vaccinated	54	19	35 > 1	
Unknown	36	16	44 > 4	0.15
Animal ages				
<3 months	4	1	25 > 0	
≥3 months	50	16	32.0	0.65
Unknown	43	18	41.8	
Number of people bitten				
1 person	81	28	34.56	0.57
≥2 people	16	7	43.7	

3.4. Concordance between DFA and RT-PCR

The comparison of the results obtained from the two diagnostic methods was performed using Cohen's Kappa coefficient, calculated from the contingency table (Table 4). The obtained Kappa value of 0.13 indicates an agreement only slightly better than that expected by chance.

Table 4. Comparative results of DFA and RT-PCR tests.

		DFA		Total
		Positive	Negative	
RT-PCR	Positive	35	0	35
	Negative	51	11	62
Total	Total	86	11	97
DFA sensibility		40.7%		
DFA specificity		100%		

Of the 97 samples analyzed, the two techniques were concordant in 46 cases, including 35 positives and 11 negatives. Among the 86 samples that tested positive by DFA, 51 (59.3%) were negative by RT-PCR. No sample that was negative by DFA yielded a positive result by RT-PCR. The calculation of Cohen's Kappa coefficient gave a value of 0.13, indicating a low level of agreement between the two methods.

4. Discussion

The present study, conducted on brain tissue samples from biting animals collected in Burkina Faso between 2018 and 2019, evaluated the diagnosis of rabies using Direct Immunofluorescence (DFA) and conventional RT-PCR. The objective was to describe the epidemiological characteristics and to compare the performance of these two diagnostic approaches. Data analysis revealed a considerable proportion of missing information regarding the geographical origin, age, and vaccination status of the animals. This lack of information, which has also been reported in other African countries [15], reflects the predominance of stray dogs, which are often culled after biting incidents without any known background information [16]. This incompleteness limits the ability to assess epidemiological patterns accurately and may introduce information bias in subgroup analyses. As a result, some associations may be underestimated or overestimated, and the generalizability of these findings to the broader animal population in Burkina Faso is potentially reduced. Most of the samples originated from Kadiogo Province, which is likely explained by its proximity to the national reference laboratory, facilitating the transport of animal heads for analysis—a pattern also observed in Namibia [17]. This geographical concentration of samples may represent a source of selection bias, potentially limiting the generalizability of the findings to the entire country. The canine species accounted for 94.8% of cases, confirming its major role as a reservoir and vector of rabies in African countries [6]. Only 4% of the animals were vaccinated, a proportion comparable to that reported in Niger [18] and Mali [19], but lower than the 13% observed in Burkina Faso by Savadogo *et al.* [20].

The overall infection rate obtained by DFA was 88.6%, similar to that reported in the Central African Republic [21] but lower than the 96% observed by Dao *et al.* [22]. The infection rate was higher among unvaccinated animals and those with unknown vaccination status compared to vaccinated animals, confirming the importance of vaccination in prevention. However, the relatively high percentage of vaccinated but positive animals suggests possible vaccine failures. Several factors may contribute to this situation, including improper vaccine administration, an incomplete or irregular vaccination schedule, poor vaccine quality, inadequate storage conditions (cold-chain breaches), or exposure to the virus shortly before or shortly after vaccination, before protective immunity could be established. A relationship between aggressiveness and infection was also observed: all animals that had bitten two or more people tested positive, compared to 86.4% of those

that had bitten only one person. This finding supports the classical description of aggressiveness as a common sign of rabies in animals. Finally, a lower infection rate was observed in young animals under three months of age, possibly due to maternal antibody protection or lower exposure risk.

The overall infection rate detected by RT-PCR was 36.1%, considerably lower than that obtained by DFA. Several factors may explain this result: degradation of viral RNA due to prolonged sample storage, exacerbated by power outages disrupting the cold chain [23], variable RNA quality, sometimes with low concentrations and insufficient purity; or a lower viral load in some vaccinated animals, leading to non-detection by PCR. The primers used targeted conserved regions of the N gene and can detect various Lyssavirus species [14], however, tissue preservation conditions appear to have had a major impact on the sensitivity of PCR under our study conditions.

The concordance between the two methods was low, with a Cohen's Kappa coefficient of 0.13. Out of 97 samples, only 46 showed concordant results (35 positive and 11 negative). More than half of the samples that tested positive by DFA were negative by PCR, although no DFA-negative sample tested positive by PCR. These findings contrast with those of De Benedictis *et al.* [14], who reported almost perfect agreement between the two methods. In our study, the discrepancy is likely attributable to sample preservation conditions and the variable quality of extracted RNA. Nevertheless, both methods showed similar trends, with higher infection rates observed in unvaccinated animals and those older than three months.

This study is noteworthy for having used both a classical method (DFA) and a molecular method (RT-PCR) to assess rabies in many samples collected over two consecutive years, thereby providing reliable documentation of virus circulation in an African context, where data remain scarce. It highlights the strong involvement of dogs as the main reservoir, the importance of vaccination, and aggressiveness as a predominant clinical sign. However, certain limitations should be noted: the lack of available information on the animals (age, origin, vaccination status), the prolonged storage of samples in a context of cold chain disruptions, and the sometimes-insufficient quality of extracted RNA likely affected the sensitivity of RT-PCR, thereby explaining the low concordance observed between the two diagnostic methods.

5. Conclusion

This study confirmed the predominant role of dogs in the transmission of rabies in Burkina Faso and revealed a high infection rate among biting animals, particularly those that were unvaccinated. Direct Immunofluorescence (DFA) demonstrated greater sensitivity than conventional RT-PCR under our working conditions, the latter likely being limited by the quality and preservation of the samples. Despite the low concordance between the two methods, the observed trends were similar, highlighting the relevance of their combined use in resource-limited settings. These findings underscore the need to strengthen vaccination programs for

domestic animals, especially dogs, to reduce the risk of human transmission. The study also emphasizes the importance of improving epidemiological data collection and sample preservation conditions to enhance the reliability of molecular diagnosis. Introducing RNA-stabilizing reagents (e.g., RNAlater® or equivalent) at the point of sample collection for tissues destined for PCR analysis would help maintain RNA integrity during transport and storage. Further investigations using real-time RT-PCR, which offers higher sensitivity, along with genetic sequencing of circulating strains, would refine the molecular epidemiology of rabies in Burkina Faso. Finally, better integration of veterinary and medical surveillance within a “One Health” approach represents a key perspective for progress toward the elimination of human rabies by 2030, in line with WHO objectives.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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