Comparison of two diagnostic tests for animal rabies in Burkina Faso

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Abstract

Various methods and tests have been developed to allow an early diagnosis for rapid management of animals bites or people who have been in contact with rabid or suspected animals. The use of a Rapid Diagnostic Test (RDT) and the direct Fluorescent Antibody Test (FAT) resulted give 50% (14/28) and 78% (22/28) of positive results for RDT and FAT respectively. 100% of the RDT positive samples were positive for FAT. The validation and availability of RDT at the decentralized level, in laboratories with a low technical platform would allow for rapid case management. However, RDT negative samples should be retested by the FAT method for confirmation.

Keywords: Rabies, Lyssavirus, Diagnosis, RDT, FAT, Burkina Faso

Comparaison de deux tests de diagnostic de la rage animale au Burkina Faso

Résumé

Diverses méthodes et tests de diagnostic de la rage ont été mis au point pour permettre un diagnostic précoce en vue d'une prise en charge rapide des morsures d'animaux ou des personnes ayant été en contact avec des animaux enragés ou suspects. L'utilisation d'un test de diagnostic rapide (TDR) et du test d'immunofluorescence direct (IFD) a donné 50 % (14/28) et 78 % (22/28) de résultats positifs respectivement pour le TDR et l'IFD. 100 % des échantillons positifs au TDR étaient positifs au IFD. La validation et la disponibilité des TDR au niveau décentralisé, dans des laboratoires disposant d'un faible plateau technique, permettraient une prise en charge rapide des cas. Les échantillons négatifs au TDR devraient être repris par l'IFD pour confirmation.

Mots-clés: Rage, Lyssavirus, Diagnostic, TDR, IFD, Burkina Faso

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Introduction

Rabies is a zoonotic disease affecting domestic and wild mammalians. It is a cosmopolitan disease present in more than 150 countries in the world. In Burkina Faso, this pathology is enzootic. Cases of bites are regularly reported. From 2015 to 2018, about 22,264 cases of bites were received at the level of the country's health structures (MS/DGESS 2016, MS/DGESS 2017, MS/DGESS 2018, MS/DGESS 2019) . During the same period, 880 samples were received at the National Livestock Laboratory for rabies diagnosis (DLNE/MRAH 2020).

Prevention and diagnosis of this pathology are essential for the implementation of rapid and effective treatment. In order to improve diagnosis and make it more accessible, the National Livestock Laboratory (LNE) is considering the use of routine rapid tests for rabies diagnosis. The rapid diagnostic test (RDT) is a rapid, inexpensive method requiring very little equipment. The aim of this study is to compare the results of the WOAH gold test, direct Fluorescent Antibody Test (FAT) with a rapid diagnostic test (RDT) "Anigen Rapid Rabies Ag Test Kit" manufactured by Bionote.

I. Materials and Methods

I.1. Study area

The country belongs to the Sudano-Sahelian climatic zone with two (02) seasons. The country's fauna population is very diversified with classified forests, parks and reserves where there are many wild carnivores that maintain the virus cycle. The samples received and analyzed came from 5 regions of Burkina Faso namely the Centre, Cascades, Centre-East, Centre-West, Centre-South and East (Figure 1).

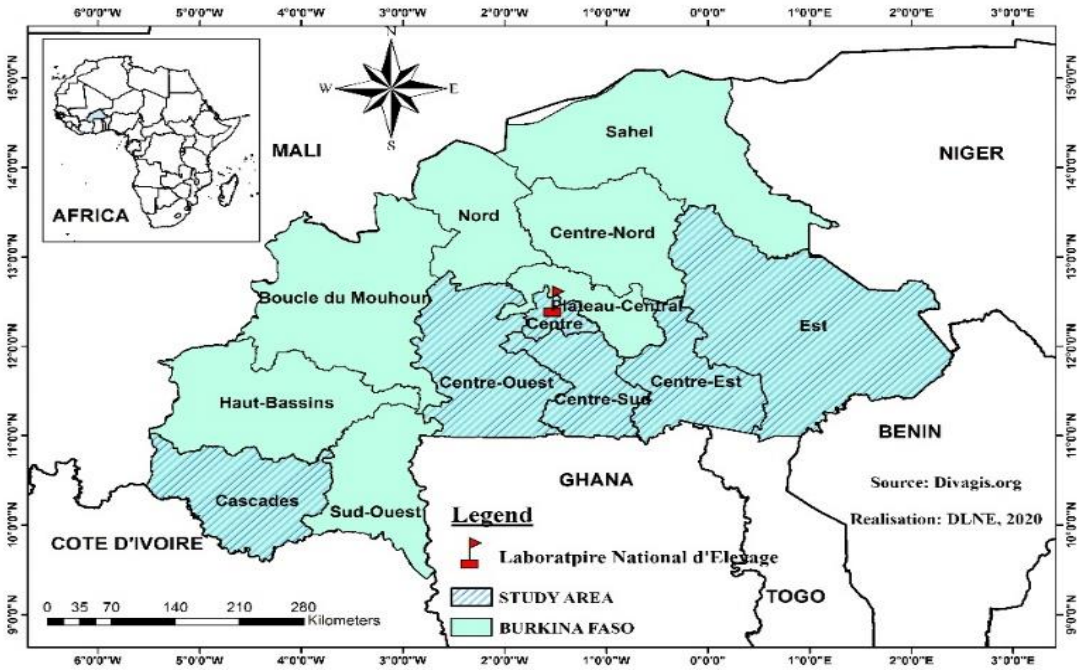

Figure 1: study area

I.2. Conditions for taking and transporting samples

The National Livestock Laboratory of Ouagadougou is the only laboratory in the country with sufficient resources to perform rabies diagnosis by FAT. As such, all samples of heads of rabies biting animals or animals suspected of having rabies are sent from the 13 regions of the country to this laboratory. These heads are **triple-packed** and transported by public transport.

I.3. Method

A random selection of the samples received by LNE over the period October 2019 to June 2020 was carried out using a 50% sampling rate applied to all samples without distinction of species. The sample size of 28 have been obtained and was composed of samples from dog (24), Cats (3) and Jackal (1).

Diagnosis by FAT is performed on brain smears (Ammon horn, cerebellum, medulla oblongata), previously fixed with acetone using fluorescein-coupled anti-nucleocapsid monoclonal antibodies, allowing the detection of all the different Lyssavirus species. A positive response results in the detection, under a fluorescence microscope, of bright green or yellow-green inclusions of varying shapes and sizes. A counter-staining with Even blue is carried out in order to facilitate reading. The slides are observed with an

immunofluorescence microscope in a dark room. Two control slides are used for reading, one positive control slide and one negative control slide. The test is completed after 2 hours.

We used a commercial RDT from Bionote for the detection of rabies virus antigen. As far as the RDT is concerned, it is a simple and quick diagnostic tool presented as an "all-in-one" kit. The brain sample is mixed directly in the tube containing the Buffer with the swab provided by the Kit for about 1 minute until most of the brain material is well dissolved and then placed on the test plate using the transfer pipette. Then about 4 drops are taken and placed on the test strip. The test is read after 10 minutes at laboratory temperature. The test is therefore validated after that a line appears in the control area "C". A positive result is obtained if there is a second strip in the test area "T" in addition to the control strip. The test is considered negative if a line appears only in the control area. The test is invalid if the control line does not appear. The test is performed according to the manufacturer's instructions.

I.4. Ethical considerations

Samples used are only from suspect animals received for disease testing or samples from animals already slaughtered for confirmation. No live specimens were used in this study and the authors did not take any samples from animals during this study**.**

II. Results

A total of 28 upstream horn samples were analyzed by both direct immunofluorescence and rapid diagnostic test methods. Dogs accounted for 86% of the animals tested. Test results showed 78% positive for FAT and 50% positive for RDT (Table I). All samples positive for RDT were also positive for FAT. From RDT negative samples, 8 were FAT-positive. The Kappa coefficient obtained by comparing these two tests shows a moderate agreement (0.43) between the results of these two tests.

Table I: percentage of positives for FAT and RDT by species.

Species	FAT	RDT	Total
Dog	79 % (19/24)	58% (14/24)	24/24
Cat	66 % (2/3)	0% (0/3)	3/3
Jackal	100% (1/1)	0% (0/1)	1/1
Total	78% (22/28)	50 % (14/28)	28/28

RDT gives a sensitivity of 64% [46-81%] compared to a specificity of 100% taking the FAT results as a reference (sick /non-sick) (Table II).

Table II: sensitivity and specificity of RDT.

		FAT	
		Positive (sick)	Negative (non-sick)
RDT	Positive	14 (Se=0,64)	0
	Negative	8	6 (Sp=1)

III. Discussion

The results obtained after the use of these two tests show that the FAT detects 78% of positive individuals against 50% for the RDT. The prevalence obtained by the FAT (78%) is similar to others studies (Savadogo, Koné *et al.* 2020). FAT is test recommended by the WOAH due to its ease of performance, its speed, the low technical platform required for its implementation as well as its high sensitivity, specificity (99.1%; 95% CI 98.7 - 99.4) (Robardet et *al.*, 2021, WOAH, 2023).

Although having good sensitivity and specificity, the results of these two tests remain highly dependent on the state of preservation of the samples (Mauti, Léchenne *et al.* 2020), which were mostly from remote areas [0-440km], involving a more or less long delay in transport by land transport. Viral concentration is a factor influencing the RDT (Klein, Fahrion *et al.* 2020). Sensitivities obtained for the bionote rabies rapid test are lower than those obtained by other study : Klein, Fahrion *et al.* (2020), Tenzin *et al.* (2020) and Certoma *et al.* (2018) (Certoma, Lunt *et al.* 2018, Tenzin, Lhamo *et al.* 2020) .

Although some authors have demonstrated the possibility of using the rapid test on species other than those intended by the manufacturer (Eggerbauer, de Benedictis *et al.* 2016, Léchenne, Naïssengar *et al.* 2016, Tenzin, Lhamo *et al.* 2020) , we obtained 100% negative results for non-recommended species (cat; jackal). The RDT demonstrated an absence of false-positivity in all 6 FAT-negative samples that were derived from various animals species, resulting in 100% specificity. RDT bionote gives results similar to those of FAT (Servat, Robardet *et al.* 2019, Klein, Fahrion *et al.* 2020). The Kappa coefficient shows a moderate agreement between these two tests. The low number of samples used in this study could also explain the disagreements obtained with the other authors.

The rapid rabies diagnostic test has many advantages, not only in terms of speed of reading but also in terms of ease of use. However, the variability in sensitivity and specificity observed in different studies should lead to further development of the test (Léchenne, Naïssengar *et al.* 2016, Klein, Fahrion *et al.* 2020, Tenzin, Lhamo *et al.* 2020)

in order to make it available in the field and in laboratories with limited resources. Therefore, a negative RDT result should be confirmed by FAT or RT-PCR in laboratories with the required equipment and technical ability.

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