UNIVERSITY OF NAIROBI

COMPARISON OF REVERSE-TRANSCRIPTION POLYMERASE CHAIN REACTION, RAPID IMMUNOCHROMATOGRAPHIC DIAGNOSTIC TEST AND FLUORESCENT ANTIBODY TEST FOR DETECTING RABIES VIRUSES CIRCULATING IN MALI

By

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DECLARATION

I hereby declare that this thesis is my original work and that it has not been submitted for examination any other university.

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DEDICATION

This thesis is dedicated to my mother, **Maréme BATHILY**. Thank you for your Prayers, Guidance and LOVE.

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LIST OF ABBREVIATIONS

ABLV	Australian bat lyssavirus
ARAV	Aravan lyssavirus
BBLV	Bokeloh bat lyssavirus
Biot	Biotinilated
BLAST	Basic Local Alignment Search Tool
BHK	Baby Hamster Kidney
cDNA	Complementary Deoxyribonucleic Acid
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CVL	Central Veterinary Laboratory
DHIS2	District Health Information Software 2
dRIT	Direct Rapid Immunohistochemical Test
DUVV	Duvenhage lyssavirus
EBLV-1	European bat lyssavirus, type 1
EBLV-2	European bat lyssavirus, type 2
ELISA	Enzyme-Linked Immunosurbent Assay
FAT	Fluorescence Antibody Test
FAVN	Fluorescence Antibody Virus Neutralization
FITC	Fluoresein Isothiocyanate

GBLV	Gannoruwa bat lyssavirus
ICTV	International Committee on Taxonomy of Viruses
IKOV	Ikoma lyssavirus
IFAT	Indirect fluorescent antibody test
IRKV	Irkut lyssavirus
KHUV	Khujand lyssavirus
LAMP	Loop-Mediated Isothermal Amplification
LBMA	Laboratory of Applied Molecular Biology
LBV	Lagos bat lyssavirus
LLEBV	Lleida bat lyssavirus
Lyssa	Lyssavirus
MAbs	Monoclonal Antibodies
ML	Maximum-Likelihood
MIT	Mousse Inoculation Test
MNT	Mouse Neutralization Test
MOKV	Mokola lyssavirus
MSA	Multiple Sequence Alignment
NASBA	Nucleic Acid Sequence-Based Amplification
NCBI	National Center for Biotechnology Information
NJ	Neighbor-Joining

OIE	World Organization for Animal Health
PAbs	Polyclonal Antibodies
PBS	Phosphate-Buffered Saline
PEP	Post-exposure Prophylaxis
PrEP	Pre-exposure Prophylaxis
qPCR	Real-Time Polymerase Chain Reaction
RABV	Rabies Virus
RFFIT	Rapid Fluorescent Focus Inhibition Test
RIDT	Rapid Immunochromatographic Diagnostic Test
RIT	Rapid Immunohistochemical Test
RNA	Ribonucleic acid
RREID	Rapid Rabies Enzyme Immunodiagnosis
RTCT	Rapid Tissue Culture Infection Test
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
SPF	Specific pathogen free
SHIBV	Shimoni bat lyssavirus
ssRNA	single stranded RNA
Taq DNA polymerase	Thermusaquaticus DNA polymerase
VI	Virus Isolation
VN	Virus Neutralization

UV	Ultraviolet
WAHIS	World Animal Health Information System
WCBV	West Caucasian bat lyssavirus
WHO	World Health Organization

ABSTRACT

In Mali, a rabies reporting procedure is in place but is efficient only in the capital city where the Central Veterinary Laboratory (CVL), which is mandated to diagnose rabies, is located. This has led to an underestimation of the diagnosis of rabies including the genetic characterization of the virus in the country. Therefore, there is need to evaluate the diagnostic methods of rabies for subsequent characterization of circulating rabies virus in Mali. In this regard, the study assessed the suitability of the Rapid Immunochromatographic Diagnostic Test (RIDT), and Reverse-Transcription Polymerase Chain Reaction (RT-PCR) for the detection and characterization rabies viruses circulating in Mali in 2017. A total of 18 samples previous submitted to the CVL in Mali were analysed for rabies virus using the lateral flow device (BioNote, Inc., Seoul, Korea) and RT-PCR. RT-PCR positive samples were sequenced using Sanger sequencing method at Inqaba Biotec and subjected to phylogenetic analysis. In order to compare to two methods, Fluorescence Antibody Test (FAT) was used as the gold standard method. Out of the 18 samples, 16 were found to be positive for rabies virus on FAT. Out of these 16 positives, only 7 (43.8%) samples were positive for the virus on RIDT while 15 (93.8%) samples were positive for the virus on RT-PCR. All the sequences analysed by Blastn shared at least 93.5% nucleotide identity to the rabies nucleoprotein gene thereby confirming rabies infection of dogs in Mali. A phylogenetic analysis revealed that all the sequences belong to the Africa 2 lineage of which five to the sub-lineage H, four to the sub-lineage F and two to the sub-lineage G. The results of RT-PCR were comparable to those of FAT. However, the positivity detection rate for RIDT was low as compared FAT. The genetic characterization of the virus confirmed previous findings of the circulation of the sub-lineages H, F and G belonging to the Africa 2 lineage in Mali. In conclusion, the RT-PCR could be used together with FAT for the detection and genetic characterization of rabies virus circulating in Mali. Further studies using large number of samples are required to validate the suitability of the new RIDT for the diagnosis of rabies in Mali.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Rabies is grave and progressive encephalitis targeting the central nervous system (CNS) as the main tissue of interest. It is caused by virus species of the Lyssavirus genus in the Rhabdoviridae family and the Mononegavirales order. It can infect animals known as warm-blooded and is recognized as approximately invariably fatal after the apparition of symptoms (Rupprecht *et al.*, 2002). In its 2013 report, the World Health Organization (WHO) estimated 61,000 human deaths in 2010 worldwide. These losses were chiefly accounted in Africa and Asia, which recorded 23,800 and 34,500 deaths, respectively (WHO, 2013). The disease also causes significant livestock loss with 11,500 cattle deaths reported annually in Africa and 21,150 deaths in Asia (Knobel *et al.*, 2005). Rabies spread to other domestic animals such as sheep, pig, and even fowl was reported (Baby *et al.*, 2015; Jiang *et al.*, 2008; Zhu *et al.*, 2011). Dog mediated rabies elimination could be achieved by mass vaccination of canine population up to 70% for five consecutive years and a proper public education (Fahrion *et al.*, 2017).

The first edition of the laboratory techniques of rabies was published more than 60 years ago. Since then, methods for investigating rabies suspected samples have changed. At that time, only the detection of Negri bodies was available. However, all that has changed for the better. Today, rabies suspected samples are investigated by detection of virus, demonstration of antigens, demonstration of antibodies and demonstration of viral nucleic acids and sequences. Among these techniques, the highly sensitive and specific, the fluorescent antibody test (FAT) also known as the direct fluorescent antibody test (DFAT), the recommended by both the WHO and World Organization for Animal Health (OIE). The FAT is a post-mortem diagnosis technique that is based on the demonstration of the nucleoprotein antigens of the rabies lyssavirus (RABV) in a brain tissue and was first introduced by Goldwasser and Kissling in the 1950s (Goldwasser *et al.*, 1959). This technique, based on the demonstration of RABV nucleoprotein antigens using fluorescein isothiocyanate (FITC)-conjugate antibodies, is the gold standard for both human laboratory as well as routine veterinary diagnosis for rabies

because of its sensitivity and specificity approaching 100%. However, the need for an expensive fluorescence microscope requiring regular maintenance, use of high-quality and high-intensity arc lamps as well as participation in proficiency testing remains the major limitations of this technique. For this reason, the FAT is not suitable for veterinary centres located in areas with limited resources in Mali due to financial burden.

In rabies endemic areas such as Asia and Africa, funds and infrastructures are most of the time insufficient to equipped veterinary services with the FAT for the definitive diagnosis of rabies where needed. Consequently, a friendly, rapid, and low cost such as the RIDT will contribute positively to reporting and the surveillance of rabies (Lechenne *et al.*, 2016). The first RIDT for the detection of RABV nucleoprotein was developed by (Kang *et al.*, 2007) was made using a purified monoclonal antibody directed against the nucleoprotein. One year later, Nishizono and colleagues developed two types of RIDT for rabies detection. While type 1 was made of a monoclonal antibody, type 2 was produced by combining two monoclonal antibodies (Nishizono *et al.*, 2008). Convinced by the high sensitivity and specificity demonstrated by these devices, the OIE recommended the use of RIDTs to diagnose the presence of the RABV nucleoprotein antigen (OIE, 2008). Nonetheless, they must go through complete validation process following the recommendations of national or international organizations to determine its characteristics such as agreement with the gold standard test, sensitivity and specificity. In addition, the OIE argued that during the validation of these RIDTs, samples should came directly from the region or country the test will be used due to possible antigenic variation. The evaluation of the anigen rapid rabies Ag test in Chad in 2016 had a sensitivity and specificity of 95.3% and 93.3% respectively (Lechenne *et al.*, 2016). Despite these promising results with the RIDT, its characteristics are unknown in Mali.

More recently, methods based on demonstration of the nucleic acids of the rabies virus are becoming more widely integrated as tools for diagnosis. Among them, the polymerase chain reaction (PCR), developed by a team led by Mullis, which has revolutionized rabies diagnosis and characterization (Mullis *et al.*, 1986). PCR is a laboratory technique using in vitro process to detect DNA sequences of the infectious agents such as rabies virus in tissues, secretions and excretions of the animals or humans infected. It involves selection of the portion of the

genome to be amplified using short oligo-nucleotide sequences called primers and a thermostable DNA polymerase (Taq plymerase). Depending on the length of the region flanked by selected forward and reverse primer, PCR products called amplicons can be revealed using gel electrophoresis technique. Rabies viruses are negative sense, single stranded RNA viruses (Rupprecht *et al.*, 2002). Consequently, a transcription of the RNA into complementary DNA (cDNA) is required before PCR amplification, giving a type PCR known as reverse transcriptase polymerase chain reaction (RT-PCR). DNA amplification of rabies virus RNA can be achieved using two-step or one-step reaction. In the first approach, the cDNA synthesis and PCR amplification occurred in different tubes, while the latter, the reverse transcription and PCR amplification take place in the same tube (WHO, 2019). RT-PCR has been used successfully on decomposed samples and offers the advantage of rabies isolates characterization. Furthermore, the characterization of these isolates from outbreaks or cases using antigenic (anti-G or anti-N monoclonal antibodies) or molecular (sequencing full or partial genome) is a powerful tool to identify the animal hosts, geographical origins and sources of infections (Streicker *et al.*, 2010). The CVL, in Bamako, is the facility equipped to perform FAT. It receives rabies suspected samples from other regions. Most of these rabies suspected specimens from other regions reach the CVL in an advanced level of decomposition making the investigation through FAT impossible.

1.2 Problem statement

Despite encouraging results of the RIDT assessment in several countries including Chad, the situation in Mali remains unknown. The anigen rapid rabies Ag test (BioNote, 2008) had a sensitivity of 95.3% and specificity of 93.3% in Chad (Lechenne *et al.*, 2016). However, the OIE recommends the use of samples directly from the region or country the test will be used during its validation to avoid decrease of sensitivity and specificity caused by antigenic variation. Consequently, the RIDT should be assessed in Mali before it adaptation as a valuable diagnosis tool in areas with limited resources.

There is an increasing idea of implementing the RT-PCR as a complementarily to the FAT especially on decomposed samples because it was used successfully to proof the presence of rabies virus from fresh samples (Biswal *et al.*, 2012), as well as decomposed and archived

specimens (Whitby *et al.*, 1997). Indeed, apart from fresh samples submitted from Bamako and surroundings, the investigation of rabies suspected specimens is impossible using FAT due to decomposition. Therefore, there is need to evaluate the RT-PCR for the investigation of decomposed samples sent to the CVL.

Given that Mali shares 4,500 miles border with seven (7) countries (Senegal, Ivory Coast, Burkina Faso, Mauritania, Guinea, Algeria, and Niger), there is a permanent risk of introduction of new rabies lyssavirus groups especially sub-lineage B and E of the Africa 2 lineage known to circulate in Guinea, and Senegal respectively. Ultimately, frequent characterization of rabies viruses circulating in Mali should be performed in order to detect any inter-country spread of the zoonose and advice on appropriates measures to policy makers. Previous studies that investigated the genetic diversity of rabies viruses circulating in the country found sub lineages G, H, and F of the Africa 2 lineage (Talbi *et al.*, 2016; Traoré *et al.*, 2016).

1.3 Justification

The use of RIDT and RT-PCR in addition to the gold standard FAT will enhance rabies diagnosis in Mali. While the first can be used to equip veterinary centres located in areas with limited resources, the later will be useful for the investigation of rabies suspected specimens unsuitable for FAT at the CVL. The characterization of lyssavirus isolates from this study will allow the determination of their geographical origins and inform on eventual inter-country spread of rabies in West Africa in general and between Mali and its neighbours in particular.

1.4 Hypothesis

There is no difference in detecting rabies virus when using RT-PCR and RIDT as compared to FAT.

There is no difference in genetic diversity of rabies viruses circulating in Mali.

1.5 Objectives

1.5.1 General objective

To investigate the suitability of the RIDT and RT-PCR for the detection and characterization rabies viruses circulating in Mali using FAT as gold standard.

1.5.2 Specific objectives

1. To detect rabies viruses circulating in dogs in Mali using RIDT.
2. To identify rabies viruses infecting dogs in Mali using RT-PCR and compare the results with the FAT.
3. To characterize the genetic diversity of rabies virus circulating in dogs in Mali.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rabies virus

2.1.1 Classification and geographical distribution

There are sixteen (16) recognized species in the genus lyssavirus based on criteria such as an 80-81% threshold of nucleotide identity. This comparison is made on either the full length of the N gene or the concatenated five (5) coding region. The consistency observed in several phylogenetic trees drawn using different evolutionary models is also used for rabies lyssavirus demarcation. These species are; Rabies lyssavirus (RABV), Lagos bat lyssavirus (LBV), Mokola lyssavirus (MOKV), Duvenhage lyssavirus (DUVV), European bat lyssavirus type 1 (EBLV-1), European bat lyssavirus type 2 (EBLV-2), Australian bat lyssavirus (ABLV), Aravan lyssavirus (ARAV), Khujand lyssavirus (KHUV), Irkut lyssavirus (IRKV), West Caucasian bat lyssavirus (WCBV), Ikoma lyssavirus (IKOV), Bokeloh bat lyssavirus (BBLV), Shimoni bat lyssavirus (SHIBV), Gannoruwa bat lyssavirus (GBLV) and Lleida bat lyssavirus (LLEBV) (Bourhy *et al.*, 2008).

Table 2.1 shows that RABV is globally distributed and is the dominant lyssavirus circulating across Africa, including Mali. The Taiwan bat lyssavirus is awaiting International Committee on Taxonomy of Viruses (ICTV) assessment. This emergence for new variants present challenges in diagnosis of rabies (Chen, 2009).

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Table 2.1: Classification of the Lyssavirus Genus (WHO, 2013)

Species	Abbreviation	Distribution
Rabies lyssavirus	RABV	World except several islands
Lagos bat lyssavirus	LBV	Sub-Sahara Africa
Mokola lyssavirus	MOKV	Sub-Sahara Africa
Duvenhage lyssavirus	DUVV	Sub-Sahara Africa
European bat lyssavirus, type 1	EBLV-1	Europe
European bat lyssavirus, type 2	EBLV-2	Europe
Australian bat lyssavirus	ABLV	Australia
Aravan lyssavirus	ARAV	Central Asia
Khujand lyssavirus	KHUV	Central Asia
Irkut lyssavirus	IRKV	Eastern Asia
West Caucasian bat lyssavirus	WCBV	South-eastern Europe
Ikoma lyssavirus	IKOV	United Republic of Tanzania
Bokeloh bat lyssavirus	BBLV	France, Germany
Shimoni bat lyssavirus	SHIBV	Kenya
Gannoruwa bat lyssavirus	GBLV	Sri Lanka
Lleida bat lyssavirus	LLEBV	Spain

These sixteen (16) species are subdivided into two (2) phylogroups based on genetic distances and serological cross-reactivity. Two (2) viruses are classified in the same phylogroup when

they have ≥74% amino acid sequence identity within the G ectodomain with presence of cross-reactivity. Viruses in the phylogroup I (RABV, ABLV, EBLV-1, EBLV-2, KHUV, ARAV, BBLV, IRKV, DUVV and GBLV) and phylogroup II (LBV, MOKV and SHIBV) are recognised with the presence of the Glycoprotein residue R333 and D333 respectively. IKOV, LLEBV and WCBV are not classified in either of these phylogroups due to long genetic distances and absence of cross-reactivity (Badrane *et al.*, 2001).

Rabies virus is globally distributed with Asia and Africa being the most affected continents where 95% of infections are caused by a dog bite. Children under the age of 15 years old are the most affected group. In the Caribbean and Latin America recent canine mass population vaccination led to a decline of rabies. Indeed, in 2004 only in only 23% of rabies reported cases involved dogs. The United States of America, Canada and Europe report few cases of rabies. Between 1980 and 2008, the United States of America reported an average of two (2) deaths yearly caused by rabies. During the same period, Canada reported an average of five (5) deaths yearly, while Europe recorded nine (9) deaths. Australia lost its status of "free-rabies" country with characterization of ABLV. A country is declared "free-rabies" by the WHO in the absence of indigenous rabies cases for at least 2 years consecutively (Chen, 2009). Figure 2.1 shows the geographical distribution of rabies.

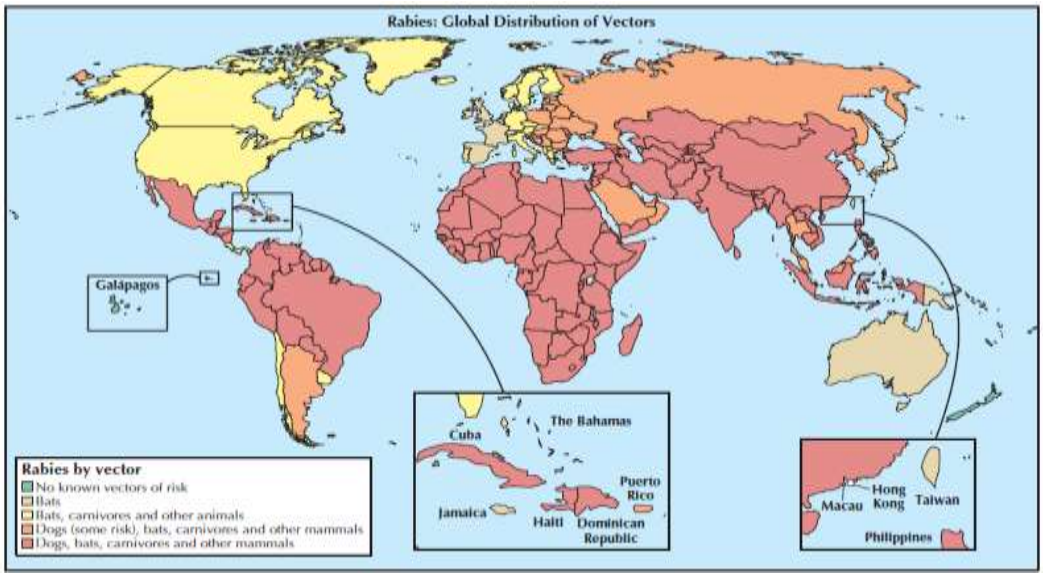

Figure 2.1: Geographical distribution of rabies. (Chen, 2009)

2.1.2 Structure

Rabies virions are bullet-shaped of around 130-250 mm long and 60-100 nm diameters. They are formed by an internal and an external unit linked together. The internal unit is composed of a nucleocapsid (NC) that includes the genomic RNA tied to the phosphoprotein (P), nucleoprotein (N), and viral polymerase (L). The external unit is formed by protruding spikes of the viral glycoprotein (G) and a bi-layer lipid envelope acquired from host cell membrane. These two (2) units are linked by the matrix protein (M) which interact with the G protein and condenses the NC (B. M. Davis *et al.*, 2015). Figure 2.2 shows the structure of rabies virus.

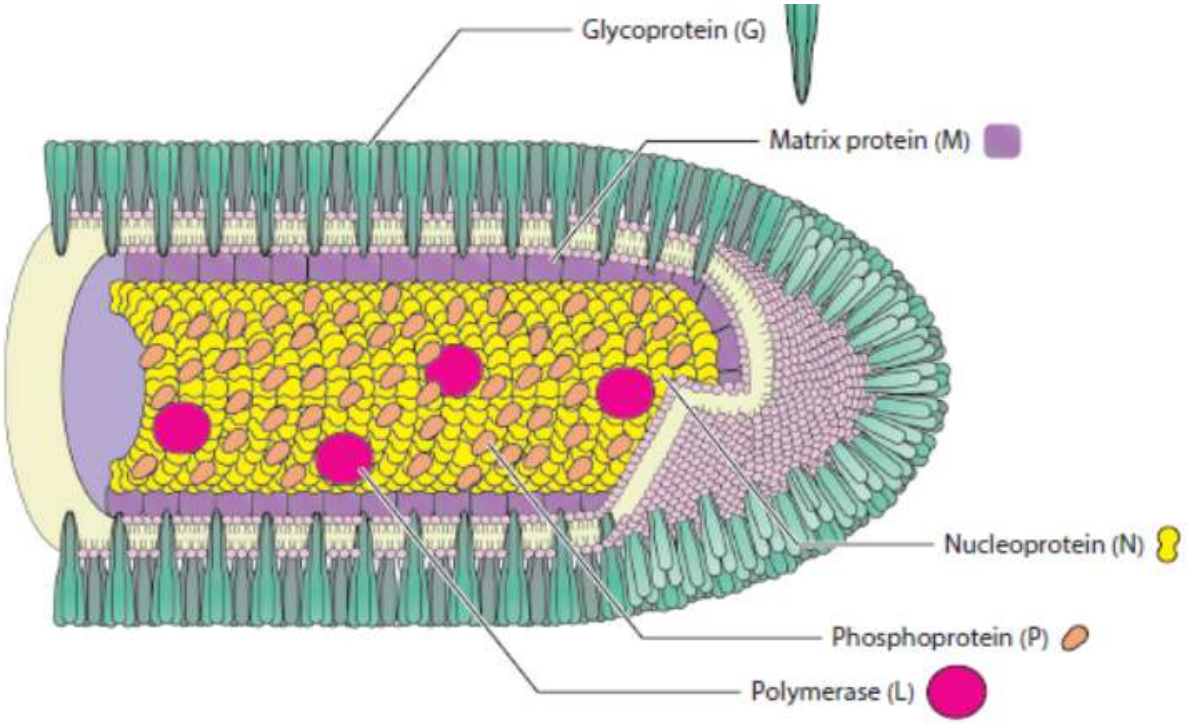

Figure 2.2: Rabies virion's schematic representation.

The figure presents the internal ribonucleoprotein (RNP) unit formed by the negative-sense, single-strand RNA genome encapsulated the virion-linked RNA polymerase (L), nucleoprotein (N) and polymerase cofactor phosphoprotein (P). The RNP in associated with the matrix protein (M) and condensed creating the bullet-shaped particle characteristic of rhabdovirures. A lipid bilayer from which the trimeric glycoprotein (G) spikes envelop the RNP-M structure (B. M. Davis *et al.*, 2015).

2.1.3 Genome

The genome of Lyssaviruses is single stranded RNA (ssRNA) measuring roughly 12 Kb. It is conserved in the specific 3'-N-P-M-G-L-5' order. Each of these five (5) genes remain flanked between a transcription initiation and termination (N Tordo *et al.*, 1988). Short untranscribed regions separate transcription units with only the G-L intergenic region reaching 400-700 nucleotides which is believed to be a remnant gene that lost functionality. The ssRNA genome has leader sequence and trailer sequence on its ends exhibiting terminal complementarity with promoter sequences for the initiation of genome and anti-genome's replication respectively. The genome of rabies viruses encodes five (5) viral proteins: nucleoprotein N (1334 base pairs), phosphoprotein P (978 base pairs), matrix protein M (840 base pairs), glycoprotein G (1674 base pairs) and polymerase L (6381 base pairs). Furthermore, the N gene being the most conserved segment of lyssaviruses genome (with the exception of some regions in the L gene). Genes which encode the ribonucleoprotein (RNP) are N, P and L while M and G genes encode for the lipid envelope that surround it. However, it is the G protein's ectodomain which exhibits the principal antigenic sites (Rupprecht *et al.*, 2002). Figure 2.3 below represents the schematic representation of rabies genome.

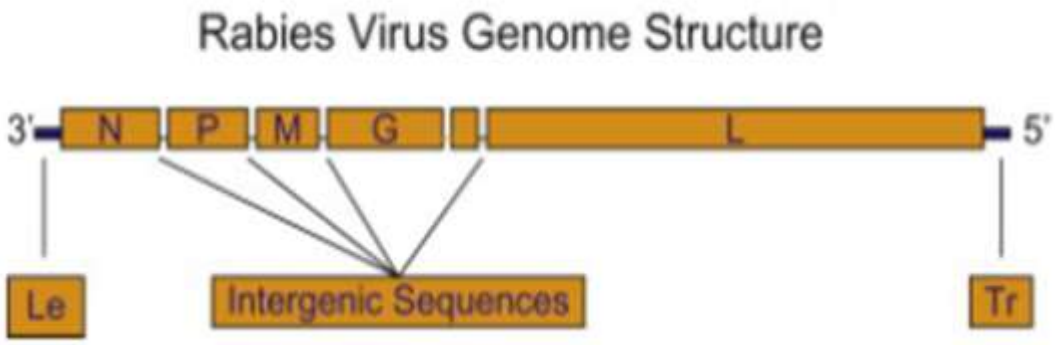

Figure 2.3: Rabies virus genome organisation.

The order nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-polymerase protein (L) genes separated by untranscribed intergenic regions and flanked by the leader (Le) and trailer (Tr) sequences (Rupprecht *et al.*, 2002).

2.1.4 Proteins

Rabies virus proteins (N, P, M, G, and L) are multifunctional. N protein ensuring the protection of the viral genome from RNAse is the major component of the NC. During transcription and replication, the N protein interacts with P and L protein. P protein participates in the replication and transcription process as a non-catalytic cofactor for the polymerase (L) as well as disrupt the host interferon (IFN)-mediated antiviral response (Rieder and Conzelmann, 2011). During N protein synthesis, P protein regulates the positioning for the polymerase on N-RNA template. It also prevents its binding to cellular RNA by acting as a chaperone. M protein linked to the domain of G protein located in the cytoplasm and NCs makes easy the budding process, apoptosis, and intercellular membrane redistribution. The G protein being the only component of the virus present on the surface is composed of the endodomain, transmembrane domain and ectodomain. The ectodomain is responsible of the binding to the receptors such as the nicotinic acetylcholine receptor in the host cell, trigger endocytosis and fusion of the viral and endosomal membranes (Lafon, 2005). During this process, the endodomain interacts with the M protein to allow virion morphogenesis and budding. The ectodomain being the only external section of the G protein provokes the synthesis of virus-neutralizing antibodies (VNA) following immune response mediated by cells. Finally, L protein with several functions and domains including RNA-dependent RNA polymerase, 3' poly (A) polymerase, cap methylation, mRNA 5' capping enzyme, and protein kinase activity (Rieder and Conzelmann, 2011).

2.2 Rabies infection

Following an exposition to rabies, the only recommended treatment is the post-exposure prophylaxis (PEP) starting with cleansing the wound before administration of rabies vaccine with/without rabies immune globulin (RIG) which must be administrated shortly following exposure. The protocol for PEP may vary depending on the status of the exposed person. Figure 2.4 shows the protocol of PEP for immunized and non-immunized patients. In case of non-respect of these guidelines, infested patients invariably die within 1 to10 days following the apparition of symptoms (Chen, 2009).

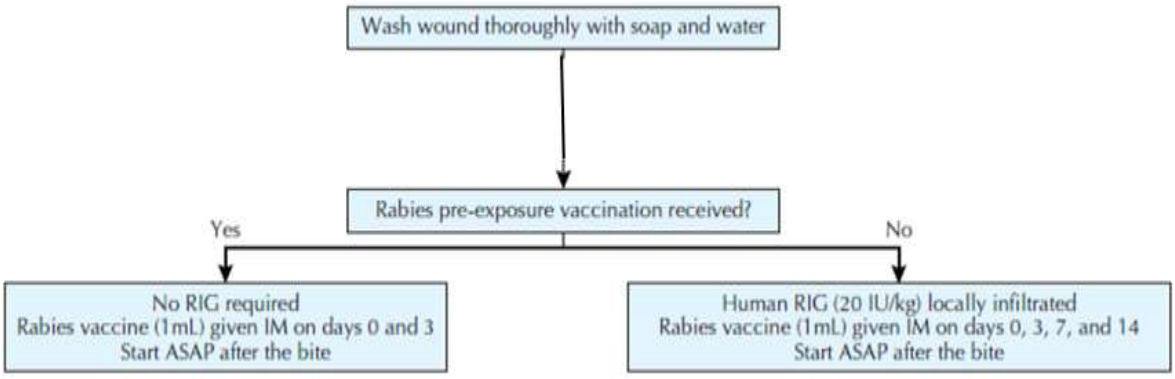

Figure 2.4: Protocol for Post Exposure Prophylaxis.
IM: Intramuscularly. RIG: Rabies immune globulin (Chen, 2009).

2.2.1 Viral pathogenesis

Rabies virions are generally delivered to the victim following a bite or scratch from a dog exposing the wound to saliva filled with RABV particles. After entry of the RABV, viruses use the motor or sensory neurons to move centripetally towards the CNS during the incubation period. Once they reach the CNS, lyssaviruses propagate rapidly to almost all sections of the CNS. The virus is then spread centrifugally from the CNS to several organs including heart, tongue, salivary glands, hair follicles, skin, and adrenal glands marking the beginning of the clinical phase of the infection. Throughout the incubation period which can last for 1-2 months on average there are non-clinical signs of the infection and diagnosis is almost impossible due uncertain location of the virus, absence of detectable immune response, and limited viral load. In contrary, the relatively short (1-2 weeks) clinical period when the affected subject develop fever, flu-like symptoms, malaise and gradually as the infection progress to encephalitis, delirium, hallucinations, hydrophobia, photophobia, aerophobia, and phonophobia. During this period, anti-N antibodies and anti-G antibodies are detectable in the cerebrospinal fluid (CSF) and serum. Death caused by either respiratory or cardiac failure occurs often within 1 to 10 days after the apparition of symptoms (Boland *et al.*, 2014). Figure 2.5 shows the mechanism of human exposure to RABV through dog bite.

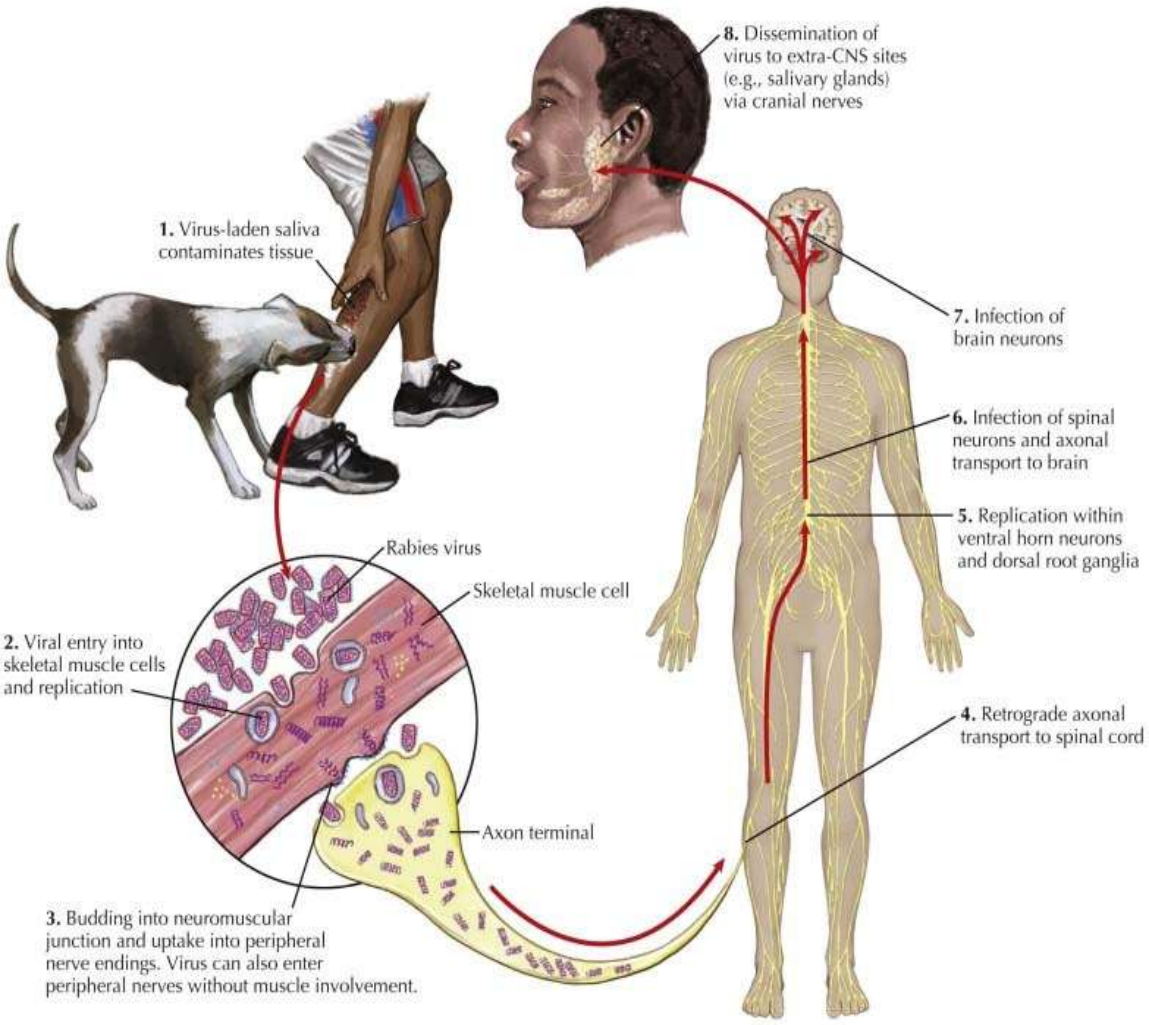

Figure 2.5: The path of rabies virus (RABV) infection following dog bite.

RABV infection starts mostly from an animal scratch or bite followed by the migration of the infection towards the central nervous system (Robertson *et al.*, 2012).

2.2.2 Clinical signs

Despite presenting a wide range of symptoms, rabies cases can be roughly divided into paralytic and furious (Singh *et al.*, 2017). Furious rabies presents classical symptoms of rabies such as severe hydrophobia and agitation during days before worsening to paralysis, impaired consciousness, coma and finally death. Paralytic rabies is marked by ascending paralysis followed by similar path leading to coma and death. Both progression of the infection has been observed in two (2) patients infected by the same dog showing that the progression is not strain-related. Rabies symptoms also present little variation based on the species involved. Indeed, small mammals (bats) express a prolonged disease progression and even recovery in some cases. In contrary, patient's recovery from clinical rabies remains controversial. In rural Peru, naturally acquired immune resistance to RABV has been documented in two (2) communities (Gilbert *et al.*, 2012). Nevertheless, these promising observations have not changed the painful outcome for patients presenting rabies symptoms no matter if the animal involved in the exposure is a small or large mammal. This confirms that RABV causes the same damage at cellular level of all mammals.

2.3 The conventional diagnostic tests for rabies

Despite the tremendous impact of rabies on agriculture and conservation biology, its greatest burden is on public health. For more than five millenniums (5000), humans have established a fine for the owner of a biting dog at around the equivalence of a half-day work due to fear of a bite from a mad dog. Today, more focus is put on the development and evaluation of diagnostic tools that are cheap and reliable to help investigate rabies suspected samples. This is the way to go if the goal set by the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health, and the WHO of zero death from rabies by 2030 is to be achieved (World Health Organization. The Tripartite's commitment, 2017).

2.3.1 Direct Microscopy

Rabies virus provokes the appearance in one corner of neuron or within the cytoplasm of the nucleus specific inclusions called Negri bodies. These inclusions are generally round, however, can assume several shapes including oval, elongate, spheroid, triangular and amoeboid. Additionally, Negri bodies can be found in different size ranging from 0.24 µm to 27 µm. However, the most important characteristic is their internal structure, which is used as essential criterion for identifying a positive sample. Negri bodies have a heterogeneous matrix containing basophilic granules organized in rosette fashion of a size ranging from 0.5 µm to 2.0 µm. They are mostly found in abundance in the brain, cerebral cortex, and cerebellum at the Ammon's horn, pyramidal cells, and Purkinje's cells respectively but also in the pons, spinal cord, thalamus and sensory glanglia in few cases. For this reason, the Ammon's horn is investigated in priority before the cerebellum and finally cerebral cortex with at least three (3) tissues samples taken from each area before to declare a sample negative for rabies (Tierkel and Atanasiu, 1996).

Despite the existence of inclusion-like bodies such as the acidophilic inclusion occasioned by the canine distemper or Rubarth's disease, there is a universal consensus that the presence of Negri bodies signals rabies infection. Additionally, a well formed Negri body can be easily differentiated from inclusion-like bodies due to its specific characteristics using Sellers' stain. There are three (3) methods for the application of brain tissues on slides; impression method, smear method and rolling method.

The impression method is performed by cutting small sections of around 2 mm to 3 mm of brain tissues with a pair of scissors and placing them, cut surface upward. A microscope slide is thereafter pressed downward on the cut surface creating a spread. Depending on the size of the section, 3 to 4 impressions are made on the slide. Finally, the slide is put during 5 to 10 seconds in Sellers' stain, rinsed using running water, and dried.

The smear method consists of the use of two slides with a small brain's section placed on one, crushed and spread with the other. The surface covered by the spread should be around three-quarter of the slide leaving a thin and homogenous film on the slide.

The rolling method is the most rapid and easiest technique consisting of cutting a section from brain tissue measuring around 5 mm. The piece is then rolled gently on surface of the slide with a wooden applicator or toothpick (Tierkel and Atanasiu, 1996).

2.3.2 Fluorescent Antibody Technique (FAT)

The Fluorescent Antibody Test, also known as Direct Fluorescent Antibody Test (DFAT), the most used diagnosis for investigating rabies suspected samples, is recommended by both the WHO and OIE. This test can be used on fresh brain tissue, cell culture, or even brain tissue of mice inoculated to investigate the presence of rabies virus antigens. When performed on fresh samples, FAT gives a reliable result in few hours in more than 95 to 99% of the cases (Dean *et al.*, 1996). Additionally, FAT can be applied to glycerol-preserved specimen, formalin-preserved specimens as well as specimens treated with proteolytic enzyme (Barnard and Voges, 1982; Umoh and Blenden, 1981). However, results become less sensitive than when performed on fresh tissue (Shankar, 2009).

The FAT procedure involve labelling antibody with fluorescein isothiocyanate (FITC) dye, allowing the antibody labelled to interact with antigen in case they are present in the sample, and thereafter visualizing the reaction's product on fluorescence microscope. The bound antigens always appear as greenish-yellow or apple-green objects when a dark background is used under ultraviolet light (U.V) in a sample with no nonspecific fluorescing material. Briefly, brain impressions prepared using different parts of the brains, are fixed using cold high-grade acetone, stained with a drop of anti-rabies fluorescent conjugate (OIE, 2008). FAT has the advantages of being highly specific allowing samples submitted to the laboratory in a morning to be processed and confirmed within the same working day. However, the need of a fluorescent microscope, rabies conjugate and well trained personnel makes the FAT an expensive technique (Barnard and Voges, 1982). Figure 2.6 shows a FAT test result.

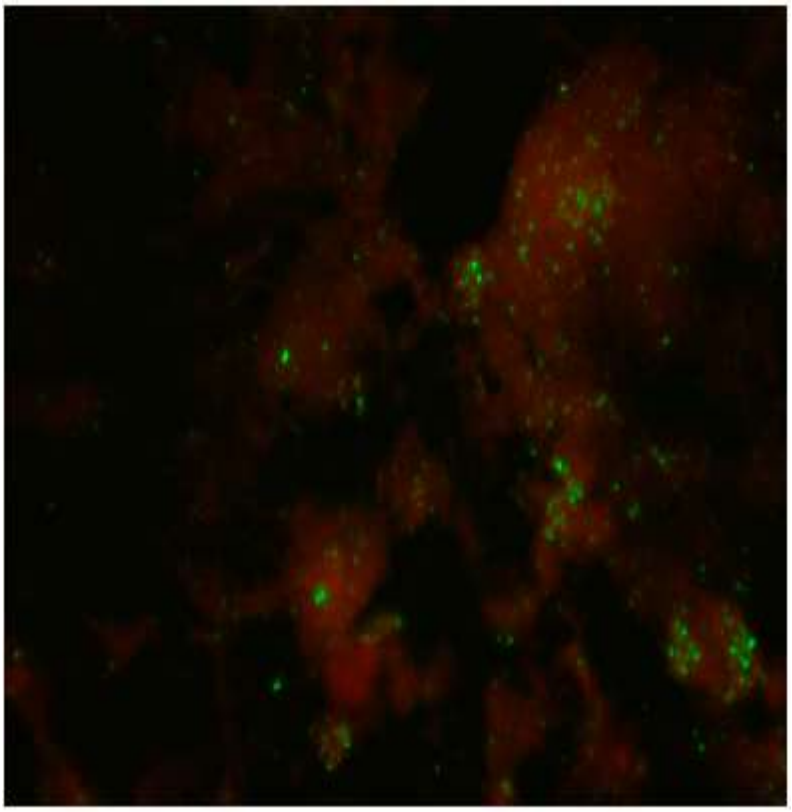

Figure 2.6: Sample of a FAT result.
Dog brain samples subjected to FAT using anti-rabies nucleocapsid protein IgG-FITC conjugate (Prabhu *et al.*, 2018).

2.3.3 Rapid Rabies Enzyme Immunodiagnosis (RREID)

Based on an enzyme-linked immunosurbent assay (ELISA) developed by Perrin and colleagues in 1986, known as rapid immunodiagnosis (RREID), two (2) more approaches have been developed; RREID-lyssa and RREID-biot. These techniques require brain tissue sample during diagnosis precluding them for antemortem diagnosis.

They involve capturing the nucleoprotein antigen using monoclonal or polyclonal anti-N antibody coated in the solid phase. While the RREID-biot and RREID use IgG directed against the PV strain only, RREID-lyssa uses several IgG against Pasteur Virus strain, European bat lyssavirus, type 1 and Mokola lyssaviruses. Furthermore, RREID-lyssa and RREID-biot employ an IgG-biotine conjugate and RREID employs IgG-peroxidase conjugate to reveal the bound antigen (Bourhy and Perrin, 1996).

The RREID test can be evaluated quantitatively using a spectrophotometer or qualitatively with the naked eye. Following addition of o-phenylenediamine and the substrate, an orange-yellow colour is visible.

The technique used to reveal the viral antigen bound differentiates RREID and RREID-biot and RREID-lyssa. While the first, uses peroxidase as a conjugate (the rabies antinucleocapside rabbit IgG with horseradish peroxidase), the latter used an IgG-biotine conjugate (the biotinylated rabies antinucleocapsid rabbit IgG with a streptavidin-peroxidase). In both techniques, negative samples appear colourless. The demarcation between positive and negative samples can be done with naked eye or by using a spectrophotometer (Bourhy and Perrin, 1996).

The measurement of the absorbance using a spectrometer should be done within the 30 minutes following the stopping of the reaction and involves three (3) steps. Firstly, carefully clean the bottom of the microtitration plate before placing in the spectrophotometer. Secondly, evaluate the optical density (OD) for 492 nm for the blank (s), the samples and the controls. Finally, determine the absorbency by deducting the OD of the blank from that of the samples and controls.

The test is valid only when the absorbency of the negative and positive controls should be below 0.1 and above 1.5 units respectively. Samples that have an absorbency of more than 0.08 units (RREID) or 0.1 (RREID-biot or RREID-lyssa) above that of the negative control are considered positive (Mani and Madhusudana, 2013). Figure 2.7 shows samples that with an orange coloration considered positive and those that are colourless negative.

Figure 2.7: Sample RREID results.

The dark brown colouration is obtained with rabies positive brains in contrast to negative brains which appear colourless (Mani and Madhusudana, 2013).

2.3.4 Virus Isolation (VI)

The purpose of Virus Isolation is to further investigate a sample involving a human exposure when the FAT or Direct Microscopy detecting Negri bodies give an uncertain result. Previous studies have shown that 10 to 15% missed by direct smear investigating Negri bodies have been proven positive by VI. It can be performed using a tissue suspension in cell culture or laboratory animals such as mouse resulting in two (2) variants of VI technique known to as Rapid Tissue Culture Infection Test (RTCIT) and Mousse Inoculation Technique (MIT) respectively (Kaprowski, 1996).

The MIT is an in-vivo test performed using five (5) to ten (10) mice of three (3) to four (4) weeks or two (2) new-born mice. These mice are inoculated using a supernatant of 20% weight/volume (w/v) homogenate of suspected brain tissue in an isotonic buffered containing antibiotic intra-cerebrally. These mice should be anesthetized before inoculation on animal welfare ground and then observed daily during 28 days. When new-born mice are used, it is possible to detect the rabies virus antigen using the FAT as earlier as 5 days after inoculation.

However, any dead occurring before day 5 is considering non-specific and might be due to bacterial infection or stress (OIE, 2008). The MIT is time consuming, expensive when Specific pathogen free (SPF) are used and should be should be avoided when an alternative technique is available (Chhabra *et al.*, 2007). It has been used successfully for testing salivary gland tissues from post-mortem as well as saliva and Cerebrospinal fluid (CSF) from living individuals (Chhabra *et al.*, 2007).

The spread of rabies virus spread toward most organs after reaching the centre nervous system (CNS) of the infected animal where it replicates efficiently. This has given laboratories the possibility to cultivate the rabies virus in large range of host cells such as murine neuroblastoma (NA-C1300) cell line. These cells share several characteristics with human neurons including the presence of neurotransmitter synthetic enzymes, a fine-structure neuron-like morphology, and excitable cell membranes with acetylcholine receptors.

Therefore, RTCIT allowing production of large quantity of virus without the use of animals has replaced MIT in several laboratories (Webster and Casey, 1996). This technique involves adding 0.5 g of mashed brain tissue to 5 ml of PBS with antibiotics for a suspension of 10% w/v concentration. This suspension is vortexed and let to settle during one hour at a temperature of 4 °C enabling the extraction of the clear upper layer. The clear upper layer is thereafter diluted at 10 fold with Eagles' minimum essential medium combined with the foetal calf serum at 10% to make a final concentration of 1% w/v suspension.

This suspension is thereafter; added to the cell suspension and incubated for at least 18 hours at 36 °C with 5% CO2 allowing a replication cycle to take place, and the presence of rabies virus antigen investigated using FAT (Rudd and Trimarchi, 1987).

2.3.5 Demonstration of Antibodies

Today, several serological techniques for detecting rabies virus antibodies have been developed including indirect fluorescent antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA). The confirmation of the presence of antibodies in either the serum or the CSF with no prior vaccination is a confirmation of rabies infection.

Considering the variability of the host immune response, the interpretation of serological testing results may be difficult but are useful for evaluating the sero-conversion after an immunization. These techniques are rarely useful for antemortem diagnosis due to high mortality rate of rabies infection but can be used in the case of paralytic rabies whereby the survival is relatively longer.

The virus neutralizing antibodies (VAN), the fluorescence antibody virus neutralization test (FAVN), mouse neutralization test (MNT), rapid fluorescent focus inhibition test (RFFIT), and the counter immunoelectrphoresis (CIE) have been developed described for controlling vaccination responses and are presented in international units which is compared with the international standard antiserum (Mani and Madhusudana, 2013).

Indeed, the quantification of a pre-exposure (PrEP) prophylaxis or post-exposure prophylaxis (PEP) can be done through antibodies titration with 0.5 IU/mL being the minimum value (WHO, 2013). The IFAT is an antigen binding assay measuring the quantity of antibodies binding from infected cells. The serum and CSF are tested separately for the presence of IgM and IgG using slides that contain fixed whole cells infected with RABV (antigen). In case RABV antibodies are present in the sample (serum or CSF), they bind with the antigen to form the complex antigen-antibody which will be revealed by using a secondary antibody, such as a fluorescein isothiocyanate (FITC)-labelled anti-human globulin (IgM or IgG) as shown in Figure 2.8.

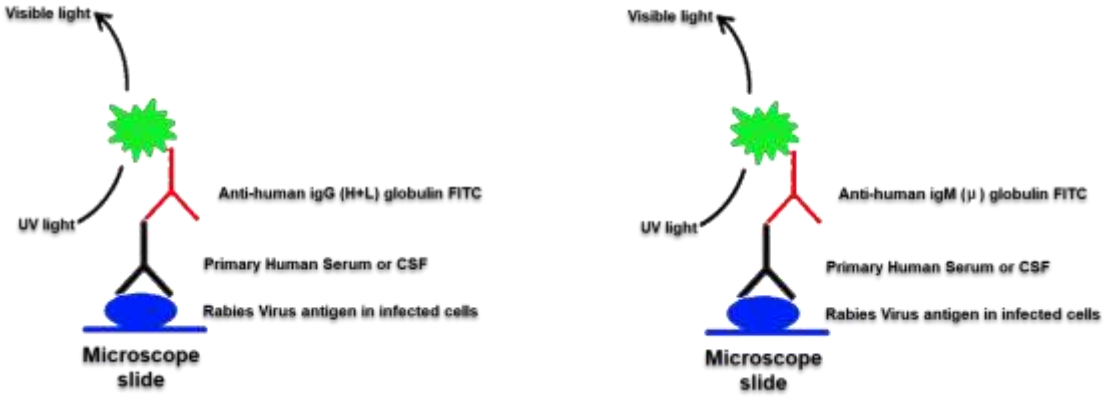

Figure 2.8: Principle of the IFA test.

CSF, Cerebrospinal fluid; FITC, Fluorescein isothiocyanate; Ig, Immunoglobulin; UV, ultraviolet. (J. B. Thomas *et al.*, 1963).

2.4 Newer diagnostic tests for rabies

New techniques for detecting rabies include Direct Rapid Immunohistochemical Test (DRIT), RIDT and Nucleic Acid Detection Techniques.

2.4.1 Direct Rapid Immunohistochemical Test (DRIT)

The American Centers for Disease Control and Prevention (CDC) developed the rapid immunohistochemical test (RIT) to detect rabies antigens, which will be modified to the ditectimmunohistochemical test (DRIT) by combining several elements of the immunoperoxidase techniques. This later could reveal rabies virus antigen using direct staining with fresh brain impressions.

A cocktail of highly concentrated and purified monoclonal antibodies coated with biotin within one (1) hour. These anti-rabies antibodies are directed specifically to the nucleoprotein. Indeed, the nucleoprotein is the viral protein synthetized in abundance during infection (Fooks *et al.*, 2009). The dRIT recognises all representative lyssaviruses and uses brain impressions but unlike FAT, the product under light microscopy where a positive result appears as magena inclusions against a blue background as shown in Figure 2.9 (Lembo *et al.*, 2006).

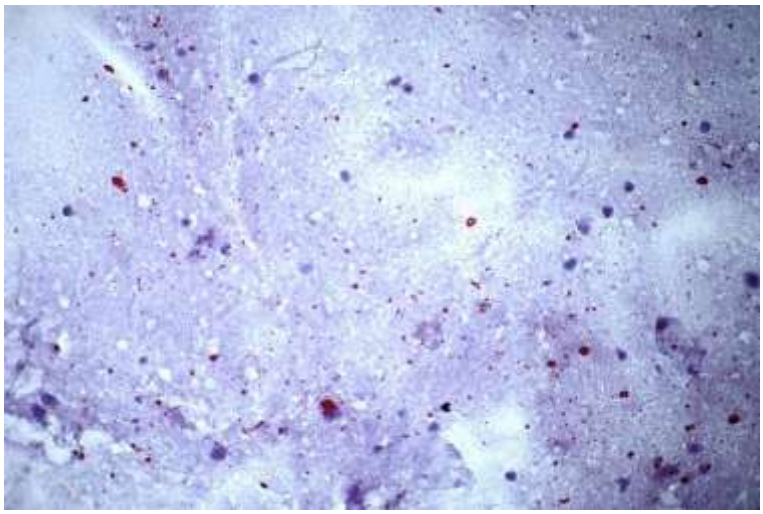

Figure 2.9: Sample of DRIT results.
Viral inclusions are perceivable with the blue background for the brain impression (Coetzer *et al.*, 2014).

2.4.2 Rapid Immunochromatographic Diagnostic Test (RIDT)

The decentralization of animal and human testing is a powerful tool to improve the reporting and surveillance of infections in areas with limited resources. These tests are useful under field conditions, present several advantages such as easy to use, fast in delivering result, room temperature's storage and are known to as lateral flow devices (LFDs), lateral flow immunoassays (LFAs), immunochromatographic strip tests or rapid immunochromatographic diagnostic tests (RIDTs).

The first commercialized RIDT was a pregnancy test in 1988 following the introduction of the idea in 1960 (Posthuma-Trumpie *et al.*, 2009). Thereafter, this technology was adopted in several areas including viral infections such as Ebola virus disease, avian influenza, and foot-and-mouth disease (Ferris *et al.*, 2012; Slomka *et al.*, 2012; Walker *et al.*, 2015).

All these devices follow the same principle whereby around four (4) drop of a liquid sample is put into the device, gold conjugated antibodies present in the pad of the device bind to the antigens if present. Thereafter, by liquid migration, the antigen-antibody complex moves through a nitrocellulose membrane before being immobilized by a second antibody located at the test line forming a visible line. The unbound conjugated antibodies will form the control line after being captured downstream.

Antigen Rapid Rabies Ag Test Kit (Bionote, Korea), Vet-o-test Rabies Ag (BioGen Technologies, Germany), Quicking Pet Rapid Test (Quicking Biotech, China), Rapid Rabies Ag Test Kit (Creative Diagnostics, USA), quickVET Rabies Antigen Rapid Test (Ubio, India), and Rabies Virus Ag Test Kit (Green Spring, China), are the six (6) commercial RIDT kits for rabies are available (Eggerbauer *et al.*, 2016).

The Antigen Rapid Rabies Ag Test Kit manufactured by Bionote is an all-in-one kit for a rapid diagnosis of rabies. Once the suspected brain sample is extracted, a sample is collected using the swab, which is then inserted into the tube containing one 1 mL of the assay diluent for about 1 minute allowing a complete dissolution of the brain materiel. Four drops are transferred into the device using the pipette provided in the kit. The test's interpretation can be done after the migration of the coloured liquid ends at.

Following the manufacturer recommendations, a positive result is designated by the presence of two lines, in the test and control zone. A result is negative when a single line is observed at the control zone. Finally, a test is invalid when a single line is observed at the test zone. The test takes around 5 to 10 minutes with no allowed interpretation after 10 minutes.

The RIDT is recommended by the manufacturer for raccon dog, dog, as well as cattle and performed immediately of collection (BioNote, 2008). It has been shown that the RIDT is a can be an efficient tool for rabies diagnosis but also can be used for the conservation of RNA. This will allow molecular detection and genotyping purposes (Lechenne *et al.*, 2016).

Recently, in 2018 in Argentina, an evaluations of Anigen Rapid Rabies Ag Test kit gave sensitivity and specificity of 97.96% and 100% respectively (Gury Dohmen *et al.*, 2018). Figure 2.10 shows a sample of RIDT kit.

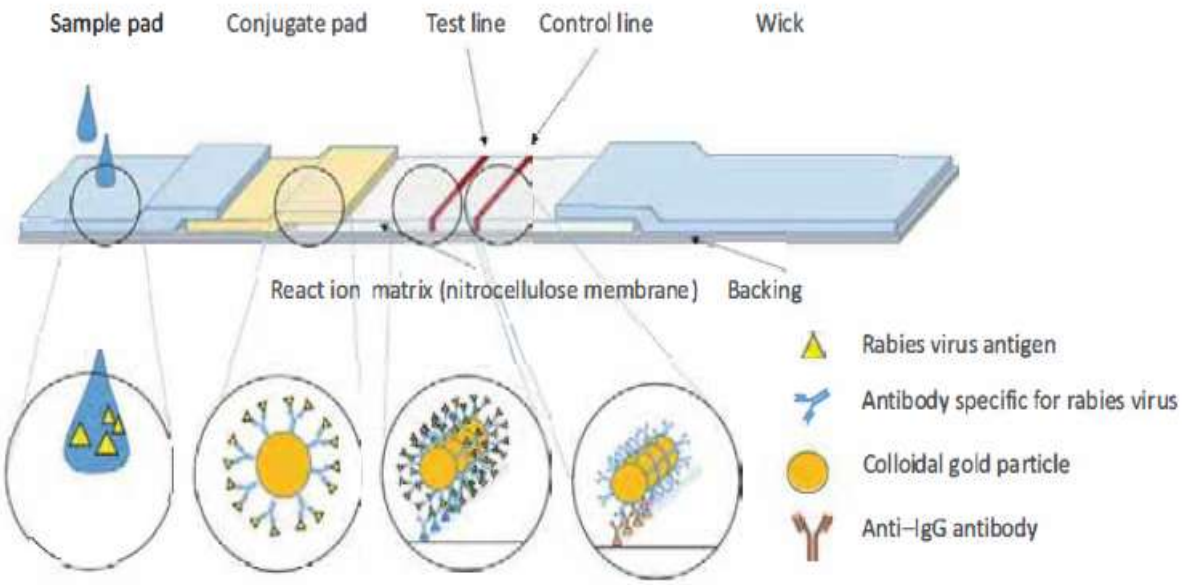

Figure 2.10: RIDT for the detection of the RABV viral nucleoprotein.
(Gury Dohmen *et al.*, 2018).

2.4.3 Nucleic Acid Detection Techniques

The cloning of the entire rabies genome has given diagnostic laboratories with nucleic acid probes for the five (5) rabies virus genes (N. Tordo *et al.*, 1996). This has led to adoption of modern techniques such as Reverse Transcription Polymerase Chain Reaction (RT-PCR),

Real-Time Polymerase Chain Reaction (qPCR), RT-Loop-Mediated Isothermal Amplification (RT-LAMP) and Nucleic Acid Sequence-Based Amplification (NASBA).

Several conventional RT-PCR protocols targeting the five lyssaviruses genes as well as generic using agarose gel electrophoresis for revelation have been published (Fooks *et al.*, 2009; Singh *et al.*, 2017). These gel-based RT-PCRs sensitivity could be improved by introducing a second round of amplification with a second pair of primers and targeting a shorter sequence within the amplicon produced by the first round.

While hemi-nested-RT-PCR uses either the forward or reverse of the pair of primers employed in the first round of amplification, nested-RT-PCR uses none of them. The nucleoprotein (N) and polymerase (L) genes of the most targeted sequences because primers are selected to target conserved segments in the genome (Heaton *et al.*, 1999).

RT-PCR is specific, rapid, highly sensitive, and contribute significantly in rabies's diagnosis for tropical countries (Junior, 2004). It was used successfully to investigate the presence of rabies in CSF, brain, neck skin, urine and saliva of infected humans (Biswal *et al.*, 2007). RT-PCR has been recommended as a confirmatory test. Indeed, RT-PCR can detect rabies infection earlier than conventional tests (Biswal *et al.*, 2012). Additionally, it is useful when the recommended techniques for rabies diagnostic FAT and MIT are not suitable due to decomposition (Araújo *et al.*, 2008).

The introduction of fluorogeneric and hydrolysis probes has allowed scientists to detect in real-time a sequence specific template. Real-time RT-PCR assays combine the amplification and detection process in one closed tube system offering a rapid and more reliable confirmation of the presence of lyssavirus genome in suspected samples.

The addition of a DNA intercalating dye (fluorochrome) or hydrolysis probes to the mix allows the detection of amplicons in real time. While fluorochromes (ResoLight or SYBER Green) emit fluorescence after it binds to the DNA double-stranded, hydrolysis probes (TaqMan probes) binds to their target region allowing the dissociation of the fluorophore and the quencher causing fluorescence.

Real-Time TaqMan-RT-PCR technology was used to detect as well as differentiate between Lyssavirus genotypes 1, 5 and 6 (Wacharapluesadee *et al.*, 2008). This technique uses a pan-Lyssavirus primer set, which amplify a large panel of representative Lyssaviruses, with probes specially designed to discriminate between European Bat Lyssaviruses type 1 and 2 and classical rabies virus. It has improved timely antemortem human rabies diagnosis, methods by detecting viral RNA (Nadin-Davis *et al.*, 2009). It has also reduced cross-contamination because of the use of the "closed-tube" nature of the assays (Hughes *et al.*, 2004). The SYBR Green real time PCR has been suggested to be specific, sensitive, and useful molecular technique for antemortem rabies detection using saliva samples (Kaw *et al.*, 2012).

First introduced in the year 2000 (Notomi *et al.*, 2000), RT-LAMP offers and cheap but also sensitive, simple and rapid method for the amplification and the detection of DNA (Yasuyoshi Mori *et al.*, 2013). This technique depends on the strategy allowing the synthesis of DNA through strand-displacement using Bst DNA polymerase occurring in an isothermal temperature without the need of DNA templates denaturation. DNA is amplified 109 to 1010 in 10 to 60 minutes resulting in products composed of stem-looped DNA with different lengths (Y Mori *et al.*, 2001). Visual inspection of the white precipitation caused by magnesium pyrophosphate or ultraviolet fluorescent dye can be used for result's revelation as shown in figure 2.11.

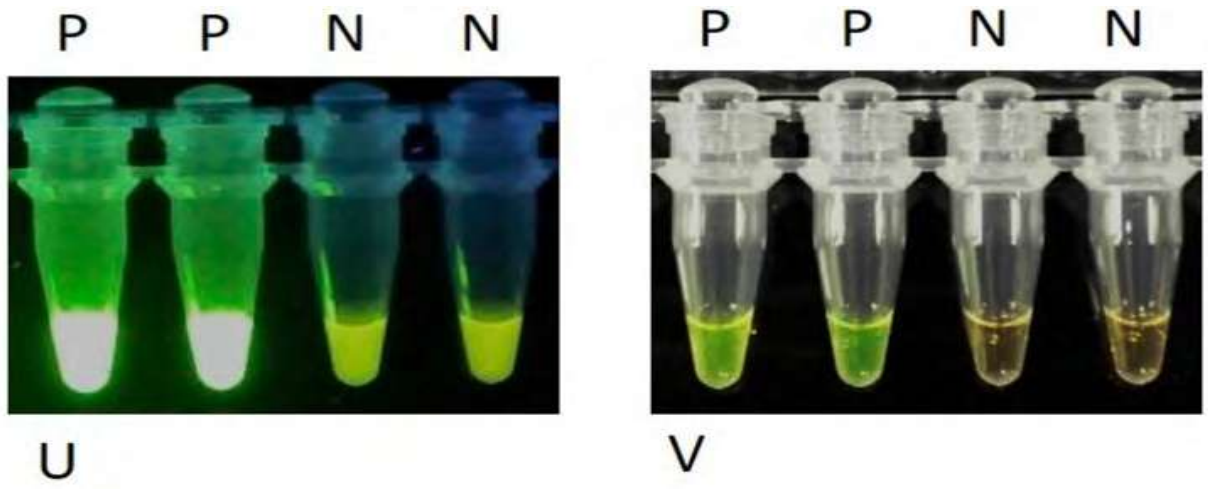

Figure 2.11: Sample of RT-LAMP results.

U and V are the results under ultraviolet and visible lights respectively (Tomita *et al.*, 2008).

RT-LAMP is an alternative DNA amplification technique to PCR for applications to the antemortem CSF, saliva, and post-mortem using the brain as sample. The target amplification can be revealed using agarose gel. However the development of RT-LAMP assay can be challenging because of sequence variation observe of the rabies genome. This is frustrating in the design of specific primer. However, attempts suggesting a combination of several primers (around 12) will lead to the amplification of RABV genomes derived from a wide geographical locations (Badrane *et al.*, 2001).

The reverse transcriptase, T7 RNA and RNase H are the three (3) enzymes involved in NASBA technique under isothermal conditions allowing the synthesis of several copies of the target RNA. Specific pair of primers with one containing the binding site for the T7 RNA polymerase, and the other having at its 5' end an electro-chemiluminescence, enabling automated detection of the amplified RNA with a reader. This technique can within four (4) hours detects rabies two (2) days following the apparition of symptoms using either CSF or saliva after collection RNA in a buffer to avoid its degradation making it a reliable ante-mortem test (Wacharapluesadee and Hemachudha, 2001). However, repeated sampling and testing of saliva and CSF are recommended (Singathia *et al.*, 2012).

2.5 Comparison of selective diagnostic tests for rabies

Today, a wide range of methods is available for rabies diagnosis leading to an inevitable comparison of the merit and demerit of each of them. Indeed, lyssaviruses are now investigated using among others, demonstration of antibodies, nucleic acid detection techniques, direct microscopy, FAT, RREID, VI, dRIT and RIDT. Appendix 1 gives a selective comparison of several rabies tests.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The present study was carried out at the rabies diagnosis and molecular biology units of the CVL and Laboratory of Applied Molecular Biology (LMBA) respectively. RIDT and RNA extraction were performed at the CVL while RT-PCR was done at the LMBA.

In 1936, the Research, Production and Sero-therapy Laboratory (LRPS) was created following the No. 2936 of 20 September 1936 ordnance. It is in 1979, by order N ° 7976 (C.M.L.N) that the name was changed to CVL of Bamako. The missions of the CVL are to ensure the production of vaccines against livestock diseases, routine diagnosis of animal diseases, research on livestock diseases and quality control of foodstuffs in Mali. The CVL also provides training national and foreign technicians as well as students in the field of laboratory techniques in the field of animal disease diagnosis.

The LBMA is an academic public research organization affiliated with the Faculty of Science and Technology of the University of Bamako. Its mission is to promote research in the field of plant and animal production as well as fight against malaria and HIV-AIDS using molecular techniques. The LBMA contributes to the modernization of university education through molecular biology. The main areas of intervention of the laboratory are: medical biotechnology and plant and animal biotechnology. The LBMA is composed of four research units: a parasitology unit, a plant and animal biotechnology unit, a virology unit and a clinical biology unit.

3.2 Collection of samples

A convenient sampling method was undertaken to collect 18 dog brain samples submitted to the CVL. Samples submitted to the CVL are routinely analysed using FAT and brains confirmed are conserved in 20% (w/v) homogenate in Phosphate Buffered Saline (PBS) and archived at -20^{o} C. They are obtained mainly from the capital city, Bamako (14) but also

Koulikoro (03), and Kayes (01) regions. Figure 3.1 shows the distribution of samples used in this study.

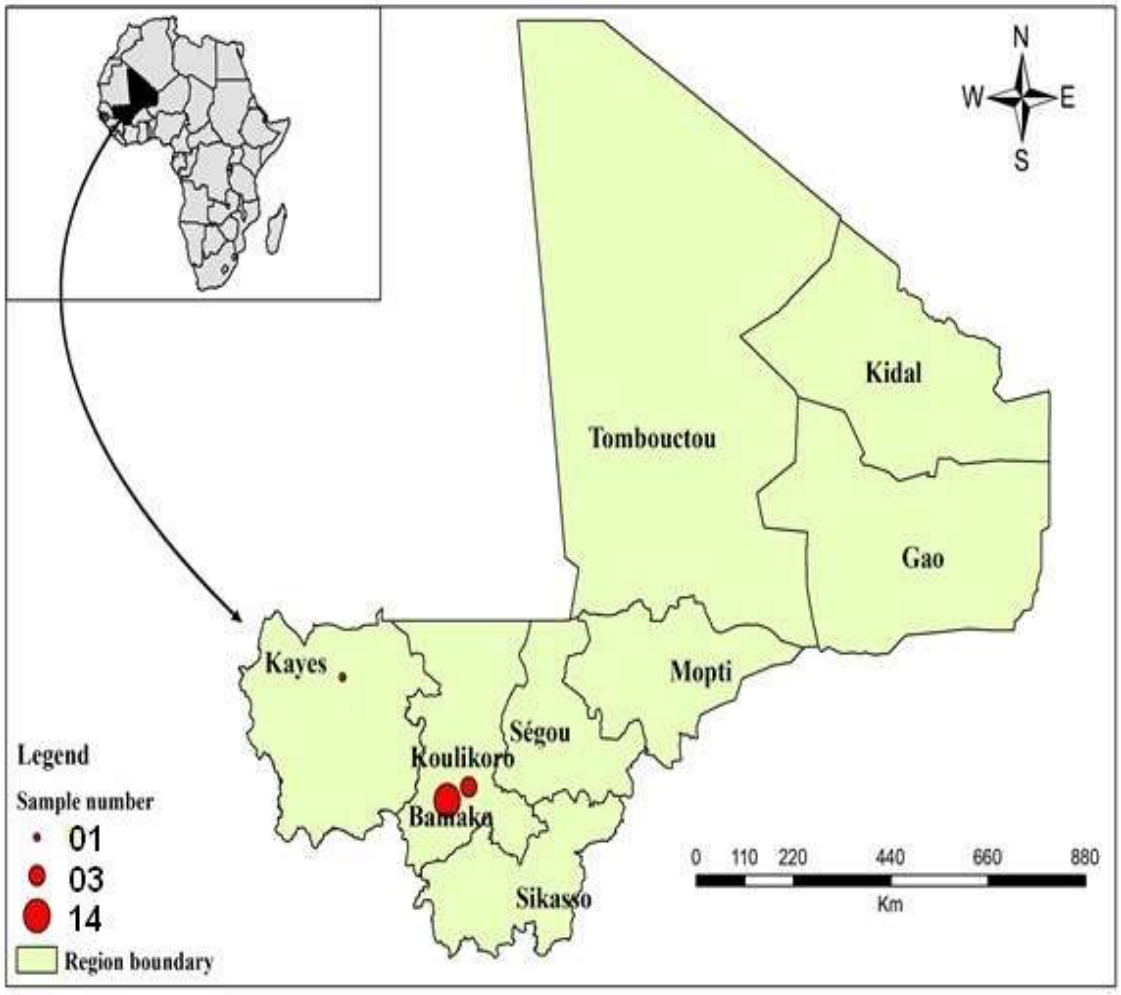

Figure 3.1: Distribution of samples used in the study in Mali

3.3 Fluorescence Antibody Test (FAT)

The FAT was performed according to the procedure described by the Office International des Epizooties (OIE, 2008) and the WHO (WHO, 2013) . Briefly, the impressions smears was prepared from different portions of brain and were fixed in chilled acetone for 2 hours. The slides were enriched around the smear and immersed in PBS (pH 7.2) for 5 minutes. They were then incubated with FITC conjugate anti-rabies antibody (BioRad) for 30 min in humidified dark chamber at 37 °C. The slides were washed with PBS thrice in slide holding glass through by creating current with magnetic stirrer. After washing, the slides were mounted and examined under fluorescence microscope at 400 nm. The presence of dusty apple green fluoresce were taken as positive signal.

3.4 Detection of rabies virus nucleoprotein antigen using RIDT

The Anigen Rapid Rabies Ag Test kit was used to diagnose rabies. Succinctly, around 1 ml of archived brain sample stored in was allowed to defrost before transferred into the specimen hole of the device, which was placed on flat, dry and clean surface and the result read within 5 to10 minutes. The interpretation of these results was done using the manufacturer's recommendations (BioNote, 2008).

Therefore, a test was considered positive only when a line appears in both the test and the control zone. However, a negative result was considered when a line appears in the control line only. Finally, a result was considered invalid in two conditions; when only one line appears in the test line or in the absence of line in both sections.

3.5 Detection and characterization of rabies virus nucleic acid using molecular technique

Total RNA was extracted using phenol and guanidinium isothiocyanate technique (Toni *et al.*, 2018). The extracted RNA was thereafter quantified to confirm its suitability for downstream applications. RT-PCR was used to amplify 202 base pairs of the N gene. Firstly, primer was design to target 202 base pairs within the nucleoprotein gene (N) which is the most conserved region of the genome. Secondly, phenol-chloroform technology was used to extract total RNA

from brain tissues before its conversion into cDNA. Thereafter, cDNA was amplified using designed primers and amplicons revealed under High Performance UV Trans illuminator. Finally, sequence determination of PCR amplicons were done through capillary sequencing principle.

3.5.1 Primer design

Several sequences covering the nucleoprotein gene of rabies viruses circulating in Mali were published in a molecular characterization of canine rabies in Mali between 2006 and 2013 (Traoré *et al.*, 2016). From these sequences, 18 were retrieved from Genbank using Bio Edit. The sequence KP976113.1was then used to design a pair of primer on NCBI/Primer-BLAST tool amplifying a region of the nucleoprotein gene covering 202 base pairs. Table 2 shows the pair of primers used.

Table 3.1: Designed primers for RT-PCR.

Primer	Nucleotide Sequences (5'-3')	Nucleotide position*	Sense	Rabies gene
S3DB2	AATGCAACTCTTTGAAGGGA	231 - 250	+	N
S3DB3	GAGCAGACCGACTAAAGATG	432 - 413	-	

*The positions are based on the Rabies virus isolate 420/2006 nucleoprotein gene, partial cds (GenBank No. KP976113.1)

3.5.2 Extraction of RNA

Total RNA from brain tissue was extracted using the TRIzol (Zymo Research Cooperation, Irvine, USA) method, following manufacturer's instructions. In brief, brain samples (100 mg) archived at -20°C was allowed to defrost and homogenized in 1 ml of TRIzol.

After incubation at room temperature during 5 minutes, 200 µl of chloroform (Sigma, USA) was added and mixed during 15 seconds by hand. Thereafter, a phase separation was performed by centrifugation during 15 minutes at 12,000×g (Minispin, Eppendorf) followed by the collection of the aqueous phase which was transferred into a new tube. 500 µl of

isopropanol was added to allow the ARN precipitation and a second round of incubation during 10 minutes at room temperature and centrifugation at 12,000 × g for 10 minutes was performed.

The supernatant was then discarded and the RNA pellet washed with 1 ml 75% ethanol by a brief vortex followed by a last round of centrifugation at 7,500 × g during 5 minutes. Finally, the supernatant was discarded and the RNA pellets thereafter dried on air for 10 to 20 minutes before to be dissolved in 25 µl of RNase-free H_2O. To determine if the RNA extracted was suitable cDNA synthesis, quantification was performed using BioPhotometer (Eppendorf) to estimate its quality and quantity.

To determine if the RNA extracted is suitable for downstream PCR application, quantification was performed using BioPhotometer (Eppendorf) to estimate its quality and quantity.

3.5.3 Amplification by RT-PCR

cDNA synthesis was performed in a PTC-100 Peltier Thermal Cycler (MJ Research, USA) using Protoscipt II first cDNA synthesis kit (BioLabsinc., New Englands) according to manufacturer's instructions. Briefly, a mixture containing 1 µl of extracted RNA with a concentration of 200ng/µl, 1 µl Forward Primer S3DB2, 1 µl Reverse Primer S3DB3, 10 µl ProtoScript II Reaction Mix (2X), 2 µl of ProtoScript II Enzyme Mix (10X) and 5 µl Nuclease-free H_2O for a final reaction volume of 20 µl.

The reaction mixture was incubated at 25°C and 42 °C during 5 minutes and 60 minutes respectively. The enzyme reverse transcriptase was then inactivated by rising the temperature at 80°C for 5 minutes. cDNA quantification was performed to determine whether the product is suitable for downstream PCR application using BioPhotometer (Eppendorf).

PCR was performed to target 202 base pairs within the nucleoprotein gene of the RABV using the pair of primers (S3DB2 and S3DB3). An amplification was performed with the reaction mixture composed of 15.375µl UltraPure Distilled Water (Invitrogen, USA), 2.5µl of PCR Buffer-10X (1X), 1.25µl of Forward Primer S3DB2-10 µM (0.5µM),1.25µl of Reverse Primer S3DB3-10 µM (0.5µM), 0.5µl of dNTP mix-10 mM (0.2 mM), 1.5 µl of MgCl2-25 mM (1.5

mM), 0.125 µl of One TaqDNA Polymerase-5U/µl (0.025 units/µl), and 2.5 µl of cDNA template.

The amplification was done in a PTC-200TM Peltier Thermal Cycler (MJ Research, USA). The following cycling conditions were adopted: initial heating at 94 °C during 3 minutes, elongation 35 cycles of 94 °C during 45 seconds, 58 °C during 60 seconds, and 72 °C during 90 seconds. A final elongation of 72 °C during 10 minutes was added.

After completion of the PCR reactions, 5 µl of the each amplicon was run on 1.5% agarose gel (0.75 g agarose in 50 ml TBE 0.5X) stained with ethidium bromide (20 µl) at 120 volt during 60 minutes. Fragments of 202base pairs were observed under High Performance UV Transilluminator.

3.5.4 Sequencing of PCR amplicons

After RT-PCR diagnosis of the eighteen (18) samples, sixteen (16) unpurified PCR products, positive with FAT, were sequenced by Inqaba Biotec. The company performed templates purification, quantification and sequencing using the ABI 3500XL Genetic analyzer (Applied Bio Inc.), POP7TM (ThermoScientific), BrillantDyeTM Terminator v3.1, for sequence determination. Briefly, capillary sequencing principle was adopted whereby fluorescently labelled dideoxynucleotides (ddNTPs) was added to the growing strand. Thereafter, the sequencing machine could read these fluorescent colours to call the bases.

3.5.5 Basic Local Alignment Search Tool on sequencing results

Forward and Reverse sequences were presented on Chromas for base calling, matched up, and aligned on Clustal W to identify the consensus region using BioEdit version 7.2.5 (A.T. Hall, 1999; A.T. Hall, 2011; Page, 1996). In order to confirm that the sequences obtained belongs to the rabies virus nucleoprotein gene, the consensus sequences were confronted with the GenBank database using Nucleotide Basic Local Alignment Search Tool (BLAST) on National Center for Biotechnology Information (NCBI).

3.5.6 Phylogenetic analysis of rabies virus isolates

Multiple sequence alignment (MSA) were performed using the Clutal X software, v2.1 (Thompson *et al.*, 1997), and sequences were trimed at 72 base pairs using Jalview version 2.11.0. Geneious Prime software 2019.2.1 (https://www.geneious.com) was used to generate the nucleotide identity matrix between the eleven (11) sequences from this study, as well as these sequences with their homologues and representatives of lyssaviruses circulating in Africa.

The Geneious software was also used to display the multiple sequence alignment performed with Clustal X software. Thereafter, molecular phylogenetic analysis was performed using Maximum-Likelihood (ML) and Neigbor-Joining (NJ) methods based on eleven (11) confirmed rabies virus sequences from this study and thirty (30) RABV representative sequences (Appendix 2) on MEGA version 7.0.18 (Kumar *et al.*, 2016).

These thirty (30) representative sequences previously published RABV nucleoprotein sequences are from Mali, Algeria, Niger, Burkina Faso, Senegal, Mauritania, Gambia, Chad, Cameroon, Nigeria, Israel, Egypt, Mozambic, Namibia, Tanzania, Marocco, and Kenyawere retrieved from GenBank (June, 2019). They represent Rabies lyssavirus species (phylogroup I), sub-lineage G, H, and F of the Africa 2 lineage, Africa 1 and 4 lineages as well as Shimoni bat lyssavirus species from Kenya (Accession No. NC_025365.1) which was used as out-group (Kuzmin *et al.*, 2010; Traoré *et al.*, 2016). Appendix shows GenBank submissions that includes lineage, sub-lineage and country of rabies virus isolate used in the first data set.

Finally, a TSC (Templeton, Crandall and Singh) haplotype network was drawn on PopART (Population Analysis with Reticulate Trees) version 1.7 from the MSA of the 11 sequences converted into Nexus sequential format using the hcv website https://hcv.lanl.gov/content/sequence/FORMAT_CONVERSION/form.html (Clement *et al.*, 2000; Leigh and Bryant, 2015)

CHAPTER FOUR

4.0 RESULTS

4.1 Rabies virus nucleoprotein (N) antigen detected by RIDT in dog brain samples

RIDT was performed on eighteen (18) samples already confirmed with FAT. The FAT showed sixteen (16) positive and two (2) negative. On RIDT, only seven (7) out of the eighteen (18) samples showed a positive result characterized by the presence of a line at both test and control zone as shown in Panel B of Figure 4.1. The eleven (11) remaining had a single line at the control zone characteristic of negative result as shown in Panel A of Figure 4.1. This shows that nine (09) samples previously positive with the FAT are now showing a negative result with the RIDT.

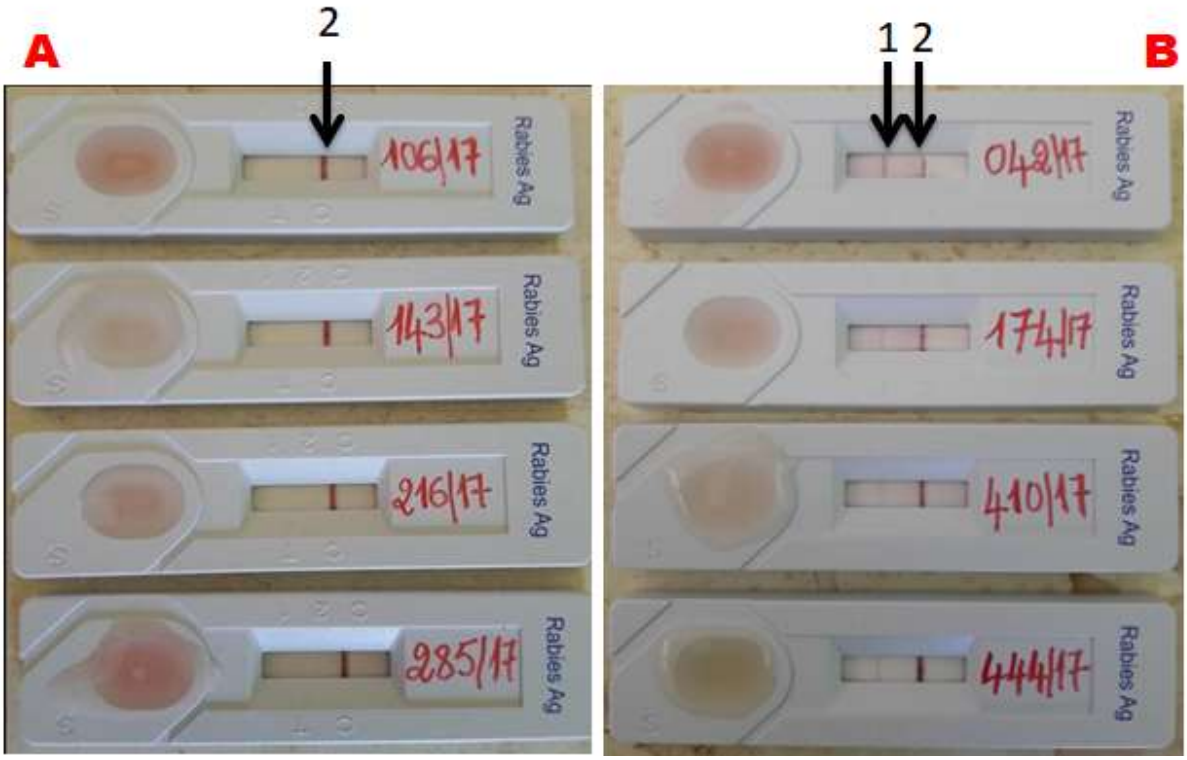

Figure 4.1: Rapid immunochromatographic diagnostic test results.

Panel A shows negative results with the appearance of a single line at the control zone (2). Panel B presenting positive results shows a line at both test (1) and control zone (2).

4.2 Quantity of rabies virus RNA extracted measured

Considering the fact that RNA is more sensible to degradation than DNA due to its structure and heat sensibility, RNA quantification was performed to make sure that samples still contain RNA suitable for cDNA synthesis.

For this reason, RNA quantification was performed following its extraction and Appendix 3 shows that the highest concentrations were recorded for isolates 029/16 and 042/17 with 5095.3 ng/µl and 4985.1 ng/µl respectively and the lowest for isolates 908/16 and 106/17 with 00 ng/µl and 0.69 ng/µl respectively therefore suitable for cDNA synthesis.

4.3 Rabies virus cDNA synthetized

Rabies virus cDNA was synthesis using gene specific primers S3DB and S3DB3 designed to amplified 202 base pairs from the nucleoprotein gene of the rabies virus from RNA extracted before subsequent amplification. The detection of cDNA at this level would indicate presence of the rabies virus is samples.

Nevertheless, further investigation name gel electrophoresis will be needed to confirm rabies virus's presence. The highest concentrations were recorded with isolates 410/17 and 216/17 with 3436 ng/µl and 3001 ng/µl respectively and the lowest for isolate 908/16 and 106/17 with 00 ng/µl as shown in Appendix 4. This indicates that the cDNA synthetized from samples were suitable for amplification.

4.4 Rabies virus nucleic acid detected by RT-PCR in dog brain samples

Analysed by RT-PCR, fifteen (15) out of eighteen (18) samples had positive result showed by the presence of 202 base pairs band on polyacrylamide gel as shown in Panels A and B of figure 4.2. This showed that one (01) sample previously positive with FAT was negative using RT-PCR.

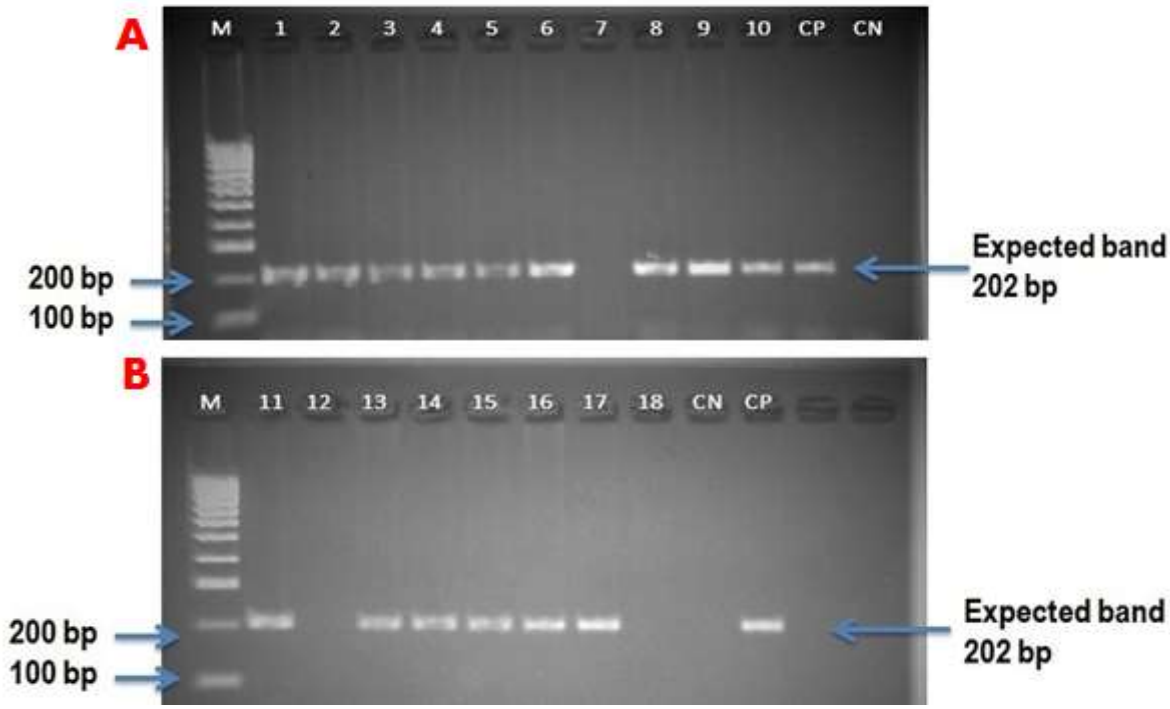

Figure 4.2: Reverse-Transcription Polymerase Chain Reaction results for detecting rabies virus in dog brain samples.

Panel A (01-10). C1 Bioline100 base pairs ladder, C2-C7 positive sample, C8 negative sample, C9-C11 positive sample, C12 positive control, C13 negative control.

Panel B (11-18). C1 Bioline100 base pairs ladder, C2 positive sample, C3 negative sample, C4-C8 positive sample, C9 negative sample, C10 negative control, C13 positive control.

4.5 Summary of diagnosis results

The detection of RABV nucleoprotein (N) antigen and nucleic acid using RIDT and RT-PCR was compared to gold standard FAT. Table 4.1 shows that RIDT has low detection rate while RT-PCR shows promising results.

Table 4.1: Comparison of RIDT and RT-PCR relative to the FAT to detect rabies virus in brain samples.

		RIDT		RT-PCR		
		+	-	+	-	Total
FAT	+	07	09	15	1	16
	-	00	02	00	02	02
	Total	07	11	15	03	18

RIDT: rapid immunochromatographic diagnostic test, FAT: fluorescent antibody test, RT-PCR: reverse-transcriptase polymerase chain reaction, +: positive, -: negative.

4.6 Genetic diversity of the rabies virus isolates

The four (4) major criteria used by the International Committee on Taxonomy of Viruses (ICTV) for the demarcation of lyssavirus includes 80-82% threshold of nucleotide identity using the complete nucleoprotein gene as well as consistency with phylogenetic trees drawn using many evolutionary models.

For this reason, Basic Local Alignment Search Tool (BLAST) was performed to confirm the obtained sequences matched with Rabies virus nucleoprotein (N) gene as well as investigate similar mutations patterns found with isolates previously published. Thereafter, lineage investigation of sequences was done using nucleotide identity matrix and phylogenetic trees based on various evolutionary models. Finally, isolates were classified into haplotypes and their geographical distribution across Africa studied using homologues genes.

Out of the sixteen (16) positive samples with FAT, eleven (11) were confirmed by sequencing and the five (05) remaining were unexploitable because of no significant similarity. Appendix shows the raw data of chromatograms of these isolates. Appendix 5-20 represents raw sequencing data for the 16 samples.

4.6.1 BLAST results confirmed rabies virus sequences

BLAST is a tool of the National Center for Biotechnology Information (NCBI) used to find sections of similarity between sequences. These sequences can be DNA, RNA or protein. The consensus sequence of the 11 confirmed sequences were compared with the GenBank database using Nucleotide BLAST on NCBI matching Rabies virus nucleoprotein (N) gene, partial or complete cds.

The best score were recorded for isolates ML921/16, ML413/16 and ML021/16 with a nucleotide identity of 100.00% and the lowest score was recorded with isolate ML023/16 with 93.48%. Figure 4.3 shows the result for isolate ML921/16.

Panel A shows nucleotide identity of 100% and 99.17% with isolates KP976118.1 and KP976119.1 respectively from Mali. Panel B presents pairwise alignment with isolates KP976118.1 (complete match) and KP976119.1 (single difference at position 42) from Mali.

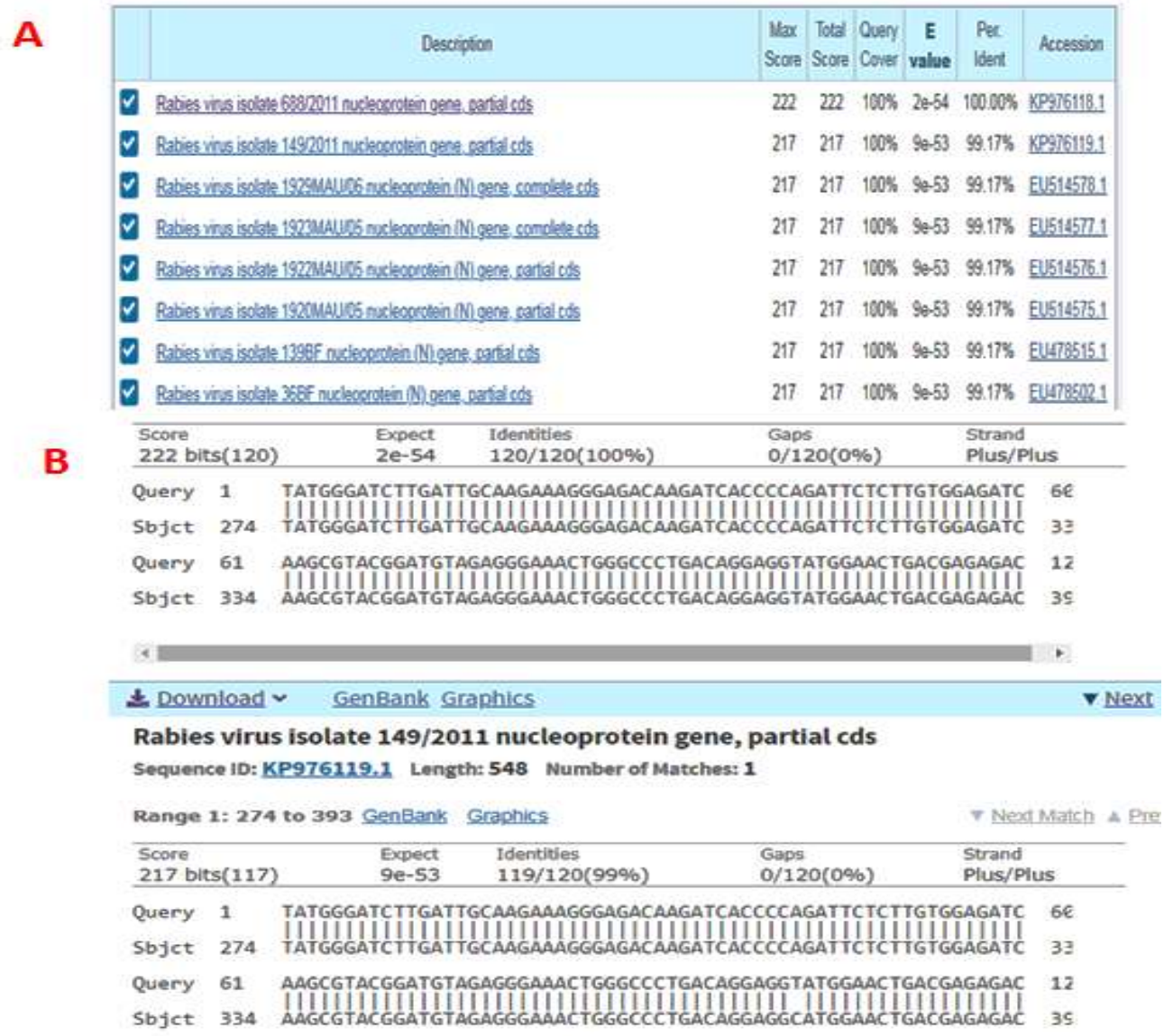

Figure 4.3: BLAST result of sequence ML921/16.

Panel A shows nucleotide identity of 100% and 99.17% with isolates KP976118.1 and KP976119.1 respectively from Mali. Panel B presents pairwise alignment with isolates KP976118.1 (complete match) and KP976119.1 (single difference at position 42) from Mali.

4.6.2 Similar mutations patterns found with isolates previously published from Mali

In order to classify sequences, homologues genes were used to perform MSA and identify conserved and non-conserved regions. Geneious software was used to display the multiple sequence alignment performed with Clustal X software based on the eleven (11) sequences from this study, 21 homologues genes, and NCBI reference sequence for Rabies lyssavirus complete genome (NC_001542.1).

Eight (8) segregating sites were found between sequences from this study. However, only three (3) segregating sites were useful for sequences demarcation. Based on this MSA, sequences from this study were categorised into three (3) groups.

The first group, composed of sequences ML216/17, ML028/16, ML410/17, and ML023/16 is characterized by the presence of at least one (1) of the following mutations; T to G at position 406 and T to C at position 433. The representative of group 1 is the sequence KP976121 from Mali belonging to sub-lineage F of the Africa 2 lineage.

The second group is formed by sequences ML021/16 and ML413/16 with the following mutation, T to C at position 404 and A to G at position 365. Group 2 is represented by sequences KP976126.1, KP976127.1, KP976128.1 and KP976130.1 from Mali of the sub-lineage G of the Africa 2 lineage. Finally, group 3 formed by sequences ML026/16, ML444/17, ML042/17, ML921/16, and ML285/17 is characterized by a several and was represented by sequences KP976118.1 and KP976119.1 from Mali classified as members of the sub-lineage H of the Africa 2 lineage.

Figure 4.4 shows the MSA result. Panel A and B show the MSA from position 360 to 420 and 420 to 480 respectively. NCBI reference sequence for Rabies lyssavirus complete genome (NC_001542.1) with a length of 11,932 base pairs was used to perform a MSA with each of the sequences involved in this study and the result is presented in Appendix 21-31.

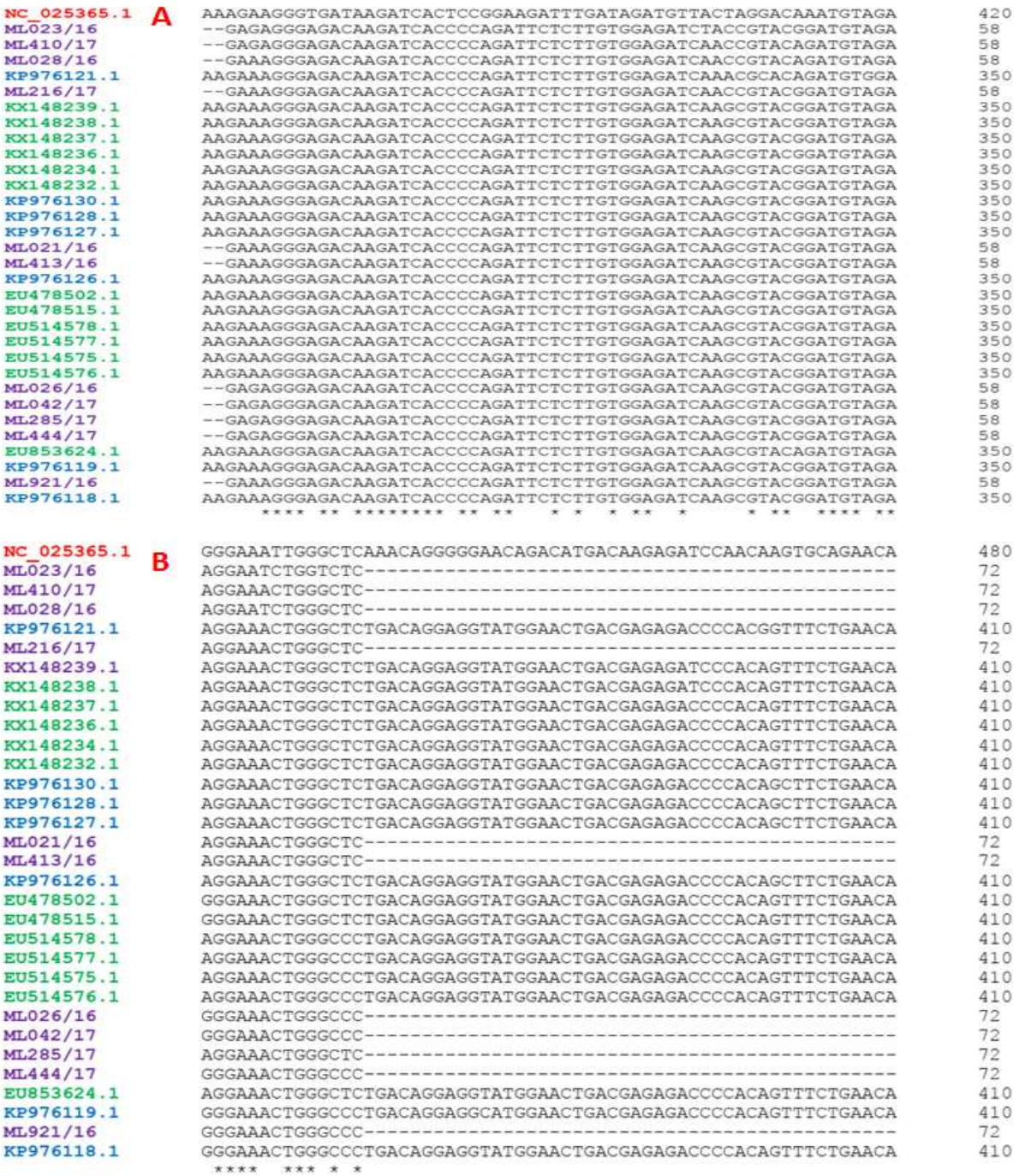

Figure 4.4: Multiple sequence alignment of the eleven sequences with homologues genes. The positions of mutations were based on the NCBI reference rabies lyssavirus isolate in red (GenBank No. NC_001542.1). Panel A and B show the MSA from position 360 to 420 and 420 to 480 respectively. Sequences from this study are in violet, previously published sequences from Mali in bleu and isolates from bordering countries in green.

4.6.3 High nucleotide identity found between isolates

Despite confirmation of rabies virus sequences, more analysis is needed to classified sequences in a phylogroup. The threshold of 80-82% set by the ICTV was used for the demarcation of sequences.

For this reason, nucleotide identity using partial cds of sequences from this study was performed. Geneious Prime was used to generate the nucleotide identity matrix between the eleven (11) sequences from this study.

These sequences show nucleotide identity ranging from 91.67% to 100% as shown in Table 4.2. This is an indication that the sequences belong to the same species (Rabies lyssavirus) and to the same phylogroup.

Table 4.2: Nucleotide identity matrix of the 11 isolates.

	ML2 16/17	ML4 13/16	ML0 21/16	ML0 26/16	ML0 42/17	ML9 21/16	ML4 44/17	ML2 85/17	ML0 23/16	ML0 28/16	ML4 10/17
ML2 16/17		98.61	98.61	94.44	94.44	95.83	94.44	97.22	94.44	97.22	97.22
ML4 13/16	98.61		100	95.83	95.83	97.22	95.83	98.61	93.06	95.83	95.83
ML0 21/16	98.61	100		95.83	95.83	97.22	95.83	98.61	93.06	95.83	95.83
ML0 26/16	94.44	95.83	95.83		100	98.61	100	97.22	91.67	91.67	94.44
ML0 42/17	94.44	95.83	95.83	100		98.61	100	97.22	91.67	91.67	94.44
ML9 21/16	95.83	97.22	97.22	98.61	98.61		98.61	95.83	90.28	93.06	93.06
ML4 44/17	94.44	95.83	95.83	100	100	98.61		97.22	91.67	91.67	94.44
ML2 85/17	97.22	98.61	98.61	97.22	97.22	95.83	97.22		94.44	94.44	97.22
ML0 23/16	94.44	93.06	93.06	91.67	91.67	90.28	91.67	94.44		94.44	94.44
ML0 28/16	97.22	95.83	95.83	91.67	91.67	93.06	91.67	94.44	94.44		97.22
ML4 10/17	97.22	95.83	95.83	94.44	94.44	93.06	94.44	97.22	94.44	97.22	

4.6.4 H, F, and G of the Africa 2 lineage found

The use of isolates previously published with known lineage is a useful tool to investigate the lineage of unknown sequences. Therefore, phylogenetic trees drawn using various evolutionary models were performed.

Maximum-Likelihood method was used to build a phylogenetic tree with the eleven (11) confirmed rabies virus sequences from this study added to 30 published sequences previously published sequences. This phylogenetic relationship were drawn with the highest log likelyhood using the Tamura 3-parameter mode (Tamura, 1992) and next to each branch is marked the percentage at which the represented sequences are found together out of 1000 replications.

The branch lengths were estimated using the number of substitutions per site between sequences. These branches were generated by using BioNJ algorithms applied to pairwise distances obtained using Maximum Composite Likelihood (MCL) method. The ML phlogenetic's tree presented in Figure 4.5 shows that the eleven (11) sequences from this study belong to Africa 2 lineage of which five (ML285/17, ML026/16, ML444/17, ML042/17, ML921/16) to sub-lineage H, four (ML216/17, ML410/17, ML023/16, ML028/16) to sub-lineage F and two (ML021/16, ML413/16) to sub-lineage G.

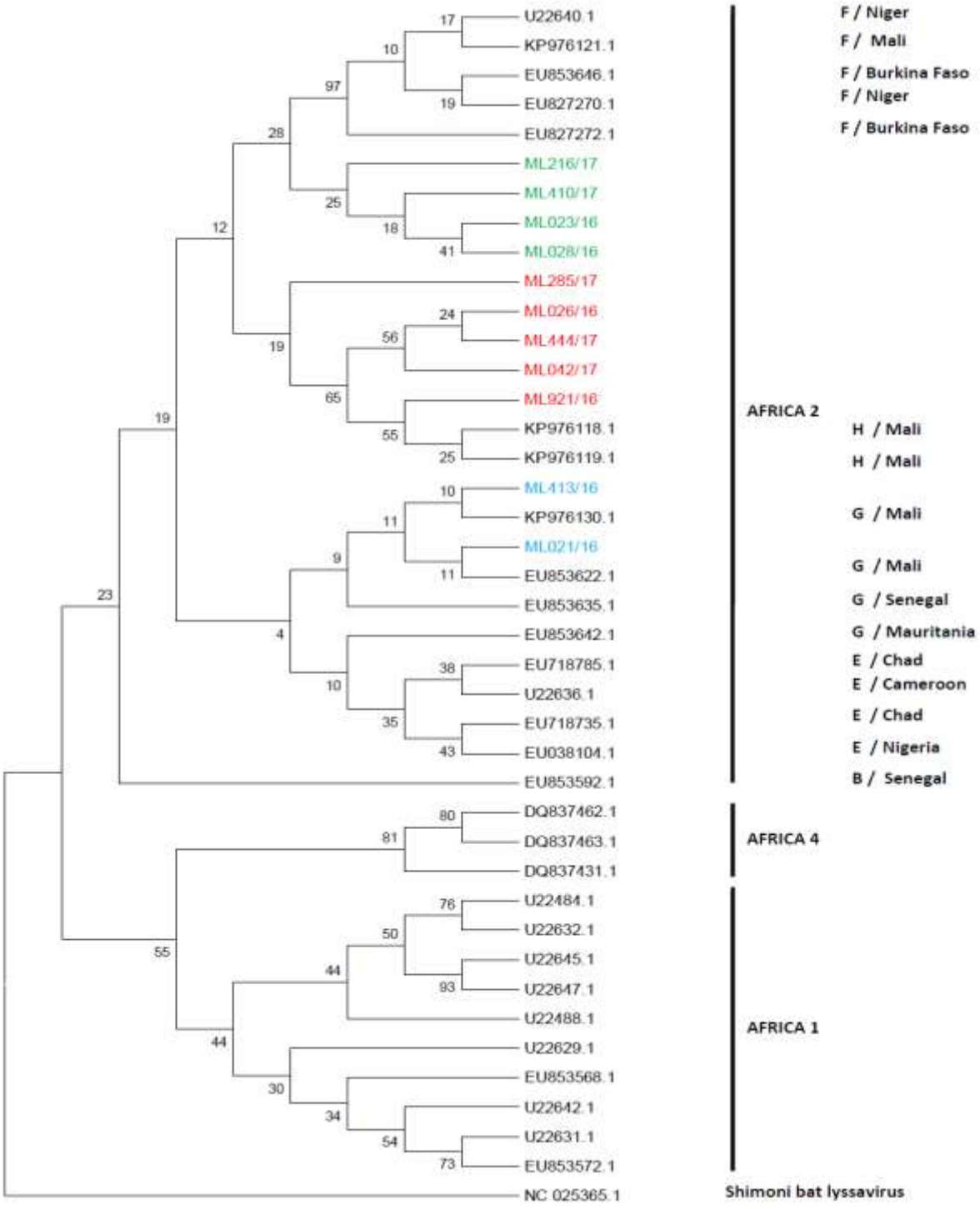

Figure 4.5: Molecular Phylogenetic analysis using Maximum-likelihood method.

This is based on eleven (11) confirmed rabies virus sequences from this study and 30 published sequences. Sequences from this study are in green (Group F), red (Group H), and bleu (Group G).

Neighbor-Joining method (Saitou and Nei, 1987) was also used to build a phylogenetic tree with the same data set. This phylogenetic tree was drawn with a bootstrap consensus calculated using 1000 replicates to estimate the evolutionary relationship of the analyzed taxa.

Next to each branch is shown the percentage in which the sequences of the represented taxa are found together out of 1000 replications (Felsenstein, 1985) with the evolutionary distance calculated using the number of difference method and expressed in number of difference between sequences unit (R. H. Thomas, 2001).

The NJ method as shown in Figure 4.6 confirmed the same distribution obtained with the ML method.

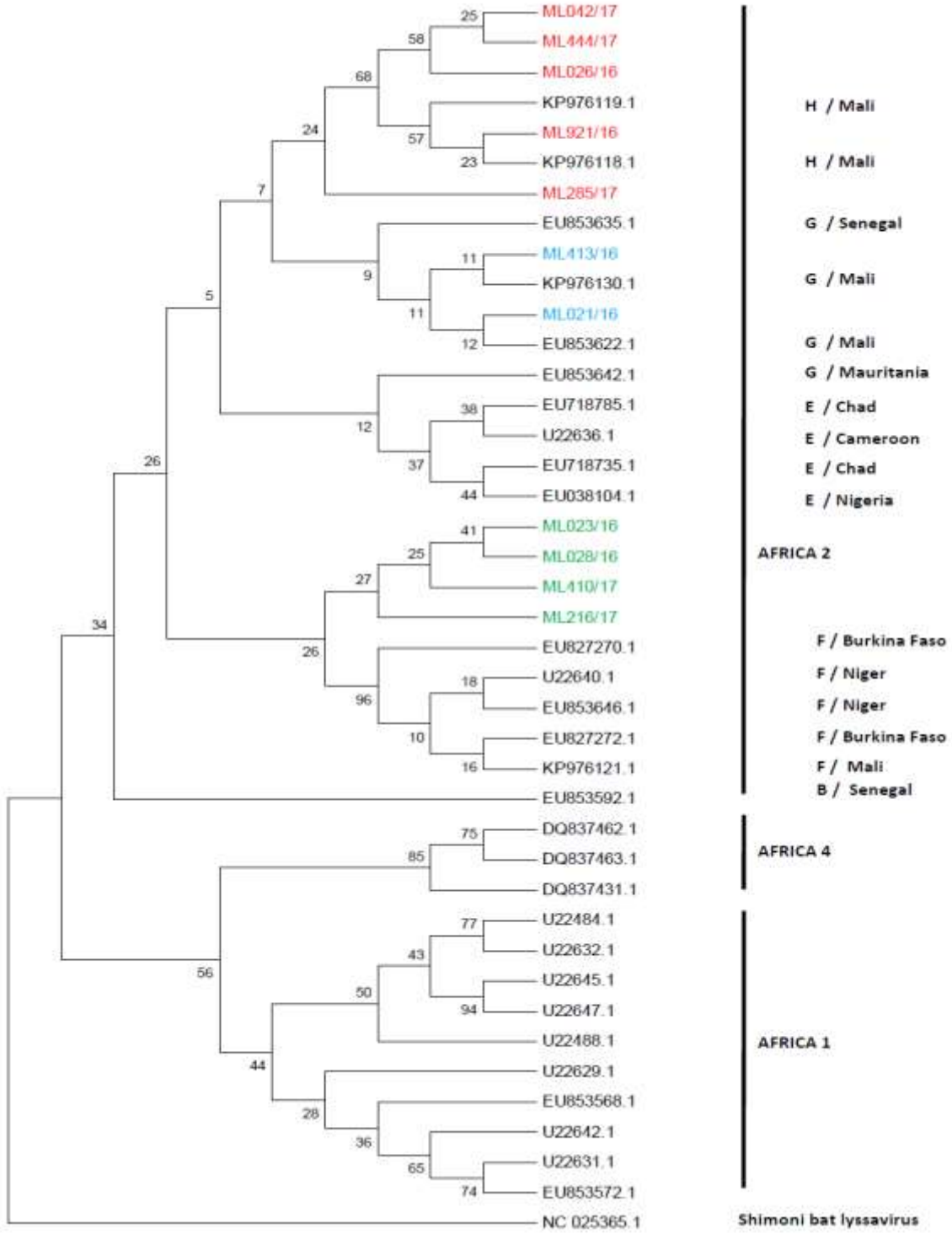

Figure 4.6: Molecular Phylogenetic analysis using Neigbor-Joining method.

This is based on eleven (11) confirmed rabies virus sequences from this study and 30 published sequences. Sequences from this study are in green (Group F), red (Group H), and bleu (Group G).

4.6.5 Isolates divided into three haplotypes

To confirm the genetic diversity of the rabies virus isolates, haplotype network was drawn using PopART. Analysis of partial cds of the nucleoprotein gene (72 base pairs) of the 11 sequences identified three (3) haplotypes with sequences ML021/16 and ML413/16 as well as ML026/16, ML042/17, and ML444/17 identical.

The number of segregating sites was 8 and parsimony-informative sites 6. The first haplotype formed by sequence ML021/16 and ML413/16. The second haplotype composed of sequences ML026/16, ML042/17, ML444/17, ML921/16, and ML285/17. Finally, haplotype 3 containing sequences ML216/17, ML410/17, ML028/16 and ML023/16. Figure 19 presents the Maximum-Parsimony Haplotype network.

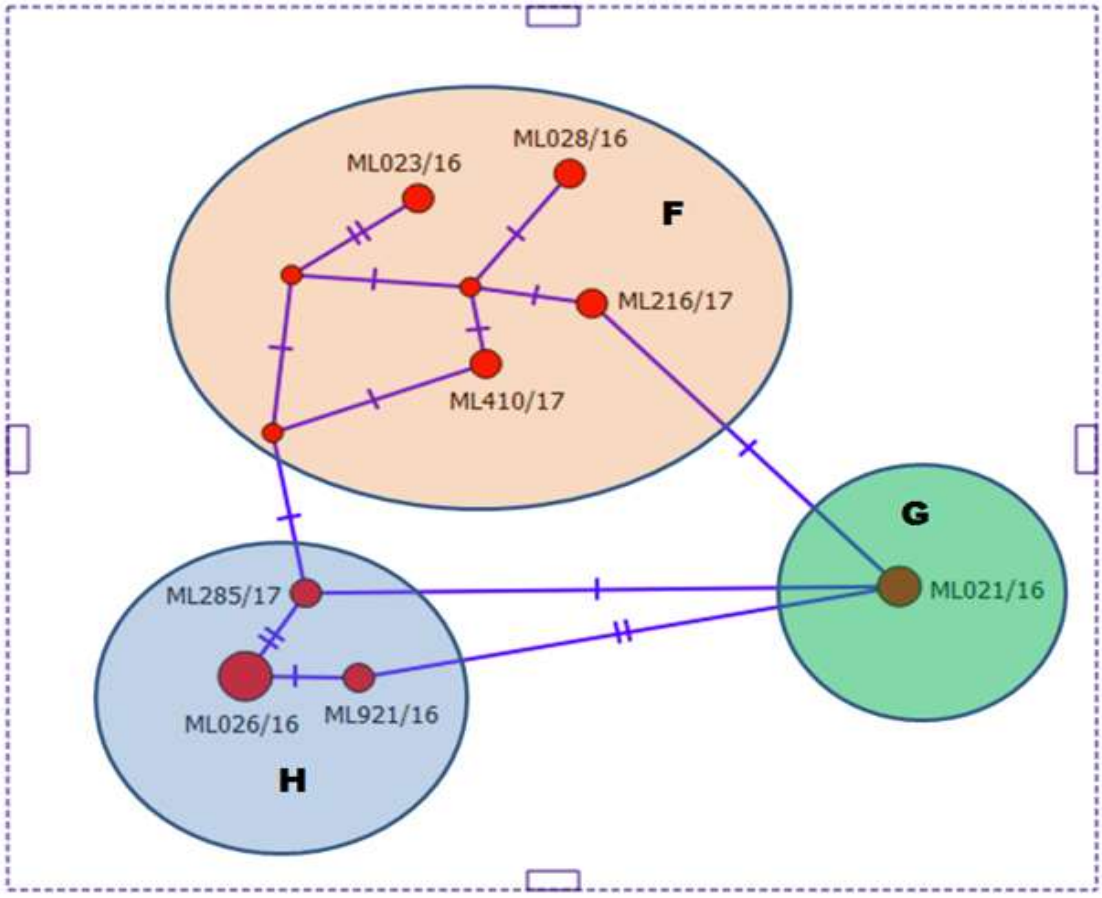

Figure 4.7: Haplotype network based on Maximum-parsimony using the 11 rabies The dot's size is proportional to the number of isolates. One line indicates one (1) mutation.

4.6.6 Geographical distribution of the homologue genes in Africa

The geographical distribution of lyssavirus isolates from this study will allow the determination of their geographical origins and inform on eventual inter-country spread of rabies in West Africa in general and between Mali and its neighbours in particular.

The geographical distribution of the homologes genes (10 highest scores) shows a relatively restraint distribution with isolates from Mali, as well as four (4) bordering countries, Ivory Coast, Mauritania, Burkina Faso, and Senegal. Appendix 32 presents GenBank submissions that includes name of the homologue, accession number, isolate and the country of origin of rabies virus isolate.

4.6.7 Summary of phylogenetic results

Based on BLAST results, similar mutations patterns found with isolates previously published from Mali, high nucleotide identity found between isolates (91.67% to 100%), consistency of phylogenetic trees drawn using different evolutionary models (Maximum-Likelihood and Neighbor-Joining), and the geographical distribution of the homologue genes in Africa, all the eleven (11) sequences from this study belong to Rabieslyssavirusspecies (phylogroup I), Africa 2 lineage of which five (ML285/17, ML026/16, ML444/17, ML042/17, ML921/16) to sub-lineage H, four (ML216/17, ML410/17, ML023/16, ML028/16) to sub-lineage F and two (ML021/16, ML413/16) to sub-lineage G.

CHAPTER FIVE

5.0 DISCUSSION

Despite all the available methods for investigating suspected samples, rabies diagnosis is still a challenge in some African countries such as Mali due to lack of funding. Indeed, the CVL located in Bamako is the only laboratory equipped to investigate rabies suspected samples using the FAT.

Samples submitted from others regions to the CVL almost invariably reached the capital city in an advanced state of decomposition and therefore unsuitable for FAT. For this reason, RIDT and RT-PCR are believed to be promising tools for solving the diagnosis burden in Mali. Furthermore, the routine characterization of PCR amplicons will inform on the animal hosts, geographical origins and sources of infections.

The CVL, diagnose rabies in Mali using the FAT, the gold standard test recognised by WHO and OIE (OIE, 2008; WHO, 2013). Its sensitivity and high specificity (95-99%) may vary in some cases due to factors such as the decomposition status of a brain, conservation of reagents such as the FITC or the technician appreciation of slides under fluorescence microscopy. Furthermore, the FAT is time consuming, and required technical skill required and expensive equipment.

Therefore, there is need for a simpler, quicker and cheaper diagnostic test, such as the RIDT. The goal of the present study was to evaluate the RIDT in order to evaluate its suitability to equip veterinary regional services located in other cities with limited resources, in order to enhance notification rabies cases to World Animal Health Information System (WAHIS) or District Health Information Software 2 (DHIS2).

In this study, the RIDT showed low performance compared with the FAT. Elsewhere, (Markotter *et al.*, 2009) found 100% specificity of the RIDT on 21 samples, representing all known African lyssavirus genotypes in comparison with the FAT. Furthermore, Kang *et al.* (2007) found a sensitivity of 91.7% and a specificity of 100% when they compared the RIDT with the FAT.

(Nishizono *et al.*, 2008)developed Type 1 and 2 lateral flow devices for rabies detection. Type 1 (monoclonal antibody), had a sensitivity of 95.5% and specificity of 88.9% while Type 2 (combination of two monoclonal antibodies), showed 93.2% and 100% of sensitivity and specificity respectively (Nishizono *et al.*, 2008).

Kasempimolporn found a sensitivity of 93.0% and specificity of 94.4% (Kasempimolporn, 2011). Servat *et al.* (2012) found 88.3% and 100% of sensitivity and specificity respectively when the RIDT was compared to the FAT. Mshelbwala and Abdullahi (2012) showed total agreement between the RIDT and FAT. Similarly, in 2016 in Chad, a sensitivity of 95.3% and specificity of 93.3% were found (Lechenne *et al.*, 2016). Finally, in 2018 in Argentina (Gury Dohmen *et al.*, 2018) found 97.96% and 100% sensitivity and specificity respectively.

In the present study, RT-PCR showed comparable results to those of the FAT. The only sample (389/17), negative with RT-PCR while showing a positive result with FAT, might be due to impurity. Elsewhere, Kulonen and colleagues using 12 samples, found an importance decrease in sensitivity from 100% (12/12) when targeting 139 base pairs to 67% (8/12) while targeting 304 base pairs (Kulonen *et al.*, 1999). In the present study, 202 base pairs were targeted and a smaller sequence targeted could lead to an increase of sensitivity.

Beltrán and colleagues were able to detect rabies virus from 14 samples conserved up to 8 years and from aliquots stored at 20°C during 120 days (Beltrán *et al.*, 2014). Similarly, Araújo *et al.* (2008) showed that the hnRT-PCR (75%) was more sensitive than RT-PCR (34.3%), and both approaches had lower sensibility with decomposed samples compared to the FAT.

In addition, Heaton and colleagues found a sensitivity of 93% and 100% for external PCR and heminested PCR respectively in a study involving samples incubated at 37°C for 360 hours (Heaton *et al.*, 1997). Cardoso Lopes *et al.* (2009) found a decline of sensitivity, 88.9% to 65.3% when samples are stored in the absence of storage medium at -20°C. Brain samples involved in this study were conserved in 20% (w/v) homogenate in PBS and archived at -20°C which can cause a decrease of sensitivity.

Finally, Aravindh and colleagues in a study, showed a sensitivity of 100% when comparing RT-PCR with the FAT (Aravindh *et al.*, 2012). These better results could be explained by the denaturation of RNAafter extraction at 100^{o} C for 5 minutes prior cDNA synthesis. Furthermore, viral RNA was extracted using QIAmp Viral RNA Kit (Germany, Hilden, Germany) instead of TRIzol method.

The present study corroborate with previous studies that shows Heminested RT-PCR technique more sensitive than the standard RT-PCR technique for detecting rabies virus. Additionally, it confirms the effect of the storage medium and a short sequence as target in the sensitivity of RT-PCR technique for detecting rabies virus.

Mali is landlocked and shares 4,500 miles border with seven (7) countries, which are Senegal (260 miles), Ivory Coast (330 miles), Burkina Faso (621 miles), Mauritania (1,390 miles), Guinea (533 miles), Algeria (855 miles), and Niger (510 miles). Sub-lineage B and E of the Africa 2 lineage are known to circulate in Guinea, and Senegal. Similarly, the Africa 1 lineage of the rabies virus has been found in Algeria. So far, only sub-lineage H, F and G of the Africa 2 lineage have been confirmed circulating in Mali by previous studies (Talbi *et al.*, 2009; Traoré *et al.*, 2016).

The aim of the present study was to investigate the genetic diversity of rabies viruses circulating in Mali and find out if there is any inter-country spread of the zoonosis. The first attempts to investigate genetic diversity for rabies viruses in Sub-Sahara Africa found three (3) phylogenetic lineages (Africa 1, 2 and 3) (Kissi *et al.*, 1995) .

Both domestic and wild canid species are reservoirs of the Africa 1 and 2 lineages. Indeed, while domestic dogs have been suggested to be the only reservoir for the transmission of rabies virus in some African countries (Lembo *et al.*, 2008), wildcanids have been found to cause rabies spill-over in South Africa, Zimbabwe, and Kenya (Bitek *et al.*, 2019; L. Nel *et al.*, 1997; Pfukenyi *et al.*, 2009). Viverrid such as Cynictispenicillata which is the most common mongoose in South Africa is believed to be the reservoir of Africa 3 lineage (P. L. Davis *et al.*, 2007).

More Recently, a novel clade Africa 4 lineage was isolated in Egypt (David *et al.*, 2007). These phylogenetic patterns have been supported by studies that investigated the distribution of rabies viruses in specific countries, across Africa and on the wildlife as well as the origin of rabies virus (Bourhy *et al.*, 2008; Hayman *et al.*, 2011; Mansfield *et al.*, 2006; L. H. Nel *et al.*, 2005; Sadeuh-Mba *et al.*, 2017; Troupin *et al.*, 2016).

This study revealed the presence of sub-lineage H, F and G of the Africa 2 lineage in Mali, satisfying the model presented for dog RABV which proposes several spatially different clusters. These clusters have limited contact among themselves (Bourhy *et al.*, 2008).

However, the result shows a spread of sub-lineage F of the Africa 2 lineage from the North to the Central part of the country. Indeed, only one (1) sample from this sub-lineage was found in Gao near the border with Niger (Traoré *et al.*, 2016). This can be explained by the important displacement of people from the North to the capital city, Bamako due to insecurity following the 2012 conflict between the national army and the Touareg rebellion.

Based on these results, there is no inter-country spread sub-lineage B from Guinea, sub-lineage E from Senegal and Africa 1 lineage from Algeria despite Mali sharing more than 4,500 miles border with its neighboring countries. Nevertheless, the surveillance at the borders should be maintained to avoid any spread of the zoonose from neighboring countries. Furthermore, Hampton and colleagues as well as Benedictis and colleagues have argued a cycles synchronized of rabies over very large special scales in Africa and inter-countries spread of the zoonose respectively (De Benedictis *et al.*, 2010).

This study, first of its type, has demonstrated the possibility of Malian's scientists to participate actively to regional studies by providing results instead of sending rabies suspected samples to laboratories located in France for molecular analysis. It has also provided the basis for strong collaboration between Malian's laboratories namely the CVL and the LMBA.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Based on the findings of this study, the following conclusions were made;

1. The RIDT detected RABV circulating in Mali but at a lower positivity rate as compared to the RT-PCR and FAT.
2. RT-PCR identified RABV nucleoprotein gene with a positivity rate comparable to that of FAT.
3. Five sub-lineages H, four sub-lineages F and two sub-lineages G belonging to the phylogroup I of the Africa 2 lineage were found circulating in Mali.

6.2 RECOMMENDATIONS

The following recommendations were made;

1. Further testing is needed for the validation of the RIDT before it could be used as a tool for further equipment of veterinary regional services located in other cities with limited resources in Mali.
2. RT-PCR can be used as a complementary test on decomposed samples for detecting rabies viruses in Mali.
3. More study is needed on the complete nucleotprotein gene as well as the ectodomain within the Glycoprotein gene to investigate any inter-country spread of rabies between Mali and its neighbours.

REFERENCES

Araújo, D. B., Langoni, H., Almeida, M. F., & Megid, J. (2008). Heminested reverse-transcriptase polymerase chain reaction (hnRT-PCR) as a tool for rabies virus detection in stored and decomposed samples. *BMC Research Notes, 1*, 17. doi:10.1186/1756-0500-1-17

Aravindh, B. R. P., Manoharan, S., Ramadass, P., & Chandran, N. D. J. (2012). Evaluation of RT-PCR assay for routine laboratory diagnosis of rabies in post mortem brain samples from different species of animals. *Indian Journal of Virology, 23*, 392-396. doi:10.1007/s13337-012-0109-9

Baby, J., Mani, R. S., Abraham, S. S., Thankappan, A. T., Pillai, P. M., Anand, A. M., & Madhusudana, S. N. (2015). Natural Rabies Infection in a Domestic Fowl (Gallus domesticus): A Report from India. 1-6. doi:10.1371/journal.pntd.0003942

Badrane, H., Bahloul, C., Perrin, P., & Tordo, N. (2001). Evidence of two Lyssavirus phylogroups with distinct pathogenicity and immunogenicity. *Journal of virology, 75*, 3268-3276. doi:10.1128/JVI.75.7.3268-3276.2001

Barnard, B. J. H., & Voges, S. F. (1982). A simple technique for the rapid diagnosis of rabies in formalin-preserved brain. *Ondersterpoort Journal of Veterinary Research, 194*, 193-194.

Beltrán, F. J., Dohmen, F. G., Del Pietro, H., & Cisterna, D. M. (2014). Diagnosis and molecular typing of rabies virus in samples stored in inadequate conditions. *Journal of infection in developing countries, 8*, 1016-1021. doi:10.3855/jidc.4136

BioNote. (2008). Inc. One-step Rabies Antigen Test [Brochure]. 2008. Available at: http://www.bionote.co.kr. 2008.

Biswal, M., Ratho, R., & Mishra, B. (2007). Usefulness of reverse transcriptase-polymerase chain reaction for detection of rabies RNA in archival samples. *Japanese Journal of Infectious Diseases, 60*, 298-299.

Biswal, M., Ratho, R. K., & Mishra, B. (2012). Role of reverse transcriptase polymerase chain reaction for the diagnosis of human rabies. *The Indian Journal of Medical Research, 135*, 837-842.

Bitek, A. O., Osoro, E., Munyua, P. M., Nanyingi, M., Muthiani, Y., Kiambi, S., . . . Thumbi, S. (2019). A hundred years of rabies in Kenya and the strategy for eliminating dog-mediated rabies by 2030. *AAS Open Research, 1*, 23. doi:10.12688/aasopenres.12872.2

Boland, T. A., McGuone, D., Jindal, J., Rocha, M., Cumming, M., Rupprecht, C. E., . . . Rosenthal, E. S. (2014). Phylogenetic and epidemiologic evidence of multiyear incubation in human rabies. *Annals of neurology, 75*, 155-160. doi:10.1002/ana.24016

Bourhy, H., & Perrin, P. (1996). Rapid rabies enzyme immunodiagnosis (RREID) for rabies antigen detection. In Kaplan MM & K. H (Eds.), *Laboratory techniques in rabies* (4th ed. ed., pp. 105–113). Geneva, Switzerland: World Health Organization.

Bourhy, H., Reynes, J. M., Dunham, E. J., Dacheux, L., Larrous, F., Huong, V. T. Q., . . . Holmes, E. C. (2008). The origin and phylogeography of dog rabies virus. *Journal of General Virology, 89*, 2673-2681. doi:10.1099/vir.0.2008/003913-0

Cardoso Lopes, M., Lima Rossignolo Venditti, L., & Queiroz, L. (2009). Comparison between RT-PCR and the mouse inoculation test for detection of rabies virus in samples kept for long periods under different conditions. *Journal of virological methods, 164*, 19-23. doi:10.1016/j.jviromet.2009.11.017

Chen, L. H. (2009). Travel Medicine, 2nd Edition. *Emerging Infectious Diseases, 15*, 511. doi:10.3201/eid1503.081591

Chhabra, M., Mittal, V., Jaiswal, R., Malik, S., Gupta, M., & Lal, S. (2007). Development and evaluation of an in vitro isolation of street rabies virus in mouse neuroblastoma cells as compared to conventional tests used for diagnosis of rabies. *Indian journal of medical microbiology, 25*, 263-266.

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology, 9*, 1657-1659. doi:10.1046/j.1365-294x.2000.01020.x

Coetzer, A., Sabeta, C. T., Markotter, W., Rupprecht, C. E., & Nel, L. H. (2014). Comparison of Biotinylated Monoclonal and Polyclonal Antibodies in an Evaluation of a Direct Rapid Immunohistochemical Test for the Routine Diagnosis of Rabies in

Southern Africa. *PLoS Neglected Tropical Diseases, 8*. doi:10.1371/journal.pntd.0003189

David, D., Hughes, G. J., Yakobson, B. A., Davidson, I., Un, H., Aylan, O., . . . Rupprecht, C. E. (2007). Identification of novel canine rabies virus clades in the Middle East and North Identification of novel canine rabies virus clades in the Middle East and North Africa. doi:10.1099/vir.0.82352-0

Davis, B. M., Rall, G. F., & Schnell, M. J. (2015). Everything You Always Wanted to Know About Rabies Virus (But Were Afraid to Ask). *Annual Review of Virology, 2*, 451-471. doi:10.1146/annurev-virology-100114-055157

Davis, P. L., Rambaut, A., Bourhy, H., & Holmes, E. C. (2007). The evolutionary dynamics of canid and mongoose rabies virus in southern Africa. *Archives of Virology, 152*, 1251-1258. doi:10.1007/s00705-007-0962-9

De Benedictis, P., Sow, A., Fusaro, A., Veggiato, C., Talbi, C., Kaboré, A., . . . Capua, I. (2010). Phylogenetic analysis of rabies viruses from Burkina Faso, 2007. *Zoonoses and Public Health, 57*. doi:10.1111/j.1863-2378.2009.01291.x

Dean, D. J., Abselseth, M. K., & Athanasiu, P. (1996). Dean, D.J., M.K. Abselseth, and P. Athanasiu (1996). The Fluorescent Antibody Test. In Meslin FX, Kaplan MM, Koprowski H, 4th ed. Laboratory techniques in rabies. World Health Organization, Geneva. pp 88-95. 1996.

Eggerbauer, E., de Benedictis, P., Hoffmann, B., Mettenleiter, T. C., Schlottau, K., Ngoepe, E. C., . . . Müller, T. (2016). Evaluation of Six Commercially Available Rapid Immunochromatographic Tests for the Diagnosis of Rabies in Brain Material. *PLoS Neglected Tropical Diseases, 10*, 1-16. doi:10.1371/journal.pntd.0004776

The Road to Dog Rabies Control and Elimination—What Keeps Us from Moving Faster?, 5 (2017).

Felsenstein, J. (1985). Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution, 39*(4), 783-791. doi:10.2307/2408678

Ferris, N. P., Clavijo, A., Yang, M., Velazquez-Salinas, L., Nordengrahn, A., Hutchings, G. H., . . . Merza, M. (2012). Development and laboratory evaluation of two lateral

flow devices for the detection of vesicular stomatitis virus in clinical samples. *Journal of virological methods, 180*, 96-100. doi:10.1016/j.jviromet.2011.12.010

Fooks, A. R., Johnson, N., Freuling, C. M., Wakeley, P. R., Banyard, A. C., McElhinney, L. M., . . . Müller, T. (2009). Emerging technologies for the detection of rabies virus: Challenges and hopes in the 21st century. *PLoS Neglected Tropical Diseases, 3*, 1-12. doi:10.1371/journal.pntd.0000530

Gilbert, A. T., Petersen, B. W., Recuenco, S., Niezgoda, M., Gomez, J., Laguna-Torres, V. A., & Rupprecht, C. (2012). Evidence of rabies virus exposure among humans in the Peruvian Amazon. *The American journal of tropical medicine and hygiene, 87*, 206-215. doi:10.4269/ajtmh.2012.11-0689

Goldwasser, R. A., Kissling, R. E., Carski, T. R., & Hosty, T. S. (1959). Fluorescent antibody staining of rabies virus antigens in the salivary glands of rabid animals. *Bulletin of the World Health Organization, 20*, 579-588.

Gury Dohmen, F., Kovacs, E., Prestrera, N. E., & Beltrán, F. J. (2018). Evaluation of a rapid immunochromatographic diagnostic test (RIDT) for diagnosis of rabies in samples from Argentina. *Journal of infection in developing countries, 12*(6), 415-421. Retrieved from https://doi.org/10.3855/jidc.9552 doi:10.3855/jidc.9552

Hall, A. T. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids.Symp.*(41), 95-98.

Hall, A. T. (2011). BioEdit : An important software for molecular biology. *GERF Bulletin of Biosciences, 2*, 60-61.

Hayman, D. T. S., Johnson, N., Horton, D. L., Hedge, J., Wakeley, P. R., Banyard, A. C., . . . Fooks, A. R. (2011). Evolutionary history of rabies in Ghana. *PLoS Neglected Tropical Diseases, 5*. doi:10.1371/journal.pntd.0001001

Heaton, P. R., Johnstone, P., McElhinney, L. M., Cowley, R., O'Sullivan, E., & Whitby, J. E. (1997). Heminested PCR assay for detection of six genotypes of rabies and rabies-related viruses. *Journal of clinical microbiology, 35*, 2762-2766.

Heaton, P. R., McElhinney, L. M., & Lowings, J. P. (1999). Detection and identification of rabies and rabies-related viruses using rapid-cycle PCR. *Journal of virological methods, 81*, 63-69. doi:10.1016/s0166-0934(99)00060-9

Hughes, G. J., Smith, J. S., Hanlon, C. A., Rupprecht, C. E., & Icrobiol, J. C. L. I. N. M. (2004). Evaluation of a TaqMan PCR Assay To Detect Rabies Virus RNA : Influence of Sequence Variation and Application to Quantification of Viral Loads. *42*, 299-306. doi:10.1128/JCM.42.1.299

An outbreak of pig rabies in Hunan province, China, 136 504-508 (2008).

Junior, D. (2004). Reverse transcription-polymerase chain reaction assay for rabies virus detection. 398-400.

Kang, B., Oh, J., Lee, C., Park, B. K., Park, Y., Hong, K., . . . Song, D. (2007). Evaluation of a rapid immunodiagnostic test kit for rabies virus. *Journal of Virological Methods, 145*, 30-36. doi:10.1016/j.jviromet.2007.05.005

Kaprowski. (1996). Koprowski H (1996). The mousse inoculation.In Meslin FX, Kaplan MM, Koprowski H, 4th ed. Laboratory techniques in rabies. World Health Organization, Geneva. pp 80-87. 1996.

Kasempimolporn, e. a. (2011). Evaluation of a rapid immunochromatographic test strip for detection of Rabies virus in dog saliva samples. *Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc, 23*, 1197-1201. doi:10.1177/1040638711425576

Kaw, A., Singh, C. K., Ramneek, Sood, N. K., Sandhu, B. S., Deka, D., & Awahan, S. (2012). Intravitam diagnosis of rabies from saliva by nested RT-PCR and real time PCR. *Brazilian Journal of Veterinary Pathology, 5*, 70-73.

Kissi, B., Tordo, N., & Bourhy, H. (1995). Genetic polymorphism in the rabies virus nucleoprotein gene. *Virology, 209*, 526-537. doi:10.1006/viro.1995.1285

Knobel, D. L., Cleaveland, S., Coleman, P. G., Fevre, E. M., Meltzer, M. I., Miranda, M. E. G., . . . Meslin, F.-X. (2005). Re-evaluating the burden of rabies in Africa and Asia. *Bulletin of the World Health Organization, 83*, 360-368. doi:/S0042-96862005000500012

Kulonen, K., Fekadu, M., Whitfield, S., & Warner, C. (1999). An Evaluation of Immunofluorescence and PCR. Methods for Detection of Rabies in Archival Carnoy-Fixed, Paraffin-Embedded Brain Tissue*. *Zentralblatt für Veterinärmedizin. Reihe B. Journal of veterinary medicine. Series B, 46*, 151-155.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular biology and evolution, 33*, 1870-1874. doi:10.1093/molbev/msw054

Kuzmin, I. V., Mayer, A. E., Niezgoda, M., Markotter, W., Agwanda, B., Breiman, R. F., & Rupprecht, C. E. (2010). Shimoni bat virus, a new representative of the Lyssavirus genus. *Virus research, 149*, 197-210. doi:10.1016/j.virusres.2010.01.018

Lafon, M. (2005). Rabies virus receptors. *Journal of neurovirology, 11*, 82-87. doi:10.1080/13550280590900427

Lechenne, M., Naissengar, K., Lampelletier, A., Alfarouk, I. O., Bourhy, H., Zinsstag, J., & Dacheux, L. (2016). Validation of a Rapid Rabies Diagnostic Tool for Field Surveillance in Developing Countries. *PLoS Neglected Tropical Disease, 10*, 1-16. doi:10.1371/journal.pntd.0005010

Leigh, J. W., & Bryant, D. (2015). popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution, 6*, 1110-1116. doi:10.1111/2041-210X.12410

Lembo, T., Hampson, K., Haydon, D. T., Craft, M., Dobson, A., Dushoff, J., . . . Cleaveland, S. (2008). Exploring reservoir dynamics: A case study of rabies in the Serengeti ecosystem. *Journal of Applied Ecology, 45*, 1246-1257. doi:10.1111/j.1365-2664.2008.01468.x

Lembo, T., Niezgoda, M., Velasco-villa, A., Cleaveland, S., Ernest, E., & Rupprecht, C. E. (2006). Evaluation of a Test for Rabies Diagnosis. *Emerging Infectious Diseases, 12*, 310-313. doi:10.3201/eid1202.050812

Mani, R. S., & Madhusudana, S. N. (2013). Laboratory diagnosis of human rabies: recent advances. *TheScientificWorldJournal, 2013*, 569712. doi:10.1155/2013/569712

Mansfield, K., McElhinney, L., Hübschle, O., Mettler, F., Sabeta, C., Nel, L. H., & Fooks, A. R. (2006). A molecular epidemiological study of rabies epizootics in kudu (Tragelaphus strepsiceros) in Namibia. *BMC Veterinary Research, 2*, 1-10. doi:10.1186/1746-6148-2-2

Markotter, W., York, D., Sabeta, C. T., Shumba, W., Zulu, G., Le Roux, K., & Nel, L. H. (2009). Evaluation of a rapid immunodiagnostic test kit for detection of African

lyssaviruses from brain material. *The Onderstepoort journal of veterinary research, 76*, 257-262.

Mori, Y., Kanda, H., & Notomi, T. (2013). Loop-mediated isothermal amplification (LAMP): recent progress in research and development. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy, 19*, 404-411. doi:10.1007/s10156-013-0590-0

Mori, Y., Nagamine, K., Tomita, N., & Notomi, T. (2001). Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochemical and biophysical research communications, 289*, 150-154. doi:10.1006/bbrc.2001.5921

Mshelbwala, P., & Abdullahi, S. (2012). Evaluation of Two Rapid Diagnostic Tests for Rabies Diagnosis under Field and Laboratory Conditions in Nigeria. *Journal of Vaccines & Vaccination, 06*, 6-10. doi:10.4172/2157-7560.1000272

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G., & Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor symposia on quantitative biology, 51 Pt 1*, 263-273. doi:10.1101/sqb.1986.051.01.032

Nadin-Davis, S. A., Sheen, M., & Wandeler, A. I. (2009). Development of real-time reverse transcriptase polymerase chain reaction methods for human rabies diagnosis. *Journal of Medical Virology, 81*, 1484-1497. doi:10.1002/jmv.21547

Nel, L., Jacobs, J., Jaftha, J., & Meredith, C. (1997). Natural spillover of a distinctly canidae-associated biotype of rabies virus into an expanded wildlife host range in southern Africa. *Virus Genes, 15*, 79-82. doi:10.1023/A:1007979502754

Nel, L. H., Sabeta, C. T., von Teichman, B., Jaftha, J. B., Rupprecht, C. E., & Bingham, J. (2005). Mongoose rabies in southern Africa: a re-evaluation based on molecular epidemiology. *Virus research, 109*, 165-173. doi:10.1016/j.virusres.2004.12.003

Nishizono, A., Khawplod, P., Ahmed, K., Goto, K., Shiota, S., Mifune, K., . . . Morimoto, K. (2008). A simple and rapid immunochromatographic test kit for rabies diagnosis. *Microbiology and immunology, 52*, 243-249. doi:10.1111/j.1348-0421.2008.00031.x

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic acids research, 28,* E63. doi:10.1093/nar/28.12.e63

OIE. (2008). WORLD ORGANISATION FOR ANIMAL HEALTH MANUAL OF DIAGNOSTIC TESTS AND VACCINES (mammals , birds and bees) Sixth Edition. *2*, Office International des Épizooties, Paris, pp. 66.

Page, R. D. M. (1996). Tree View: An application to display phylogenetic trees on personal computers. *Bioinformatics, 12,* 357-358. doi:10.1093/bioinformatics/12.4.357

Pfukenyi, D. M., Pawandiwa, D., Makaya, P. V., & Ushewokunze-Obatolu, U. (2009). A retrospective study of wildlife rabies in Zimbabwe, between 1992 and 2003. *Tropical Animal Health and Production, 41*, 565-572. doi:10.1007/s11250-008-9224-4

Posthuma-Trumpie, Geertruida, A., Korf, J., & van Amerongen, A. (2009). Lateral flow (immuno)assay: its strengths, weaknesses, opportunities and threats. A literature survey. *Analytical and Bioanalytical Chemistry, 393*, 569-582. doi:10.1007/s00216-008-2287-2

Prabhu, K. N., Isloor, S., Veeresh, B. H., Rathnamma, D., Sharada, R., Das, L. J., . . . Rahman, S. A. (2018). Application and Comparative Evaluation of Fluorescent Antibody, Immunohistochemistry and Reverse Transcription Polymerase Chain Reaction Tests for the Detection of Rabies Virus Antigen or Nucleic Acid in Brain Samples of Animals Suspected of Rabies in India. *Veterinary Sciences, 5*(1). doi:10.3390/vetsci5010024

Rieder, M., & Conzelmann, K.-K. (2011). Interferon in rabies virus infection. *Advances in virus research, 79*, 91-114. doi:10.1016/B978-0-12-387040-7.00006-8

Robertson, K., Marano, N., & Johnson, K. J. (2012). Rabies. In *Netter's Infectious Diseases* (pp. 411-418): W.B. Saunders.

Rudd, R. J., & Trimarchi, C. V. (1987). Comparison of sensitivity of BHK-21 and murine neuroblastoma cells in the isolation of a street strain rabies virus. *Journal of Clinical Microbiology, 25*, 1456-1458.

Rupprecht, C. E., Hanlon, C. A., & Hemachudha, T. (2002). Rabies re-examined. *Lancet Infectious Diseases, 2*, 327-343. doi:10.1016/S1473-3099(02)00287-6

Sadeuh-Mba, S. A., Momo, J. B., Besong, L., Loul, S., & Njouom, R. (2017). Molecular characterization and phylogenetic relatedness of dog-derived Rabies Viruses circulating in Cameroon between 2010 and 2016. *PLoS Neglected Tropical Diseases, 11*. doi:10.1371/journal.pntd.0006041

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*, 406-425. doi:10.1093/oxfordjournals.molbev.a040454

Servat, A., Picard-Meyer, E., Robardet, E., Muzniece, Z., Must, K., & Cliquet, F. (2012). Evaluation of a Rapid Immunochromatographic Diagnostic Test for the detection of rabies from brain material of European mammals. *Biologicals : journal of the International Association of Biological Standardization, 40*, 61-66. doi:10.1016/j.biologicals.2011.12.011

Shankar, B. P. (2009). Advances in diagnosis of rabies. *Veterinary World, 2*, 74-78.

Singathia, R., Dutta, P., Yadav, R., Gupta, S. R., Gangil, R., & Gattani, A. (2012). Current Update on Rabies Diagnosis. *Articles from International Journal for Agro Veterinary and Medical Sciences, 6*, 229-240. doi:10.5455/ijavms.

Singh, R., Singh, K. P., Cherian, S., Saminathan, M., Kapoor, S., Manjunatha Reddy, G. B., . . . Dhama, K. (2017). Rabies - epidemiology, pathogenesis, public health concerns and advances in diagnosis and control: a comprehensive review. *The Veterinary quarterly, 37*, 212-251. doi:10.1080/01652176.2017.1343516

Slomka, M. J., To, T. L., Tong, H. H., Coward, V. J., Mawhinney, I. C., Banks, J., & Brown, I. H. (2012). Evaluation of lateral flow devices for identification of infected poultry by testing swab and feather specimens during H5N1 highly pathogenic avian influenza outbreaks in Vietnam. *Influenza and other respiratory viruses, 6*, 318-327. doi:10.1111/j.1750-2659.2011.00317.x

Streicker, D. G., Turmelle, A. S., Vonhof, M. J., Kuzmin, I. V., McCracken, G. F., & Rupprecht, C. E. (2010). Host phylogeny constrains cross-species emergence and establishment of rabies virus in bats. *Science (New York, N.Y.), 329*, 676-679. doi:10.1126/science.1188836

Talbi, C., Holmes, E. C., Benedictis, P. D., Faye, O., Gamatie, D., Diarra, A., . . . Sall, A. A. (2016). Evolutionary history and dynamics of dog rabies virus in western and central Africa. 783-791. doi:10.1099/vir.0.007765-0

Talbi, C., Holmes, E. C., de Benedictis, P., Faye, O., Nakouné, E., Gamatié, D., . . . Bourhy, H. (2009). Evolutionary history and dynamics of dog rabies virus in western and central Africa. *Journal of General Virology, 90*, 783-791. doi:10.1099/vir.0.007765-0

Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. *Molecular biology and evolution, 9*, 678-687. doi:10.1093/oxfordjournals.molbev.a040752

Thomas, J. B., Sikes, R. K., & Ricker, A. S. (1963). Evaluation of Indirect Fluorescent Antibody Technique for Detection of Rabies Antibody in Human Sera. *Journal of immunology (Baltimore, Md. : 1950), 91*, 721-723.

Thomas, R. H. (2001). Molecular Evolution and Phylogenetics. Masatoshi Nei and Sudhir Kumar. Oxford University Press, Oxford. 2000. pp. 333. Price £65.00, hardback. ISBN 0 19 513584 9. *Heredity, 86*, 385. doi:10.1046/j.1365-2540.2001.0923a.x

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic acids research, 25*, 4876-4882. doi:10.1093/nar/25.24.4876

Tierkel, E. S., & Atanasiu. (1996). Tierkel E.S & Atanasiu (1996). Rapid microscopic examination for Negri bodies and preparation of specimens ffor biological tests. In Meslin FX, Kaplan MM, Koprowski H, 4th ed. Laboratory techniques in rabies. World Health Organization, Geneva. pp 55-60. 1996.

Tomita, N., Mori, Y., Kanda, H., & Notomi, T. (2008). Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nature protocols, 3*, 877-882. doi:10.1038/nprot.2008.57

Toni, L. S., Garcia, A. M., Jeffrey, D. A., Jiang, X., Stauffer, B. L., Miyamoto, S. D., & Sucharov, C. C. (2018). Optimization of phenol-chloroform RNA extraction. *MethodsX, 5*, 599-608. doi:10.1016/j.mex.2018.05.011

Tordo, N., Poch, O., Ermine, A., Keith, G., & Rougeon, F. (1988). Completion of the rabies virus genome sequence determination: highly conserved domains among the L (polymerase) proteins of unsegmented negative-strand RNA viruses. *Virology, 165*, 565-576. doi:10.1016/0042-6822(88)90600-9

Tordo, N., Sacramento, D., & Bourhy, H. (1996). In Meslin FX, Kaplan MM, Koprowski H, 4th ed. Laboratory techniques in rabies. World Health Organization, Geneva. pp 157-168 Umoh, J.U. & Blenden, D.C., 1981. Immunofluorescent staining of rabies virus antigen *59*, 1996.

Traoré, A., Picard-Meyer, E., Mauti, S., Biarnais, M., Balmer, O., Samaké, K., . . . Cliquet, F. (2016). Molecular characterization of canine rabies virus, Mali, 2006–2013. *Emerging Infectious Diseases, 22*, 866-870. doi:10.3201/eid2205.150470

Troupin, C., Dacheux, L., Tanguy, M., Sabeta, C., Blanc, H., Bouchier, C., . . . Bourhy, H. (2016). Large-Scale Phylogenomic Analysis Reveals the Complex Evolutionary History of Rabies Virus in Multiple Carnivore Hosts. *PLoS Pathogens, 12*, 1-20. doi:10.1371/journal.ppat.1006041

Umoh, J. U., & Blenden, D. C. (1981). Immunofluorescent staining of rabies virus antigen in formalin-fixed tissue after treatment with trypsin. *Bulletin of the World Health Organization, 59*, 737-744.

Wacharapluesadee, S., & Hemachudha, T. (2001). Nucleic-acid sequence based amplification in the rapid diagnosis of rabies. *Lancet, 358*, 892-893. doi:10.1016/S0140-6736(01)06041-X

Wacharapluesadee, S., Sutipanya, J., Damrongwatanapokin, S., Phumesin, P., Chamnanpood, P., Leowijuk, C., & Hemachudha, T. (2008). Development of a TaqMan real-time RT-PCR assay for the detection of rabies virus. *Journal of Virological Methods, 151*, 317-320. doi:10.1016/j.jviromet.2008.05.004

Walker, N. F., Brown, C. S., Youkee, D., Baker, P., Williams, N., Kalawa, A., . . . Brooks, T. (2015). Evaluation of a point-of-care blood test for identification of Ebola virus disease at Ebola holding units, Western Area, Sierra Leone, January to February 2015. *Eurosurveillance, 20*. doi:https://doi.org/10.2807/1560-7917.ES2015.20.12.21073

Webster, W. A., & Casey, G. A. (1996). Virus Isolation in neuroblastoma cell culture. In Meslin FX, Kaplan MM, Koprowski H, 4th ed. Laboratory techniques in rabies. World Health Organization, Geneva. pp 96-101. 1996.

Whitby, J. E., Johnstone, P., & Sillero-Zubiri, C. (1997). Rabies virus in the decomposed brain of an Ethiopian wolf detected by nested reverse transcription-polymerase chain reaction. *Journal of wildlife diseases, 33*, 912-915. doi:10.7589/0090-3558-33.4.912

WHO. (2013). WHO Expert Consultation on Rabies.

WHO. (2019). *Laboratory techniques in rabies* (C. E. Rupprecht, A. R. Fooks, & B. Abela-Ridder Eds. Fifth Edition ed. Vol. 1 & 2).

World Health Organization. The Tripartite's commitment (Producer). (2017, September 2, 2020). Providing multi-sectoral, collaborative leadership in addressing health challenges. Retrieved from http://www.who.int/zoonoses/tripartite_oct2017.pdf?ua=1

Zhu, Y., Zhang, G., Shao, M., Lei, Y., Jiang, Y., & Tu, C. (2011). An outbreak of sheep rabies in Shanxi province, China. *Epidemiology and Infection, 139*, 1453-1456. doi:10.1017/S0950268811001348

APPENDICES

Appendix 1: Selective comparison of selective rabies diagnosis methods

	Test	Passive surveillance	Active surveillance	Clinical diagnosis	Sample types	Notes
Molecular	Reverse transcriptase polymerase chain reaction (RT-PCR)	++	++	+++	Brain Skin biopsy Saliva	Primary and confirmatory diagnostic test used in combination with sequencing
	Hemi-nested RT-PCR	++	++	+++	Brain Skin biopsy Saliva	Primary and confirmatory diagnostic test used in combination with sequencing
	Real-time RT-PCR	+	++	+++	Brain Skin biopsy Saliva	Primary diagnostic test
Antigen	Direct fluorescent antibody test (DFAT)	+++	+++	+++	Brain Skin biopsy Saliva	Primary ‘gold standard’ test Recognized by WHO and OIE
	Direct rapid immune-chemistry test (DRIT)	+++	+++	+++	Brain Skin biopsy	Primary ‘gold standard’ test Recognized by WHO and OIE

Antigen	Rapid immune-chromatographic test (RIDT)	-	+	+	Brain	Low-cost Transportable Variability in term of sensitivity and specificity
Antibody	Rapid fluorescence inhibition test (RFFIT)	-	-	+++	Serum CSF	Useful for checking vaccination responses and disease activity
	Fluorescent antibody virus neutralization test (FAVN)					
	Competitive ELISA	-	-	++	Serum CSF	Used in wildlife immunization program for immunogenicity studies

OIE, World Organisation for Animal Health; WHO, World Health Organisation; CSF, Cerebrospinal fluid

+++, high recommendation, can be used for primary or confirmatory testing; ++, moderate recommendation, can be used for primary or confirmatory testing; +, low recommendation, can be used for confirmatory testing; - not recommended

Appendix 2: Isolates of RABV used for the phylogenetic analysis

GenBank Accession Number	Country	Lineage	Sub-lineage
KP976118	Mali	Africa 2	H
KP976119	Mali	Africa 2	H
EU853622	Mali	Africa 2	G
KP976130	Mali	Africa 2	G
EU853642	Mauritania	Africa 2	G
EU853635	Senegal	Africa 2	G
KP976121	Mali	Africa 2	F
U22640	Niger	Africa 2	F
EU827270	Burkina Faso	Africa 2	F
EU853646	Niger	Africa 2	F
EU827272	Burkina Faso	Africa 2	F
EU718785	Chad	Africa 2	E
U22636	Cameroon	Africa 2	E
EU038104	Nigeria	Africa 2	E
EU718735	Chad	Africa 2	E
EU853592	Senegal	Africa 2	B
DQ837431	Israel	Africa 4	
DQ837462	Egypt	Africa 4	
DQ837463	Egypt	Africa 4	
U22484	Mozambic	Africa 1	
U22632	Namibia	Africa 1	
U22645	Tanzania	Africa 1	
U22488	Nigeria	Africa 1	
U22629	Gambia	Africa 1	
EU853568	Algeria	Africa 1	
U22642	Marocco	Africa 1	
U22647	Tanzania	Africa 1	

GenBank Accession Number	Country	Lineage	Sub-lineage
EU853572	Marocco	Africa 1	
U22631	Marocco	Africa 1	
NC_025365.1	Kenya	Shimoni bat lyssairus	

Appendix 3: RNA quantification results

Order	Sample	Sample ID	(ng/µl)	A260/A280	260/A230
1	03/16	029/16	5095.3	1.75	1.65
2	04/16	921/16	2474	1.70	0.66
3	05/16	089/16	449.4	1.37	0.22
4	09/16	023/16	3645	1.62	0.49
5	18/16	028/16	1269.8	1.72	0.64
6	01/17	174/17	2829	1.71	0.78
7	02/17	389/17	2366.2	1.79	0.83
8	04/17	042/17	4985.1	1.68	1.07
9	08/17	444/17	3987	1.75	1.14
10	09/17	143/17	4513.2	1.83	1.24
11	01/16	145/16	1030.2	1.62	0.48
12	02/16	908/16	00	00	00
13	15/16	413/16	3330.8	1.66	1.40
14	17/16	021/16	1256.4	1.47	0.75
15	03/17	410/17	4906.4	1.68	1.22
16	05/17	285/17	3191.9	1.84	1.07
17	12/17	216/17	4426	1.68	1.38
18	17/17	106/17	0.69	00.5	00.8

Appendix 4: cDNA quantification results

Order	Sample	Sample ID	(ng/µl)	A260/A280	260/A230
1	03/16	026/16	2949	1.70	3.69
2	04/16	921/16	1571	1.69	1.82
3	05/16	089/16	1821	1.69	7.82
4	09/16	023/16	1000	1.73	_
5	18/16	028/16	1143	1.66	1.81
6	01/17	174/17	2002	1.65	1.80
7	02/17	389/17	923	1.64	14.9
8	04/17	042/17	2649	1.67	1.92
9	08/17	444/17	1066	1.61	1.69
10	09/17	143/17	2589	1.65	1.76
11	01/16	145/16	1858	1.68	1.83
12	02/16	908/16	00	00	00
13	15/16	413/16	1180	1.70	1.80
14	17/16	021/16	2848	1.67	1.69
15	03/17	410/17	3436	1.70	2.06
16	05/17	285/17	2796	1.70	2.06
17	12/17	216/17	3001	1.69	1.82
18	17/17	106/17	00	00	00

Appendix 5: List of specimens used in the study.

ORDER	SAMPLE ID	FAT	RIDT	RT-PCR	SPECIES	LOCATION
1	029/16	Positive	Positive	Positive	Dog	Bamako
2	921/16	Positive	Positive	Positive	Dog	Bamako
3	089/16	Positive	Positive	Positive	Dog	Bamako
4	023/16	Positive	Positive	Positive	Dog	Bamako
5	028/16	Positive	Positive	Positive	Dog	Bamako
6	174/17	Positive	Positive	Positive	Dog	Kayes
7	389/17	Positive	Negative	Negative	Dog	Bamako
8	042/17	Positive	Positive	Positive	Dog	Bamako
9	444/17	Positive	Negative	Positive	Dog	Koulikoro
10	143/17	Positive	Negative	Positive	Dog	Koulikoro
11	145/16	Positive	Negative	Positive	Dog	Bamako
12	908/16	Negative	Negative	Negative	Dog	Bamako
13	413/16	Positive	Negative	Positive	Dog	Bamako
14	021/16	Positive	Negative	Positive	Dog	Bamako
15	410/17	Positive	Negative	Positive	Dog	Bamako
16	285/17	Positive	Negative	Positive	Dog	Koulikoro
17	216/17	Positive	Negative	Positive	Dog	Bamako
18	106/17	Negative	Negative	Negative	Dog	Bamako

Appendix 6: Sequencing Results

nucl1_AF_E05_14

CCCCGAGCAGCAGCTATGGGAKCTTGATTGCAGAGAGGGAGACAAGATCACCCC
AGATTCTCTTGKGGAGATCAAGCGTACGGATGTAGAGGGAAACTGGGCCCTGACA
GGAGGTATGGAACTGACGAGAGACCCCACAGTTTCTGAACATGCATCTTTAGTCR
GTCTGCTCA

nucl1_AR_E07_13

CCGTCTGCAGCGCCCTCGTCAGCTCGTCGTCCTGGGGGGAAAGATCTCCCTATATT
CTGTAGGGTAGATAACACAAGAGAAGCTGAGGGAAACTGGGCCCTGTCTCGAGG
TATGGAAATGACGAGACACCCCCCAGCTTCTGAACATGCATCTTTAAACAGTCTG
CT

nucl2_AF_F05_17

GRGGGGMWTMYATGGGATCTTGATTGCAAGARAGGGRGACAAGATCACCCCAK
ATTCTCTTGTGGAGATCAAGCGTACRGATGTAGAGGGAAACTGGGCCCTGACAGG
AGGTATGGAAMTGACGAGAGACCCCACAGYTTCTGAACATGCATCTTTAGTCRGT
CTGCTCAAGG

nucl2_AR_F07_16

CGACGGGCAGTCTCTCGTCAGTTCCATACCTCCTGTCAGGGMCCAGWTTCCCTCT
ACATCCGTACGCTTGATCTCCACAAGAGAATCTGGGGTGATCTTGTCTCCCTTTCT
TGSAATCAARATCCCATAGCTGGYCCAGTCTTCAGGACATGTCCCTTCAAAGAGTT
GCATTA

nucl3_AF_G05_20

AAATGGTCAGCATGGGTCATTATTAGGCTTTCCCCTCTWATGTSGKCMTGGACAG
SGAGGCACTAAGCRTTCACTGAGCAAATTTGTTATTATATCTCTTTAAATTCTTTCC
AGGYGSTCTACATGGTACARAACTTCAACATCTTTAGTCGGTCTGCTCAA

nucl3_AR_G07_19

AGAAMAGCAGCCCTGTGCTRGMCGTACCAGGCTCTGACTC

nucl4_AF_H05_23

TAYTGGCTTCCATGGGATCTTGATTGCACSAGGGGASACAGATCWCCCCMKATTC
TCTTGKGGAGATCTCCGTACRGATGYAGAAGGAATTGGTCTCTGTTTCTAGGWAT
GGAAATGCCRTAASTGCCCMMRKCTTCWGGACATGYCCCTTTARWCAGTCTGCTT
A

nucl4_AR_H07_22

AGGATTGAGTAGATGGTCAKCGTCATACRTCCWGYCRGAGMCCAGATWCCTTCK
AWTCCGTACGSTTGATCTCCACWAGAGAATCTGGGGGGATCTTGTCTCCCTTTCTT
GGWATGRARATGCCATAASTGGYCCMRKCTTCWGGACATGYCCCTTTARASAGTY
GGMTTA

nucl5_AF_A06_03

CGGWAGTCTATGGGATCTTGATTGCACSAKAGGGASACAAGATCACCCCAGATTC
TCTTGKGGAGATAACCGWACAGATGYAGAAGGAATCTGGGCTCTGTTTCTAGGW
ATGGAAATGCCRTRASAGCCCMCRKCTTCTGGACATGCATCTTTARWCAGTCTGC
TTA

nucl5_AR_A08_02

CWGGTGGTCGCTGGTCAGTGTCATACATCCTGYCWGAGCCCAGATACCTTCGACA
TCCGTACGGTTGATCTCCACWACAGAATCTGGGGGGAGTTGTCTCCCTTTCTTGY
WATCRAAATCCCATARSTGGYCCMRKCTTCWGGACATGYCCCTTTARASAGTYGG
MTTA

nucl6_AF_B06_06

CGTGACTGCTCGATGAGGGACTTGWCRCGRSSTSKTCTYTGG

nucl6_AR_B08_05

CCGAAGAGCATACTCACGATGAATGAGGTGCTTAACAGCCCTGWCTTMGACGGT
GGYWCGTCTGMGTTGAGACAAGGRGMCAGWAGCATTAAKGCTCATCCCTTCARG
AGTTGCATTA

nucl7_AF_C06_09

ARRGKGGMWYAYYATGGGATCTTGATTGCAAGARAGGGAGACAAGATCACCCCA
GATTCTCTTGTGGAGATCAAGCGTACGGATGTAGAGGGAAACTGGGCCCTGACAG
GAGGTATGGAACTGACGAGAGACCCCACAGTTTCTGAACATGCATCTTTAGTCGG
TCTGCTCA

nucl7_AR_C08_08

CGGCGGGCAGTCTCTCGTCAGTTCCATACCTCCTGTCRGGGMCCAGWTTCCCTCT
ACATCCGTACGCTTGATCTCCACAAGAGAATCTGGGGTGATCTTGTCTCCCTTTCT
TGCAATCAARATCCCATAGCTGGYCCAGTCTTCAGGACATGTCCCTTCAAAGAGTT
GCATT

nucl8_AF_D06_12

GRGGTGGSRKKMYATGGGATCTTGATTGCASAGAGGGAGACAAGATCACCCCAK
ATTCTCTTGTGGAGATCAAGCGTACRGATGYAGAGGGAAACTGGGCCCTGACAGG
AGGWATGGAAMTGACGAGAGACCCCMCRKYTTCTGAACATGCMTCTTTARWCA
GTCTGCTT

nucl8_AR_D08_11

CGAGTGCAGTATMATCGTCAKYTCCATACCTCCTGTCRGGGMCCAGATTTCCCTC
TACATCCGTACGSTTGATCTCCACAASAGAATCTGGGGKGATCTTGKCTCCCTTTC
TTGSAATSRARATCCCATAGCTGSYCCARKCTTCWGGACATGYCCCTTCAAASAGT
YGSATTA

nucl9_AF_E06_15

AGGGGWAGCTATCCTGAGTCGACTTTCCKATCTTATGTACTCMTYGACYSGTYCS
ACTAAGCCTTCCCTGAKYCCCTGGAAACATATCTCTTTATCTCTTTCCAGGCGGTC
CCCATGGTACAGAACTTCAACATCTTTAGTCGGTCTGCTCA

nucl9_AR_E08_14

GGGGATCACCGAATGAGCACTCCTGTCTCCAGACTCMCACWGASACGCTYGCWG
CATGTGTMAKGCCTCTGCTTYGCCTGATACATTAAGGMGCACCAATACTGAGATG
CATTAAATGYATTGGACAAATTCCTCCCTTCAAAGAGYTGCATTCAA

nucl10_AF_F06_18

ARSGGTCTCATATTCTGAGTCGGCTTTTCCCTCTWGTGTKCTCTGRG

nucl10_AR_F08_17

CTTCCAACAGCCGCTCAGCTCTCCCGTACCACGCACCMGACAGATKCGCTGGCMG
CATGTGTARGGACGCAGCTTYGCCAGATTTATTAAGGTGTTCCCTTAATGACTTGC
WTTAGTTGCATTGGGRAATTCCTCCCTTCGGGAGGTCCATTCMAAT

nucl11_AF_G06_21

GAYYGGTAGCATATTCTGAGKCGGCTTGCCCCTCTWGTGTMCTCTGRGACY

nucl11_AR_G08_20

GGGGCGAACAGCCCCCTGAGCTGGTWCGTACCACGCTCCTGYCTGATTCGCYSGC
TGCRYGTGTMRGCTCGTTAAAGGGGCRAGAACATTGCAGGMGCATCCCTTACTCC
CTTCAWTTAGATGCATTRRACAAMTTCCTCCCTTCAAAGAGTTCCRTTCMAA

nucl12_AF_H06_24

AAAGTGTMTTACATGGGATCTTGATTGCAMGARAGGGAGACAAGATCACCCCAK
ATTCTCTTGKGGAGATCAASCGTACGGATGTAGAAGGAAACTGGGCTCTGWCAGG
AGGWATGGAAMTGACGARAGASCCCMCAGCTTCTGRACATGCATCTTTARWCRG
TCTGCTCA

nucl12_AR_H08_23

GGAGCTGCAGTCCTSGTCAGCTCCATACCTCCTGKCRGAGMCCAGWTTCCTTCTA
CATCCGTACGSTTGATCTCCACAAGAGAATCTGGGGKGATCTTGTCTCCCTTTCTT
GSAATSRARATSCCATAGSTGGYCCAGKCTTCAGGACATGTCCCTTCAAASAGTYG
SATTA

nucl13_AF_A07_01

TRGGGCWTMYATGGGATCTTGATTGCAMGAAAGGGAGACAAGATCACCCCAGAT
TCTCTTGKGGAGATCAASCGTACGGATGTAGAAGGAAACTGGGCTCTGACAGGAG
GTATGGAAMTGACGAGAGACCCCACAGCTTCTGAACATGCATCTTTAGTCRGTCT
GCTCA

nucl13_AR_A09_03

GAGKTGCAGTCTCTCGTCAGTTCCATACCTCCTGTCAGAGCCCAGWTTCCTTCTAC
ATCCGTACGCTTGATCTCCACAAGAGAATCTGGGGKGATCTTGTCTCCCTTTCTTG
CAATCAARATCCCATAGCTGGTCCAGTCTTCAGGACATGTCCCTTCAAAGAGTTGC
ATTA

nucl14_AF_B07_04

CCGCGTCAGCTATGGGAKCTTGATTGCAGAGAGGGAGACAAGATCACCCCAGATT
CTCTTGKGGAGATCAASCGTACRGATGTAGAAGGAAACTGGGCTCTGACAGGAGG
TATGGAACTGACGAGAGACCCCMCAGCTTCTGAACATGCATCTTTAGWCRGTCTG
CTCA

nucl14_AR_B09_06

TTGACCTCAGCGATCGTCACTCATTGTCCTGKGGAGACCAGATWCCTTCTAWTCC
GTACGGTTGATCTCCACWASAGAATCTGGGGGGATCTTGTCTCCCTTTCTTGGWA
TSRAAATCCCATARSTGCCCCMRKCTTCWGGACATGYCCCTTCAAASAGTYGCMT

nucl15_AF_C07_07

GRAATGGCMMGTACYTGGGATCTTGATTGCMSARAGGGAGACAAGATCACCCCA
GATTCTCTTGKGGAGATCAASCGTACGGATGTAGAAGGAAACTGGGCTCTGACAG
GAGGTATGGAAMTGACGARAGASCCCMCAGCTTCTGRACATGCATCTTTARWCR
GTCTGCTCA

nucl15_AR_C09_09

GAAGTGCAGGTCCTCGTCAGTTCCATACCTCCTGTCRGAGCCCAGWTTCCTTCTAC
ATCCGTACGSTTGATCTCCACAAGAGAAKCTGGGGKGATCTTGTCTCCCTTTCTTG
SAATSRARATSCCATAGCTGGYCCAGTCTTCAGGACATGTCCCTTCAAAGAGTTGS
ATTA

nucl16_AF_D07_10

AAAGTGCATTATTATGGGATCTTGATTGCAMGARAGGGASACAAGATCACCCCAG
ATTCTCTTGKGGAGATCAASCGTACRGATGYAGAAGGAAACTGGGCTCTGACAGG

AGGTATGGAACTGACRARAGACCCCMCAGCTTCTGAACATGCATCTTTAGTCRGT
CTGCTCA

nucl16_AR_D09_12

GAAGTGCAGCCTGGGAKCTCATACCTCCTGKGGAGMCCAGATWCCTTCTAWTCC
GTACGGTTGATCTCCACAAGAGAATCTGGGGGGATCTTGTCTCCCTTTCTTGSAAT
SRAAATSCCATARSTGGYCCAGKCTTCWGGACATGTCCCTTCAAASAGTYGSATTA

Appendix 7: Nucleotide alignments

> Nucleotide alignment, Rabies isolate ML026/16nucleoprotein gene, partial cds

```
             ....|....| ....|....| ....|....| ....|....| ....|....|
                 5          15         25         35         45
NC_001542.   ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                 55         65         75         85         95
NC_001542.   AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                105        115        125        135        145
NC_001542.   AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                155        165        175        185        195
NC_001542.   GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                205        215        225        235        245
NC_001542.   GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                255        265        275        285        295
NC_001542.   AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                305        315        325        335        345
```

```
NC_001542.   AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                355        365        375        385        395
NC_001542.   TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML026/16     ---------- --GAGAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co                **.*.*** ** ******* ******.*** *** ******

             ....|....| ....|....| ....|....| ....|....| ....|....|
                405        415        425        435        445
NC_001542.   ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML026/16     ATCAAGCGTA CGGATGTAGA GGGAAACTGG GCCCTGACAG GAGGTATGGA
Clustal Co   **.**.**** * ******** .**.** *** ** ******* **** *****

             ....|....| ....|....| ....|....| ....|....| ....|....|
                455        465        475        485        495
NC_001542.   ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML026/16     ACTGACGAGA GACC------ ---------- ---------- ----------
Clustal Co   ******.*** ****

             ....|....| ....|....| ....|....| ....|....| ....|....|
                505        515        525        535        545
NC_001542.   TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                555        565        575        585        595
NC_001542.   TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                605        615        625        635        645
NC_001542.   TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co
             ....|....| ....|....| ....|....| ....|....| ....|....|
                655        665        675        685        695
NC_001542.   GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                705        715        725        735        745
NC_001542.   GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
```

```
               755        765        775        785        795
NC_001542.   CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                805        815        825        835        845
NC_001542.   GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML026/16     ---------- ---------- ---------- ---------- ----------
Clustal Co
```

> Nucleotide alignment, Rabies isolate ML921/16nucleoprotein gene, partial cds

```
             ....|....| ....|....| ....|....| ....|....| ....|....|
                 5          15         25         35         45
NC_001542.   ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML921/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                 55         65         75         85         95
NC_001542.   AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML921/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                105        115        125        135        145
NC_001542.   AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML921/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                155        165        175        185        195
NC_001542.   GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML921/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                205        215        225        235        245
NC_001542.   GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML921/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                255        265        275        285        295
NC_001542.   AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML921/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
```

```
                305        315        325        335        345
NC_001542.  AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML921/16    ---------- ---------- ---------- ---------- ---TATGGGA
Clustal Co                                                *****.*

            ....|....| ....|....| ....|....| ....|....| ....|....|
               355        365        375        385        395
NC_001542.  TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML921/16    TCTTGATTGC AAGAAAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co  ** ******* *.****.*** ** ******* ******.*** *** ******


            ....|....| ....|....| ....|....| ....|....| ....|....|
               405        415        425        435        445
NC_001542.  ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML921/16    ATCAAGCGTA CGGATGTAGA GGGAAACTGG GCCCTGACAG GAGGTATGGA
Clustal Co  **.**.**** * ******** .**.** *** ** ******* **** *****

            ....|....| ....|....| ....|....| ....|....| ....|....|
               455        465        475        485        495
NC_001542.  ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML921/16    ACTGACGAGA GAC------- ---------- ---------- ----------
Clustal Co  ******.*** ***

            ....|....| ....|....| ....|....| ....|....| ....|....|
               505        515        525        535        545
NC_001542.  TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML921/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               555        565        575        585        595
NC_001542.  TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML921/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               605        615        625        635        645
NC_001542.  TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML921/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               655        665        675        685        695
NC_001542.  GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
ML921/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               705        715        725        735        745
NC_001542.  GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
ML921/16    ---------- ---------- ---------- ---------- ----------
Clustal Co
```

```
            ....|....| ....|....| ....|....| ....|....| ....|....|
               755        765        775        785        795
NC_001542.  CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML921/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               805        815        825        835        845
NC_001542.  GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML921/16    ---------- ---------- ---------- ---------- ----------
Clustal Co
```

>Nucleotide alignment, Rabies isolate ML023/16nucleoprotein gene, partial cds

```
            ....|....| ....|....| ....|....| ....|....| ....|....|
                5         15         25         35         45
NC_001542.  ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML023/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               55         65         75         85         95
NC_001542.  AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML023/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               105        115        125        135        145
NC_001542.  AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML023/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               155        165        175        185        195
NC_001542.  GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML023/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               205        215        225        235        245
NC_001542.  GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML023/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               255        265        275        285        295
NC_001542.  AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML023/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               305        315        325        335        345
```

```
NC_001542.   AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML023/16     ---------- ---------- ---------- ---------- ----ATGGGA
Clustal Co                                                  ****.*

             ....|....| ....|....| ....|....| ....|....| ....|....|
                355        365        375        385        395
NC_001542.   TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML023/16     TCTTGATTGC ACGAGAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co   ** ******* ****.*.*** ** ******* ******.*** *** ******


             ....|....| ....|....| ....|....| ....|....| ....|....|
                405        415        425        435        445
NC_001542.   ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML023/16     ATCTACCGTA CGGATGTAGA AGGAATCTGG TCTCTGTTTC TAGGTATGGA
Clustal Co   **.:*.**** * ******** ***.*: ***  *****: :   *** *****

             ....|....| ....|....| ....|....| ....|....| ....|....|
                455        465        475        485        495
NC_001542.   ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML023/16     AATGCCATA- ---------- ---------- ---------- ----------
Clustal Co   *.**.**:.

             ....|....| ....|....| ....|....| ....|....| ....|....|
                505        515        525        535        545
NC_001542.   TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML023/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                555        565        575        585        595
NC_001542.   TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML023/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                605        615        625        635        645
NC_001542.   TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML023/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                655        665        675        685        695
NC_001542.   GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
ML023/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                705        715        725        735        745
NC_001542.   GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
ML023/16     ---------- ---------- ---------- ---------- ----------
Clustal Co
```

```
            ....|....| ....|....| ....|....| ....|....| ....|....|
               755        765        775        785        795
NC_001542.  CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML023/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               805        815        825        835        845
NC_001542.  GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML023/16    ---------- ---------- ---------- ---------- ----------
Clustal Co
```

> Nucleotide alignment, Rabies isolate ML028/16 nucleoprotein gene, partial cds

```
            ....|....| ....|....| ....|....| ....|....| ....|....|
                5          15         25         35         45
NC_001542.  ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML028/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               55         65         75         85         95
NC_001542.  AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML028/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               105        115        125        135        145
NC_001542.  AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML028/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               155        165        175        185        195
NC_001542.  GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML028/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               205        215        225        235        245
NC_001542.  GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML028/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               255        265        275        285        295
NC_001542.  AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML028/16    ---------- ---------- ---------- ---------- ----------
Clustal Co
```

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                   305        315        325        335        345
NC_001542.   AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML028/16     ---------- ---------- ---------- ---------- ---TATGGGA
Clustal Co                                                    *****.*

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   355        365        375        385        395
NC_001542.   TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML028/16     TCTTGATTGC ACGAAAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co   ** ******* ******.*** ** ******* ******.*** *** ******


                ....|....| ....|....| ....|....| ....|....| ....|....|
                   405        415        425        435        445
NC_001542.   ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML028/16     ATCAACCGTA CAGATGTAGA AGGAATCTGG GCTCTGTTTC TAGGTATGGA
Clustal Co   **.**.**** *:******** ***.*: *** ******: :   *** *****

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   455        465        475        485        495
NC_001542.   ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML028/16     AATGCCATA- ---------- ---------- ---------- ----------
Clustal Co   *.**.**:.

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   505        515        525        535        545
NC_001542.   TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML028/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   555        565        575        585        595
NC_001542.   TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML028/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   605        615        625        635        645
NC_001542.   TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML028/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   655        665        675        685        695
NC_001542.   GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
ML028/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   705        715        725        735        745
NC_001542.   GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
```

```
ML028/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                755        765        775        785        795
NC_001542.   CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML028/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                805        815        825        835        845
NC_001542.   GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML028/16     ---------- ---------- ---------- ---------- ----------
Clustal Co
```

>Nucleotide alignment, Rabies isolate ML042/17nucleoprotein gene, partial cds

```
             ....|....| ....|....| ....|....| ....|....| ....|....|
                 5          15         25         35         45
NC_001542.   ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML042/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                 55         65         75         85         95
NC_001542.   AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML042/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                105        115        125        135        145
NC_001542.   AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML042/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                155        165        175        185        195
NC_001542.   GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML042/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                205        215        225        235        245
NC_001542.   GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML042/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                255        265        275        285        295
NC_001542.   AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML042/17     ---------- ---------- ---------- ---------- ----------
Clustal Co
```

```
            ....|....| ....|....| ....|....| ....|....| ....|....|
               305        315        325        335        345
NC_001542.  AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML042/17    ---------- ---------- ---------- ---------- --CTATGGGA
Clustal Co                                                ******.*

            ....|....| ....|....| ....|....| ....|....| ....|....|
               355        365        375        385        395
NC_001542.  TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML042/17    TCTTGATTGC AAGAGAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co  ** ******* *.**.*.*** ** ******* ******.*** *** ******

            ....|....| ....|....| ....|....| ....|....| ....|....|
               405        415        425        435        445
NC_001542.  ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML042/17    ATCAAGCGTA CGGATGTAGA GGGAAACTGG GCCCTGACAG GAGGTATGGA
Clustal Co  **.**.**** * ******** .**.** *** ** ******* **** *****

            ....|....| ....|....| ....|....| ....|....| ....|....|
               455        465        475        485        495
NC_001542.  ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML042/17    ACTGACGAGA GAC------- ---------- ---------- ----------
Clustal Co  ******.*** ***

            ....|....| ....|....| ....|....| ....|....| ....|....|
               505        515        525        535        545
NC_001542.  TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML042/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               555        565        575        585        595
NC_001542.  TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML042/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               605        615        625        635        645
NC_001542.  TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML042/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               655        665        675        685        695
NC_001542.  GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
ML042/17    ---------- ---------- ---------- ---------- ----------
Clustal Co
```

```
             ....|....| ....|....| ....|....| ....|....| ....|....|
                705        715        725        735        745
NC_001542.   GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
ML042/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                755        765        775        785        795
NC_001542.   CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML042/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                805        815        825        835        845
NC_001542.   GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML042/17     ---------- ---------- ---------- ---------- ----------
Clustal Co
```

>Nucleotide alignment, Rabies isolate ML444/17nucleoprotein gene, partial cds

```
             ....|....| ....|....| ....|....| ....|....| ....|....|
                5          15         25         35         45
NC_001542.   ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML444/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                55         65         75         85         95
NC_001542.   AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML444/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                105        115        125        135        145
NC_001542.   AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML444/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                155        165        175        185        195
NC_001542.   GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML444/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                205        215        225        235        245
NC_001542.   GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML444/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                255        265        275        285        295
```

```
NC_001542.   AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML444/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                305        315        325        335        345
NC_001542.   AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML444/17     ---------- ---------- ---------- ---------- --CTATGGGA
Clustal Co                                                 ******.*

             ....|....| ....|....| ....|....| ....|....| ....|....|
                355        365        375        385        395
NC_001542.   TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML444/17     TCTTGATTGC AAGAGAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co   ** ******* *.**.*.*** ** ******* ******.*** *** ******



             ....|....| ....|....| ....|....| ....|....| ....|....|
                405        415        425        435        445
NC_001542.   ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML444/17     ATCAAGCGTA CGGATGTAGA GGGAAACTGG GCCCTGACAG GAGGTATGGA
Clustal Co   **.**.**** * ******** .**.** *** ** ******* **** *****

             ....|....| ....|....| ....|....| ....|....| ....|....|
                455        465        475        485        495
NC_001542.   ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML444/17     ACTGACGA-- ---------- ---------- ---------- ----------
Clustal Co   ******.*

             ....|....| ....|....| ....|....| ....|....| ....|....|
                505        515        525        535        545
NC_001542.   TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML444/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                555        565        575        585        595
NC_001542.   TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML444/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                605        615        625        635        645
NC_001542.   TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML444/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                655        665        675        685        695
NC_001542.   GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
ML444/17     ---------- ---------- ---------- ---------- ----------
```

```
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               705        715        725        735        745
NC_001542.  GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
ML444/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               755        765        775        785        795
NC_001542.  CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML444/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               805        815        825        835        845
NC_001542.  GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML444/17    ---------- ---------- ---------- ---------- ----------
Clustal Co
```

>Nucleotide alignment, Rabies isolate ML413/16 nucleoprotein gene, partial cds

```
            ....|....| ....|....| ....|....| ....|....| ....|....|
                5         15         25         35         45
NC_001542.  ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML413/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               55         65         75         85         95
NC_001542.  AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML413/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               105        115        125        135        145
NC_001542.  AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML413/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               155        165        175        185        195
NC_001542.  GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML413/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               205        215        225        235        245
NC_001542.  GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML413/16    ---------- ---------- ---------- ---------- ----------
Clustal Co
```

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                   255        265        275        285        295
NC_001542.      AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML413/16        ---------- ---------- ---------- ---------- ----------
Clustal Co

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   305        315        325        335        345
NC_001542.      AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML413/16        ---------- ---------- ---------- ---------- ----ATGGGA
Clustal Co                                                     ****.*

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   355        365        375        385        395
NC_001542.      TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML413/16        TCTTGATTGC AAGAAAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co      ** ******* *.****.*** ** ******* ******.*** *** ******


                ....|....| ....|....| ....|....| ....|....| ....|....|
                   405        415        425        435        445
NC_001542.      ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML413/16        ATCAAGCGTA CGGATGTAGA AGGAAACTGG GCTCTGACAG GAGGTATGGA
Clustal Co      **.**.**** * ******** ***.** *** ********** **** *****

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   455        465        475        485        495
NC_001542.      ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML413/16        ACTGACGAG- ---------- ---------- ---------- ----------
Clustal Co      ******.**

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   505        515        525        535        545
NC_001542.      TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML413/16        ---------- ---------- ---------- ---------- ----------
Clustal Co

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   555        565        575        585        595
NC_001542.      TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML413/16        ---------- ---------- ---------- ---------- ----------
Clustal Co

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   605        615        625        635        645
NC_001542.      TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML413/16        ---------- ---------- ---------- ---------- ----------
Clustal Co

                ....|....| ....|....| ....|....| ....|....| ....|....|
                   655        665        675        685        695
NC_001542.      GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
ML413/16        ---------- ---------- ---------- ---------- ----------
```

```
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               705        715        725        735        745
NC_001542.  GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
ML413/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               755        765        775        785        795
NC_001542.  CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML413/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               805        815        825        835        845
NC_001542.  GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML413/16    ---------- ---------- ---------- ---------- ----------
Clustal Co
```

>Nucleotide alignment, Rabies isolate ML021/16 nucleoprotein gene, partial cds

```
            ....|....| ....|....| ....|....| ....|....| ....|....|
                5         15         25         35         45
NC_001542.  ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML021/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
                55         65         75         85         95
NC_001542.  AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML021/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               105        115        125        135        145
NC_001542.  AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML021/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               155        165        175        185        195
NC_001542.  GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML021/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               205        215        225        235        245
NC_001542.  GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML021/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
```

```
                255        265        275        285        295
NC_001542.   AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML021/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                305        315        325        335        345
NC_001542.   AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML021/16     ---------- ---------- ---------- ---------- ---TATGGGA
Clustal Co                                                 *****.*

             ....|....| ....|....| ....|....| ....|....| ....|....|
                355        365        375        385        395
NC_001542.   TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML021/16     TCTTGATTGC AAGAAAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co   ** ******* *.****.*** ** ******* ******.*** *** ******


             ....|....| ....|....| ....|....| ....|....| ....|....|
                405        415        425        435        445
NC_001542.   ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML021/16     ATCAAGCGTA CGGATGTAGA AGGAAACTGG GCTCTGACAG GAGGTATGGA
Clustal Co   **.**.**** * ******** ***.** *** ********** **** *****

             ....|....| ....|....| ....|....| ....|....| ....|....|
                455        465        475        485        495
NC_001542.   ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML021/16     ACTGACGAGA GA-------- ---------- ---------- ----------
Clustal Co   ******.*** **

             ....|....| ....|....| ....|....| ....|....| ....|....|
                505        515        525        535        545
NC_001542.   TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML021/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                555        565        575        585        595
NC_001542.   TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML021/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                605        615        625        635        645
NC_001542.   TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML021/16     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                655        665        675        685        695
NC_001542.   GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
ML021/16     ---------- ---------- ---------- ---------- ----------
```

```
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               705        715        725        735        745
NC_001542.  GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
ML021/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               755        765        775        785        795
NC_001542.  CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML021/16    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               805        815        825        835        845
NC_001542.  GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML021/16    ---------- ---------- ---------- ---------- ----------
Clustal Co
```

>Nucleotide alignment, Rabies isolate ML410/17 nucleoprotein gene, partial cds

```
            ....|....| ....|....| ....|....| ....|....| ....|....|
                5         15         25         35         45
NC_001542.  ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML410/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               55         65         75         85         95
NC_001542.  AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML410/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               105        115        125        135        145
NC_001542.  AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML410/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               155        165        175        185        195
NC_001542.  GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML410/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               205        215        225        235        245
NC_001542.  GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML410/17    ---------- ---------- ---------- ---------- ----------
```

```
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                255        265        275        285        295
NC_001542.   AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML410/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                305        315        325        335        345
NC_001542.   AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML410/17     ---------- ---------- ---------- ---------C AGCTATGGGA
Clustal Co                                             * ********.*

             ....|....| ....|....| ....|....| ....|....| ....|....|
                355        365        375        385        395
NC_001542.   TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML410/17     TCTTGATTGC AAGAGAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co   ** ******* *.**.*.*** ** ******* ******.*** *** ******


             ....|....| ....|....| ....|....| ....|....| ....|....|
                405        415        425        435        445
NC_001542.   ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML410/17     ATCAACCGTA CAGATGTAGA AGGAAACTGG GCTC------ ----------
Clustal Co   **.**.**** *:******** ***.** *** ****

             ....|....| ....|....| ....|....| ....|....| ....|....|
                455        465        475        485        495
NC_001542.   ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML410/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                505        515        525        535        545
NC_001542.   TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML410/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                555        565        575        585        595
NC_001542.   TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML410/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                605        615        625        635        645
NC_001542.   TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML410/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
```

```
                655        665        675        685        695
NC_001542.  GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
ML410/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               705        715        725        735        745
NC_001542.  GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
ML410/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               755        765        775        785        795
NC_001542.  CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML410/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               805        815        825        835        845
NC_001542.  GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML410/17    ---------- ---------- ---------- ---------- ----------
Clustal Co
```

>Nucleotide alignment, Rabies isolate ML285/17 nucleoprotein gene partial cds

```
            ....|....| ....|....| ....|....| ....|....| ....|....|
                5          15         25         35         45
NC_001542.  ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML285/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               55         65         75         85         95
NC_001542.  AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML285/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               105        115        125        135        145
NC_001542.  AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML285/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               155        165        175        185        195
NC_001542.  GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML285/17    ---------- ---------- ---------- ---------- ----------
Clustal Co

            ....|....| ....|....| ....|....| ....|....| ....|....|
               205        215        225        235        245
NC_001542.  GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML285/17    ---------- ---------- ---------- ---------- ----------
```

```
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                255        265        275        285        295
NC_001542.   AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML285/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                305        315        325        335        345
NC_001542.   AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML285/17     ---------- ---------- ---------- ---------- ----TTGGGA
Clustal Co                                                  :***.*

             ....|....| ....|....| ....|....| ....|....| ....|....|
                355        365        375        385        395
NC_001542.   TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML285/17     TCTTGATTGC AAGAGAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co   ** ******* *.**.*.*** ** ******* ******.*** *** ******

             ....|....| ....|....| ....|....| ....|....| ....|....|
                405        415        425        435        445
NC_001542.   ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML285/17     ATCAAGCGTA CGGATGTAGA AGGAAACTGG GCTCTGACAG GAGGTATGGA
Clustal Co   **.**.**** * ******** ***.** *** ********** **** *****

             ....|....| ....|....| ....|....| ....|....| ....|....|
                455        465        475        485        495
NC_001542.   ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML285/17     ACTGACGAG- ---------- ---------- ---------- ----------
Clustal Co   ******.**

             ....|....| ....|....| ....|....| ....|....| ....|....|
                505        515        525        535        545
NC_001542.   TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML285/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                555        565        575        585        595
NC_001542.   TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML285/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                605        615        625        635        645
NC_001542.   TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML285/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                655        665        675        685        695
NC_001542.   GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
```

```
ML285/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                705        715        725        735        745
NC_001542.   GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
ML285/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                755        765        775        785        795
NC_001542.   CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML285/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                805        815        825        835        845
NC_001542.   GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML285/17     ---------- ---------- ---------- ---------- ----------
Clustal Co
```

>Nucleotide alignment, Rabies isolate ML216/17 nucleoprotein gene partial cds

```
            ....|....| ....|....| ....|....| ....|....| ....|....|
                 5          15         25         35         45
NC_001542.   ACGCTTAACA ACCAGATCAA AGAAAAAACA GACAGCGTCA ATGGCAGAGC
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                 55         65         75         85         95
NC_001542.   AAAAATGTAA CACCTCTACA ATGGATGCCG ACAAGATTGT ATTCAAAGTC
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                105        115        125        135        145
NC_001542.   AATAATCAGG TGGTCTCTTT GAAGCCTGAG ATTATCGTGG ATCAATATGA
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                155        165        175        185        195
NC_001542.   GTACAAGTAC CCTGCCATCA AAGATTTGAA AAAGCCCTGT ATAACTCTAG
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                205        215        225        235        245
NC_001542.   GAAAGGCTCC CGATTTAAAT AAAGCATACA AGTCAGTTTT ATCATGCATG
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co
```

```
             ....|....| ....|....| ....|....| ....|....| ....|....|
                255        265        275        285        295
NC_001542.   AGCGCCGCCA AACTTGATCC TGACGATGTA TGTTCCTATT TGGCGGCGGC
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                305        315        325        335        345
NC_001542.   AATGCAGTTT TTTGAGGGGA CATGTCCGGA AGACTGGACC AGCTATGGAA
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                355        365        375        385        395
NC_001542.   TCGTGATTGC ACGAAAAGGA GATAAGATCA CCCCAGGTTC TCTGGTGGAG
ML216/17     --TTGATTGC AAGAAAGGGA GACAAGATCA CCCCAGATTC TCTTGTGGAG
Clustal Co      ******* *.****.*** ** ******* ******.*** *** ******

             ....|....| ....|....| ....|....| ....|....| ....|....|
                405        415        425        435        445
NC_001542.   ATAAAACGTA CTGATGTAGA AGGGAATTGG GCTCTGACAG GAGGCATGGA
ML216/17     ATCAACCGTA CGGATGTAGA AGGAAACTGG GCTCTGACAG GAGGTATGGA
Clustal Co   **.**.**** * ******** ***.** *** ********** **** *****

             ....|....| ....|....| ....|....| ....|....| ....|....|
                455        465        475        485        495
NC_001542.   ACTGACAAGA GACCCCACTG TCCCTGAGCA TGCGTCCTTA GTCGGTCTTC
ML216/17     AC-------- ---------- ---------- ---------- ----------
Clustal Co   **

             ....|....| ....|....| ....|....| ....|....| ....|....|
                505        515        525        535        545
NC_001542.   TCTTGAGTCT GTATAGGTTG AGCAAAATAT CCGGGCAAAG CACTGGTAAC
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                555        565        575        585        595
NC_001542.   TATAAGACAA ACATTGCAGA CAGGATAGAG CAGATTTTTG AGACAGCCCC
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                605        615        625        635        645
NC_001542.   TTTTGTTAAA ATCGTGGAAC ACCATACTCT AATGACAACT CACAAAATGT
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                655        665        675        685        695
NC_001542.   GTGCTAATTG GAGTACTATA CCAAACTTCA GATTTTTGGC CGGAACCTAT
```

```
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                705        715        725        735        745
NC_001542.   GACATGTTTT TCTCCCGGAT TGAGCATCTA TATTCAGCAA TCAGAGTGGG
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                755        765        775        785        795
NC_001542.   CACAGTTGTC ACTGCTTATG AAGACTGTTC AGGACTGGTG TCATTTACTG
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co

             ....|....| ....|....| ....|....| ....|....| ....|....|
                805        815        825        835        845
NC_001542.   GGTTCATAAA ACAAATCAAT CTCACCGCTA GAGAGGCAAT ACTATATTTC
ML216/17     ---------- ---------- ---------- ---------- ----------
Clustal Co
```

Appendix 8: List of homologue genes

Isolate	Homologue gene	% nucleotide identity	GenBank accession No	Country of origin
ML026/16				
688/2011	Rabies nucleoprotein gene	99.02%	KP976118.1	Mali
149/2011	Rabies nucleoprotein gene	98.04%	KP976119.1	Mali
1929MAU/06	Rabies nucleoprotein gene	98.04%	EU514578.1	Mauritania
1923MAU/05	Rabies nucleoprotein gene	98.04%	EU514577.1	Mauritania
1922MAU/05	Rabies nucleoprotein gene	98.04%	EU514576.1	Mauritania
1920MAU/05	Rabies nucleoprotein gene	98.04%	EU514575.1	Mauritania
139BF	Rabies nucleoprotein gene	98.04%	EU478515.1	Burkina Faso
36BF	Rabies nucleoprotein gene	98.04%	EU478502.1	Burkina Faso
93012MAU	Rabies nucleoprotein gene	97.06%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	97.06%	KX148236.1	Mauritania
1923MAU/05	Rabies nucleoprotein gene	98.35%	EU514577.1	Mauritania
1922MAU/05	Rabies nucleoprotein gene	98.35%	EU514576.1	Mauritania
ML042/17				
688/2011	Rabies nucleoprotein gene	99.17%	KP976118.1	Mali
149/2011	Rabies nucleoprotein gene	98.35%	KP976119.1	Mali
1929MAU/06	Rabies nucleoprotein gene	98.35%	EU514578.1	Mauritania
1923MAU/05	Rabies nucleoprotein gene	98.35%	EU514577.1	Mauritania
1922MAU/05	Rabies nucleoprotein gene	98.35%	EU514576.1	Mauritania
1920MAU/05	Rabies nucleoprotein gene	98.35%	EU514575.1	Mauritania
139BF	Rabies nucleoprotein gene	98.35%	EU478515.1	Burkina Faso
36BF	Rabies nucleoprotein gene	98.35%	EU478502.1	Burkina Faso
93012MAU	Rabies nucleoprotein gene	97.52%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	97.52%	KX148236.1	Mauritania
93003SEN	Rabies nucleoprotein gene	100.00%	KX148238.1	Senegal
93012MAU	Rabies nucleoprotein gene	100.00%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	100.00%	KX148236.1	Mauritania

86036HAV	Rabies nucleoprotein gene	100.00%	KX148234.1	Burkina Faso
ML444/17				
688/2011	Rabies nucleoprotein gene	99.14%	KP976118.1	Mali
149/2011	Rabies nucleoprotein gene	98.28%	KP976119.1	Mali
1929MAU/06	Rabies nucleoprotein gene	98.28%	EU514578.1	Mauritania
1923MAU/05	Rabies nucleoprotein gene	98.28%	EU514577.1	Mauritania
1922MAU/05	Rabies nucleoprotein gene	98.28%	EU514576.1	Mauritania
1920MAU/05	Rabies nucleoprotein gene	98.28%	EU514575.1	Mauritania
139BF	Rabies nucleoprotein gene	98.28%	EU478515.1	Burkina Faso
36BF	Rabies nucleoprotein gene	98.28%	EU478502.1	Burkina Faso
93005SSEN	Rabies nucleoprotein gene	97.41%	KX148239.1	Senegal
93003SEN	Rabies nucleoprotein gene	97.41%	KX148238.1	Senegal
ML921/16				
688/2011	Rabies nucleoprotein gene	100.00%	KP976118.1	Mali
149/2011	Rabies nucleoprotein gene	99.17%	KP976119.1	Mali
1929MAU/06	Rabies nucleoprotein gene	99.17%	EU514578.1	Mauritania
1923MAU/05	Rabies nucleoprotein gene	99.17%	EU514577.1	Mauritania
1922MAU/05	Rabies nucleoprotein gene	99.17%	EU514576.1	Mauritania
1920MAU/05	Rabies nucleoprotein gene	99.17%	EU514575.1	Mauritania
139BF	Rabies nucleoprotein gene	99.17%	EU478515.1	Burkina Faso
36BF	Rabies nucleoprotein gene	99.17%	EU478502.1	Burkina Faso
93012MAU	Rabies genome	98.33%	KX148237.1	Mauritania
93011MAU	Rabies genome	98.33%	KX148236.1	Mauritania
ML413/16				
93005SEN	Rabies nucleoprotein gene	100.00%	KX148239.1	Senegal
93003SEN	Rabies nucleoprotein gene	100.00%	KX148238.1	Senegal
93012MAU	Rabies nucleoprotein gene	100.00%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	100.00%	KX148236.1	Mauritania
86036HAV	Rabies nucleoprotein gene	100.00%	KX148234.1	Burkina Faso
92037CI	Rabies nucleoprotein gene	100.00%	KX148232.1	Ivory Coast

352/2007	Rabies nucleoprotein gene	100.00%	KP976130.1	Mali
100/2013	Rabies nucleoprotein gene	100.00%	KP976128.1	Mali
357/2011	Rabies nucleoprotein gene	100.00%	KP976127.1	Mali
146/2008	Rabies nucleoprotein gene	100.00%	KP976126.1	Mali
93011MAU	Rabies nucleoprotein gene	100.00%	KX148236.1	Mauritania
86036HAV	Rabies nucleoprotein gene	100.00%	KX148234.1	Burkina Faso
ML021/16				
93005SEN	Rabies nucleoprotein gene	100.00%	KX148239.1	Senegal
93003SEN	Rabies nucleoprotein gene	100.00%	KX148238.1	Senegal
93012MAU	Rabies nucleoprotein gene	100.00%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	100.00%	KX148236.1	Mauritania
86036HAV	Rabies nucleoprotein gene	100.00%	KX148234.1	Burkina Faso
92037CI	Rabies nucleoprotein gene	100.00%	KX148232.1	Ivory Coast
357/2007	Rabies nucleoprotein gene	100.00%	KP976130.1	Mali
100/2013	Rabies nucleoprotein gene	100.00%	KP976128.1	Mali
357/2011	Rabies nucleoprotein gene	100.00%	KP976127.1	Mali
146/2008	Rabies nucleoprotein gene	100.00%	KP976126.1	Mali
ML023/16				
93005SEN	Rabies nucleoprotein gene	93.48%	KX148239.1	Senegal
93003SEN	Rabies nucleoprotein gene	93.48%	KX148238.1	Senegal
93012MAU	Rabies nucleoprotein gene	93.48%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	93.48%	KX148236.1	Mauritania
86036HAV	Rabies nucleoprotein gene	93.48%	KX148234.1	Burkina Faso
92037CI	Rabies nucleoprotein gene	93.48%	KX148232.1	Ivory Coast
352/2007	Rabies nucleoprotein gene	93.48%	KP976130.1	Mali
100/2013	Rabies nucleoprotein gene	93.48%	KP976128.1	Mali
357/2011	Rabies nucleoprotein gene	93.48%	KP976127.1	Mali
146/2008	Rabies nucleoprotein gene	93.48%	KP976126.1	Mali
93012MAU	Rabies nucleoprotein gene	95.70%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	95.70%	KX148236.1	Mauritania

ML028/16				
120MAU	Rabies nucleoprotein gene	96.77%	EU853624.1	Mauritania
93005SEN	Rabies nucleoprotein gene	95.70%	KX148239.1	Senegal
93003SEN	Rabies nucleoprotein gene	95.70%	KX148238.1	Senegal
93012MAU	Rabies nucleoprotein gene	95.70%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	95.70%	KX148236.1	Mauritania
86036HAV	Rabies nucleoprotein gene	95.70%	KX148234.1	Burkina Faso
92037CI	Rabies nucleoprotein gene	95.70%	KX148232.1	Ivory Coast
352/2007	Rabies nucleoprotein gene	95.70%	KP976130.1	Mali
100/2013	Rabies nucleoprotein gene	95.70%	KP976128.1	Mali
357/2011	Rabies nucleoprotein gene	95.70%	KP976127.1	Mali
ML410/17				
9120MAU	Rabies nucleoprotein gene	97.89%	EU853624.1	Mauritania
93005SEN	Rabies nucleoprotein gene	96.84%	KX148239.1	Senegal
93003SEN	Rabies nucleoprotein gene	96.84%	KX148238.1	Senegal
93012MAU	Rabies nucleoprotein gene	96.84%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	96.84%	KX148236.1	Mauritania
86036HAV	Rabies nucleoprotein gene	96.84%	KX148234.1	Burkina Faso
92037	Rabies nucleoprotein gene	96.84%	KX148232.1	Ivory Coast
352/2007	Rabies nucleoprotein gene	96.84%	KP976130.1	Mali
100/2013	Rabies nucleoprotein gene	96.84%	KP976128.1	Mali
357/2011	Rabies nucleoprotein gene	96.84%	KP976127.1	Mali
93011MAU	Rabies nucleoprotein gene	99.12%	KX148236.1	Mauritania
86036HAV	Rabies nucleoprotein gene	99.12%	KX148234.1	Burkina Faso
ML216/17				
93005SEN	Rabies nucleoprotein gene	99.00%	KX148239.1	Senegal
93003SEN	Rabies nucleoprotein gene	99.00%	KX148238.1	Senegal
93012MAU	Rabies nucleoprotein gene	99.00%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	99.00%	KX148236.1	Mauritania
86036HAV	Rabies nucleoprotein gene	99.00%	KX148234.	Burkina Faso

92037CI	Rabies nucleoprotein gene	99.00%	KX148232.1	Ivory Coast
352/2007	Rabies nucleoprotein gene	99.00%	KP976130.1	Mali
100/2013	Rabies nucleoprotein gene	99.00%	KP976128.1	Mali
357/2011	Rabies nucleoprotein gene	99.00%	KP976127.1	Mali
146/2008	Rabies nucleoprotein gene	99.00%	KP976126.1	Mali
ML285/17				
93005SEN	Rabies nucleoprotein gene	99.12%	KX148239.1	Senegal
93003SEN	Rabies nucleoprotein gene	99.12%	KX148238.1	Senegal
93012MAU	Rabies nucleoprotein gene	99.12%	KX148237.1	Mauritania
93011MAU	Rabies nucleoprotein gene	99.12%	KX148236.1	Mauritania
86036HAV	Rabies nucleoprotein gene	99.12%	KX148234.1	Burkina Faso
92037CI	Rabies nucleoprotein gene	99.12%	KX148232.1	Ivory Coast
352/2007	Rabies nucleoprotein gene	99.12%	KP976130.1	Mali
100/2013	Rabies nucleoprotein gene	99.12%	KP976128.1	Mali
357/2011	Rabies nucleoprotein gene	99.12%	KP976127.1	Mali
146/2008	Rabies nucleoprotein gene	99.12%	KP976126.1	Mali