

EPIDEMIOLOGY OF RABIES IN ETHIOPIA AND EVALUATION OF THE RAPID IMMUNODIAGNOSTIC TEST KIT FOR RABIES DIAGNOSIS

BY

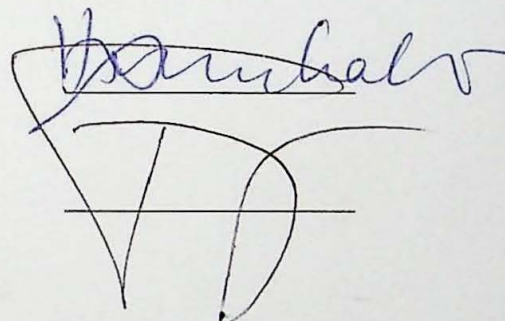
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A handwritten signature in blue ink, appearing to read 'Reto Tesfaye', is written over a horizontal line. Below this line is another horizontal line, and below that is a large, stylized, handwritten 'V' or 'D' shape.

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LIST OF ABBREVIATIONS

AD	After Death of Mrs. Christ
AFRFS	The African Rivers Export Forum
BC	Bayer Clinic
CDC	Center for Disease Control
CFIA	Canada Food Inspection Agency
CSPH	Center for Food Security and Public Health
CI	Confidence Interval
CNS	Central Nervous System
CMA	Central Medical Agency
DAAD	Deutscher Akademischer Austausch Dienst
DIRT	Direct Indirect Immunological Test
EHLV	European Herpetological Society
EHNRI	European Health and Nutrition Research Institute
ELISA	Enzyme-linked Immunosorbent Assay
EU	European Union
FAT	Fluorescent Antibody Test
FTC	Fluorescent Antibody Test
ELI	Ernst-Löwenstein-Institut
EU	European Union
ICIV	International Committee on Taxonomy of Viruses
IR	International River
EL	Enzyme
LD	Laboratory
MMA	Molecular Microbiology
MT	Mouse Tissue Culture
mRNA	Messenger Ribonucleic Acid
WHO	World Health Organization
FBS	Fetal Bovine Serum
PCR	Polymerase Chain Reaction

LIST OF ABBREVIATIONS

AD	After Birth of Jesus Christ
AfroREB	The African Rabies Expert Bureau
BC	Before Christ
CDC	Center for Disease Control
CFIA	Canadian Food Inspection Agency
CFSPH	Center for Food Security and Public Health
CI	Confidence Interval
CNS.....	Central Nervous System
CSA.....	Central Statistical Agency
DAAD	Deutscher Akademischer Austausch Dienst
dRIT	Direct Immuno-histochemical Test
EBLV	European Bat <i>Lyssavirus</i>
EHNRI	Ethiopian Health and Nutrition Research Institute
ELISA	Enzyme-linked Immunosorbent Assay
EU	European Union
FAT.....	Fluorescent Antibody Test
FITC.....	Fluorescein Isothiocyanate
FLI	Friedrich-Loeffler-Institute
FU	Freie Universität
ICTV	International Committee on Taxonomy of Viruses
IU	International Unit
Kb.....	Kilobyte
LD	Lethal Dose
MAbs	Monoclonal Antibody
MIT	Mouse Inoculation Test
mRNAs	Messenger Ribonucleic Acid
OIE.....	World organization for animal health
PBS	Phosphate-buffered Saline
PCR.....	Polymerase Chain Reaction

PEP.....	Post-exposure Prophylaxis
RABV	Rabies Virus
RIDT	Rapid Immunodiagnostic Test
RIG.....	Rabies Immunoglobulins
RNA	Ribonucleic Acid
RTCIT	Rabies Tissues Culture Inoculation Test
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
Se	Sensitivity
SEARG	Southern and Eastern African Rabies Group
SNNP	Southern Nations, Nationalities and People
Sp	Specificity
UAACP	Urban Agriculture Extension Core Process
UN OCHA.....	United Nations Office for the Coordination of Humanitarian affairs
USA	United States of America
VN.....	Virus Neutralization
WHO.....	World Health Organization

ABSTRACT

The aims of this study were to investigate the epidemiology of rabies in humans and animals and to evaluate the diagnostic performances of the Anigen Rapid Rabies Ag Test Kit. Retrospective data of 2008-2011 and 2009/10 were used to analyze rabies in animals and humans, respectively, mainly in and around Addis Ababa. A questionnaire was developed and used to get additional epidemiological information during the study period (March to September, 2011). A total of 935 brain samples were examined during 2008-2011. Of this total number, 77.6% (N=726) samples were positive for rabies. The highest proportion, 87.2% (N=633) of the positive cases was recorded in dogs followed by cats, 5.1% (N=37). The frequencies of occurrence of rabies in other species were very limited. This showed that dogs remain the dominant species in maintenance and transmission of rabies to humans and other animals in Ethiopia. Further analysis of characteristics of rabid dogs showed that owned, adult, non-vaccinated, male dogs were most frequently diagnosed positive for rabies virus. Furious form of rabies was the most commonly reported form of the disease in rabid dogs. Rabies cases were reported throughout the year with slight variations among the months of the year. Its occurrence showed variation among administrative regions depending on their distance from the laboratory. A total of 2345 people received post-exposure prophylaxis in 2010/2011 against rabies due to exposure to rabid or rabies suspected animals and humans. The greater proportion of post-exposure prophylaxis was given to humans exposed to rabid or rabies suspected dogs. Fermi type vaccine was the most common, 81% (1887/2337), vaccine type used for post-exposure prophylaxis. The majority, 72.3% (1653/2286), of the post-exposure prophylaxis was given to humans bitten by animals with an unknown rabies status. The mean number of exposed humans per rabid animal was about 3 with a range of 0 to 15 persons. The gold standard test for rabies diagnosis is the fluorescent antibody test, requiring a fluorescent microscope and additional laboratory equipment. Recently, a rapid and easy immunodiagnostic test was developed which needs no specialized laboratory equipment and needs less expertise. Anigen Rapid Rabies Ag Test Kit was evaluated using 115 brain samples collected from dogs, cats and bovines. The sensitivity and specificity of the rapid immunodiagnostic test were 96.5 (95% CI: 90.0 – 99.3%) and 100% (95% CI: 88.4 – 100%), respectively compared to the fluorescent antibody test. This test could be useful for field use particularly in rabies surveillance and also for targeted post-exposure prophylaxis where the

standard test is not available. Strategy for control and elimination of rabies should be in place and implemented in Ethiopia. Furthermore, the rapid immunodiagnostic test kit should be evaluated at larger scale using samples from different species of animals. Strategies for the application of the rapid immunodiagnostic test for the improvement of rabies surveillance and subsequent post-exposure prophylaxis should also be studied.

Key words: Anigen Rapid Rabies Ag Test Kit, Epidemiology, Ethiopia, Fluorescent antibody test, Rabies.

1. INTRODUCTION

Rabies is an acute viral disease of the central nervous system (CNS) that affects humans and other mammals but is most common in carnivores. It is almost invariably fatal once the clinical signs develop (Radostits *et al.*, 2007; CFSPH, 2009). The causative agent of rabies is a negative stranded RNA-virus belonging to the genus *Lyssavirus*, family *Rhabdoviridae* of the order *Mononegavirales*. The genus *Lyssavirus* includes 11 different genotypes and further 1 genotype has been recently proposed to be included in the genus (Kuzmin *et al.*, 2010; ICTV, 2011).

Depending on the animal species which is present locally as a main vector and reservoir of infection, wildlife and dog mediated rabies transmission cycles have been differentiated. Wildlife rabies is characterized by the establishment of transmission cycles in one or more carnivorous wildlife species. Bat species have been incriminated as a rabies virus reservoir and vectors, too. The disease is transmitted to humans and other mammalian species mainly through the bite of rabid carnivores and/or by the bite of bats. Dog mediated rabies is by far more important from public health view point. It is sustained by dogs and cats, whereby in 90% dogs are the main vectors. The final links of the chain of infection are other domestic animals, especially cattle and humans (Seifert, 1996; WHO, 2011a).

Rabies is widely distributed throughout the world and is present in all continents except Antarctica. Dog mediated rabies is the most important form in Asia and Africa. Wildlife rabies is endemic in Asia, Africa, the Americas, Australia and most of Europe (Warrell, 2004). Each year, rabies kills large numbers of humans, dogs, cattle and other animal species. But the exact burden of the disease is not known. In humans, it is estimated that rabies causes about 55, 000 deaths annually in Asia and Africa. People most at risk of rabies live in rural areas of Africa and Asia. An estimated 31, 000 people die from dog rabies in Asia each year. In Africa the annual death toll is 24, 000 (Knobel *et al.*, 2005). Rabies is most common in children aged less than 15 years; on average 40 % of post-exposure prophylaxis regimens are given to children aged 5–14 years. Children often play with animals and are less likely to report bites or scratches. In addition, dog rabies was shown to be responsible for more than 15 million courses of post-exposure treatment to prevent the onset of the disease. The economic burden of rabies in the developing countries

also takes a large toll (WHO, 2011b). It has thus been clear that rabies is one of the most feared public health concerns in Asia and Africa.

In Ethiopia, rabies is one of the most severe infectious diseases. It has been diagnosed from various parts of the country. In addition to classical rabies virus, two rabies related viruses, Mokola virus (genotype 3) and Lagos bat virus (genotype 2) have been isolated from domestic animals in Ethiopia (Mebatsion *et al.*, 1992). Dog is the species most responsible for human exposure, with over 92% of the human cases and vaccinations due to the bite of rabid or rabies suspected dogs (Yimer *et al.*, 2002). Cats are known to be the source of infections next to dogs. The disease is also diagnosed in many different species of domestic and wild animals including cattle, sheep, goats, jackals, mongoose, cerval cat, cheetah, hyena, monkey and red fox (Fekadu, 1982; Tefera *et al.*, 2002; Randall *et al.*, 2004; Newayesilassie *et al.*, 2004; Ali *et al.*, 2010; Deressa *et al.*, 2010). Many people receive anti-rabies post-exposure treatments annually due to the wide spread nature of dog rabies. Annual post-exposure prophylaxis (PEP) for human rabies ranges between 1026 and 1580 in Addis Ababa (capital city of Ethiopia) alone during the period of 2001–2009. Within the same period 386 deaths of humans with annual range of 35 to 58 was recorded (Deressa *et al.*, 2010). There is, however, scarcity of epidemiological information to scale the magnitude of rabies among both in animals and humans in the country (Yimer *et al.*, 2002).

Several laboratory techniques can be used for rabies diagnosis. However, the most commonly used and the gold-standard diagnostic test is the Fluorescent Antibody Test (FAT) which detects virus antigen in the brain using fluorescent labeled anti-rabies virus antibodies. This test is recommended by both WHO and OIE (WHO, 2005; OIE, 2008). Other methods for diagnosis and research include: the Mouse Inoculation Test (MIT), Rabies Tissues Culture Inoculation Test (RTCIT), Enzyme-linked Immunosorbent Assays (ELISAs), Electron Microscopy, Histological examination, Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Immunohistochemistry (David *et al.*, 2002; McElhinney *et al.*, 2008; OIE, 2008; Fooks *et al.*, 2009). However, these methods have limited utility under field conditions because they are time consuming and need highly qualified personnel with higher technical skill and equipment (Helmick, 1983; Fishbein *et al.*, 1991).

Rabies diagnostic laboratory under the auspices of Ethiopian Health and Nutrition Research Institute (EHNRI) is the only laboratory in Ethiopia that performs rabies diagnosis from brain samples of animals and humans. Fluorescent antibody test has been the method of choice for detection of rabies virus in the specimen. The use of the fluorescent-antibody assay in different parts of the country is limited by the costs of acquiring and maintaining a fluorescent microscope, its need for trained expertise and equipment (Ali *et al.*, 2010; Deressa *et al.*, 2010). The absence of a confirmatory test can result in the inappropriate management of animal bite injuries, with human deaths a potential consequence due to delays in rabies PEP and unnecessary administration of PEP. The latter is a particular concern, given the scarcity and costs of human rabies vaccines and immunoglobulin in many parts of the world. In addition, the true public health impact of rabies cannot be determined and this contributes for lack of political commitment for its control (WHO, 2005).

Recently Rapid Immunodiagnostic Test (RIDT) for detecting rabies virus in brain tissue and saliva has been developed. This test is based on the principles of immunochromatography (Kang *et al.*, 2007). This lateral flow strip test is a one-step test that facilitates low-cost and rapid identification of viruses (Zhang *et al.*, 2009). Validation studies by kang *et al.* (2007) using 51 clinical samples and 4 isolates of rabies virus showed that the RIDT had a sensitivity of 91.7% and specificity of 100% (95.8% CI). Markotter *et al.* (2009) also reported 100% sensitivity and specificity in his study of African isolates. This method of rabies diagnostic technique was shown to be not cross-reactive with non-rabies virus microbes (Kang *et al.*, 2007). Furthermore, this test was shown to be a very simple test that can be performed in less than 10 min without any specialized equipment, infrastructure, or high level of training. There are no draw backs to field use such as cold storage, since the test kit contains everything required for the diagnosis and is stable at ambient temperatures (Kang *et al.*, 2007; Markotter *et al.*, 2009). The use of such tests would be crucial to understand the epidemiological situation of rabies in countries like Ethiopia where there has been no laboratories to diagnose rabies in many parts of the country.

Suspected samples (mainly heads of dead or killed animals) have to be submitted to EHNRI for diagnosis. The diagnostic accuracy may be affected by improper sample storage and shipment. Lack of sensitive, specific, simple and inexpensive diagnostic tests for field use has caused most

of the rabies cases go undiagnosed in the majority of the rural areas of Ethiopia. This has become bottle neck for the better understanding of the epidemiological patterns of the disease. Better knowledge of the epidemiology is pre-requisite for effective prevention and control programs. Therefore, a rapid and simple diagnostic tool needs to be validated for the diagnosis of rabies cases in Ethiopia, where rabies diagnostic laboratory is almost unavailable. In addition to this, epidemiological information including characteristics of the dominant maintenance and transmitter of rabies should be identified to see their contribution in the occurrence of the disease. The knowledge of distribution of rabies at sub-city and zone level is important in better understanding of rabies distribution in the study area. Furthermore, identifying category of animals responsible for PEP is also important in planning to enhance targeted PEP. In general, analysis of retrospective and current epidemiological data is useful to have better understanding of current rabies situation and for any intervention program in the area in attempt to control the disease.

General objectives:

To investigate the epidemiology of rabies in animals and humans and evaluate the diagnostic performances of the Anigen Rapid Rabies Ag Test Kit

Specific objectives:

- To determine spatial and temporal distribution of rabies confirmed cases.
- To identify the distribution of rabies among species of animals and determine the characteristics of the dominant species.
- To identify species and situation of animals responsible for PEP, type of vaccines used in humans and quantify number of humans exposed to a rabid case.
- To evaluate the diagnostic performances of the Anigen Rapid Rabies Ag Test Kit using FAT as a reference test in Ethiopian condition.

2. LITERATURE REVIEW

2.1. History

Rabies in dogs and the importance of saliva in its transmission may have been recognized in pharaonic times and in China seven centuries before Christ (BC). Aristotle (322 BC) described rabies in animals but seemed to deny that humans could be infected or could die from the disease (Warrell, 2004). In the 1st century AD, Cornelius Celsus coined the term “hydrophobia”, which referred to the fear of water, and he described a case of human rabies. Girolamo Fracastoro, in his treatise “The Incurable Wound” dated 1584, described how humans are not only susceptible to rabies, but also that they invariably die once clinical symptoms occur. After Fracastoro, rabies research was relatively quiescent until the 1800s (Fu, 1997). In 1793, John Hunter suggested that the transmission of rabies should be studied by inoculating saliva from rabid animals and humans into dogs and that attempts should be made to inactivate the ‘poison’ in the saliva. These ideas may have inspired the animal experiments on transmission by Zinke, and Magendie and Breschet (Warrell, 2004). Zinke infected rabbits and chickens with the saliva of a rabid dog, and then Francois Magendie and Gilbert Breschet transmitted rabies to dogs from the saliva of a human patient (Fu, 1997).

Galtier (1979) found that rabbits could be infected with rabies virus and were more convenient experimental animals than dogs. In 1881, he first demonstrated specific immunization against the disease (Warrell, 2004). Pasteur adopted the use of rabbits in his studies of rabies, beginning in the 1880s. He was the first to recognize that the major site of infection was the CNS. Pasteur was able to protect dogs from challenge by immunizing them with a virus attenuated in desiccated rabbit spinal cord and in 1885 he used this as a vaccine for the first time in Joseph Meister, who had been severely bitten by rabid dogs (Baer, 2007). In 1903 Negri described his diagnostic inclusion body which allowed the post-mortem laboratory diagnosis of rabies. The introduction of the more specific and sensitive immunofluorescence method in 1958 has since replaced Seller’s stain for Negri bodies. Remlinger, in 1903, showed that rabies was caused by a filterable agent. It was not until 1936 that the size of the virus was established, and it was first seen by electron microscopy in 1962 (Warrell, 2004).

Early nineteenth-century travelers, the first to discuss the incidence of rabies in Ethiopia, suggested that rabies was fairly widespread. Thus in the 1830s the German explorer Eduard Riippell, who reports seeing a mad dog at Adwa, goes on to declare that rabies was common in Ethiopia. The French traveler, Rochet d'Hericourt a decade or so later also indicated the existence of rabies in the country. At about the same time the careful French investigator, Antoine d'Abbadie, noted that 'hydrophobia is not rare'. The Frenchman's belief was that the disease was more widespread than in other Middle Eastern countries (Pankhurst, 1970).

The first recorded rabies outbreak occurred in Addis Ababa in August 1903, and is reported by Lincoln De Castro, a physician at the Italian legation. He indicated that the outbreak lasted for a few months and then disappeared. Later writers in 1940, towards the end of the fascist period, also described that rabies was much diffused in Ethiopia among both wild and domestic carnivores. Arne Barkhuus, an early post-war member of a United States technical project mission, declared in 1947 that 'the number of rabid dogs in Ethiopia was 'very large', though 'little' was known as to the number of people actually bitten (Pankhurst, 1970). From 1964 to 1975 a total of 1597 cases of animal rabies were diagnosed at EHNRI. During this period deaths of 488 people due to rabies was recorded (Fekadu, 1982). A total of 885 animals were confirmed positive from animals presented to rabies diagnostic laboratory (EHNRI) during 1979 to 1987. Reported number of deaths of humans was 320 during this period (Tefera *et al.*, 2002). A retrospective review on situation of rabies in Ethiopia (1990-2000) showed 2171 confirmed positive cases and 322 reported human deaths due to rabies (Yimer *et al.*, 2002). Furthermore, a review from 2000 to 2009 has indicated 2593 confirmed positive cases and 386 fatal human cases (Deressa *et al.*, 2010). Rabies has been recorded in wide range of domestic and wild animals in the country. However, it has been indicated that dogs are the principal recognized vector of rabies. The reported cases of animals and humans reflect mainly rabies situation in and around 40 km radius of Addis Ababa, the capital city of Ethiopia (Deressa *et al.*, 2010). The distribution of the disease in other parts of the country has not been indicated.

2.2. Etiology

Rabies virus belongs to the genus *Lyssavirus* within the *Rhabdoviridae* family and order *Mononegavirales*. The virus is bullet-shaped, measuring 180 nm in length and 80 nm in diameter. It contains an envelope with short spikes (6 nm to 7 nm in length). The virus has a negative-sense single-stranded RNA genome of approximately 12 kb. The five structural proteins of the virus particle (virion) include a nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase or large protein (L). The virus attaches to the cell through the G-glycoprotein. Antibodies against this protein exhibit neutralizing activity and studies of these antibodies have revealed several antigenic variants of rabies virus (Bjorvatn and Haukenes, 2002).

The genus *Lyssavirus* comprises rabies virus (RABV) and closely related viruses, including Mokola virus (MOKV), Lagos bat virus (LBV), Duvenhage virus (DUVV), European bat viruses 1 (EBLV-1), European bat viruses 2 (EBLV-2) and Australian bat *lyssavirus* (ABL). Four other *lyssaviruses* have been incorporated into the genus: Aravan virus (ARAV), Khujand virus (KHUV), Irkut virus (IRKV), and west Caucasian bat virus (WCBV) (Kuzmin *et al.*, 2008; ICTV, 2011). A new lyssavirus, named Shimoni bat virus (SHIBV), was isolated from the brain of a dead Commerson's leaf-nosed bat (*Hipposideros commersoni*), found in a cave in the coastal region of Kenya. Genetic distances and phylogenetic reconstructions, demonstrated that SHIBV is an independent species within phylogroup II of the *Lyssavirus* genus, most similar to Lagos bat virus (LBV) (Kuzmin *et al.*, 2010).

Isolation and characterization of 115 isolates of rabies virus from different animal species in Ethiopia indicated that 113 isolates were classic street rabies viruses (serotype 1). An isolate of feline origin (Eth-16) was a Mokola virus and another isolate of dog origin (Eth-58) was Lagos bat virus (Mebatsion *et al.*, 1992). In 2003, rabies virus was diagnosed from 13 of 15 wolf brain samples collected from Bale Mountain, Ethiopia. The nucleoprotein (N) gene sequences were identical for all 13 samples. Comparison with other N gene demonstrated that the virus belongs to the overall group associated with domestic dogs in different regions throughout northern and central Africa. Case trace back in this outbreak suggests rabies may have been brought into wolf

habitat by an unvaccinated immigrant dog accompanying people and livestock searching for seasonal grazing (Randall *et al.*, 2004).

2.2.1. Virus replication

Viral entry into its host cell occurs via fusion of the viral envelope with the cell membrane; all replication steps occur in the cytoplasm (Murphy *et al.*, 1999). The *lyssavirus* RNA genome, which is a non-segmented, single-strand RNA molecule with negative-sense polarity cannot be translated directly into protein (i.e. it is not infectious). The first event in the process of rabies virus replication, therefore, is to transcribe (copy) the genome RNA to produce complementary (positive strand) monocistronic mRNAs representing each of the viral genes or cistrons in the genome. The monocistronic mRNAs are then translated to produce the viral proteins. Translation, the process of building a protein from the codons in the RNA message, involves the cellular ribosome complex, initiation and elongation factors and transfer RNA charged with individual amino acids. The organization and specific features of the RABV genome RNA are critical to the transcription and replication process (Wunner, 2007).

2.2.2. Resistance to physical and chemical agents

Rabies virus is heat-labile and is susceptible to degradation by radiation, strong acids, alkalis, most disinfectants, lipid solvents, and anionic solvents (Sommardahl, 2004). The virus may persist in infected brain tissue for up to 10 days at room temperature (Zee, 1999). Rabies virus in a PBS buffer solution is rapidly inactivated by heat: at 56 °C the half-life is <1 minute; at 37°C the half-life is prolonged to about 3 hours in moist conditions and at 4 °C to 3–4 days experimentally. The stabilizing presence of serum or other buffer solutions enhances survival. The lipid coat of the virion renders it vulnerable to disruption by detergents such as simple 1% soap solution. Ethanol (45%), iodine solutions (with 1/10 000 available iodine), 3% sodium hydroxide, 1% benzalkonium chloride, 3% formalin, neat chloroform and acetone all inactivate the virus. Hypochlorite and glutaraldehyde solutions are suitable for laboratory use with the normal precautions, but phenol is not an effective virucidal compound. Although benzylkonium chloride and other quaternary ammonium compounds are virucidal experimentally, they are not recommended for cleaning rabies-infected wounds at the concentrations in normal clinical use

because they are inactivated by the presence of soap, which is recommended as first aid treatment of animal bites (Warrell, 2004).

2.3. Epidemiology

2.3.1. Host range and susceptibility

All mammals are susceptible, but to varying degrees, and are regarded as the ultimate sources of all rabies infections. Trends in species susceptibility are greatly influenced by species attributes and, to a lesser extent, by individual host factors, such as immunologic status and age. Capable rabies reservoir species are found among members of the order Carnivora in the families, Canidae (i.e. dogs, foxes, coyotes and jackals), Mephitidae (e.g. mongoose species), Procyonidae (i.e. the common raccoon and its relatives), Mephitidae (i.e. the skunks) and bats. Species at moderate to low risk for natural rabies infection consist of members of Felidae, Hyaenidae, Perissodactyla (equids) and Artiodactyla (pigs, camels, cervids, antelopes, giraffes and bovids). Primates appear to be at moderate to low risk as well (Hanlon *et al.*, 2007).

Infected wildlife species, including bats, can transmit rabies to humans, but the total number of such cases remains limited compared with the annual number of human deaths caused by dog-transmitted rabies. By contrast, canine rabies predominates in most of the developing countries of central and South America, Africa and Asia, where the greater burden of human rabies falls. More than 90% of cases of human rabies are transmitted by dogs; most deaths occur in Asia and Africa (WHO, 2011a). Several species-adapted strains of rabies virus have been described. Strains affecting a particular species are transmitted more readily to members of that species than to other animal species. In a given geographical region, rabies is usually maintained and transmitted by particular mammalian reservoir hosts. Two epidemiologically important infectious cycles are recognized, dog mediated rabies and sylvatic or wildlife rabies (Radostits *et al.*, 2007).

2.3.2. Temporal patterns of dog rabies

Eleven years (1986-1996) of wildlife and domestic rabies data studied in Namibia indicated cyclical variation in rabies incidence. Three to four years cyclical fluctuations were identified in domestic ruminants and black-backed jackals. The black-backed jackal time-series variable was a significant predictor of the domestic-ruminant and dog time-series variables (Courtin, *et al.*, 2000). In Bolivia, a 5 to 6 year cycle in canine rabies was observed during the interval between 1972 and 1997. Similarly, a 5 year cycle in Chile between 1950 and 1960 was indicated (Ernst and Fabrega, 1989; Widdowson *et al.*, 2002). As the incidence of canine rabies declined in Chile during the 1960s and 1970s, the cyclic pattern to canine rabies disappeared (Ernst and Fabrega, 1989).

Peaks in canine rabies reports associated with seasonal reproductive pattern of dogs, measured by household surveys or observation of the proportion of pups in the canine population, have been demonstrated from several countries, including Nigeria, Chile and Namibia (Ezeokoli and Umoh, 1987; Ernst and Fabrega 1989; Courtin *et al.*, 2000). In Ethiopia seasonal peak of rabies incidence in rainy season, June to July was recorded (Ali *et al.*, 2010).

2.3.3. Spatial distribution

Rabies is widely distributed across the globe, with only a few countries (mainly islands and peninsulas) being free of the disease. Many animal species are involved in the maintenance and transmission of the disease in nature. Fox rabies has been brought under control in Western Europe, but skunk, raccoon and fox rabies remain prevalent in parts of Canada and the United States. Jackals, bat-eared foxes and mongoose are involved in rabies transmission in Africa, particularly in the south-eastern part of the continent. A variety of bat species have been shown to harbor rabies or rabies-related viruses in Africa, Australia, central and south-east Asia, Europe and most of the Americas (WHO, 2011b). The spatial distribution of rabies is depicted in Figure 1.

Africa

In Africa, the primary vector is the domestic dog (*Canis familiaris*) which accounts for most of the human cases every year. However, jackals (*Canis spp.*), spotted hyenas (*Crocuta crocuta*), domestic and wild cats (*Felis spp.*) and bat-eared foxes (*Otocyon megalotis*) are also major vectors on the continent. Rabies is thought to have been present in North Africa for hundreds of years; however, in sub-Saharan Africa it has only recently become epizootic. The cosmopolitan rabies lineage, which today is the most widespread and dominant rabies virus globally, is thought to have been introduced into sub-Saharan Africa in the twentieth century from Europe, the Middle East and North Africa. This virus is spread in domestic dogs and canid wildlife of the entire sub-continent. In addition, a mongoose rabies virus variant, unique to southern Africa, circulates in non-canid populations including members of the *Herpestidae* and *Viverridae* families such as genets, badgers, mongooses and suricates, but has also been shown to infect a wide range of other wildlife species. In another unique situation in Namibia, kudu antelope (*Tragelaphus strepsiceros*) are known to be able to maintain epidemiological cycles of rabies, and is at present the only known herbivorous species to do so (SEARG, 2011).

In Ethiopia rabies is widespread disease of domestic animals particularly dog. It has been diagnosed from various species of animals including cattle, sheep, goats, jackals, mongoose, cervical cat, cheetah, hyena, monkey, and red fox (Tefera *et al.*, 2002; Newayesilassie *et al.*, 2004; Ali *et al.*, 2010; Deressa *et al.*, 2010). All available evidence suggests that domestic dogs are the main reservoir for rabies in Ethiopia. They are the species most responsible for rabies cases and post-exposure vaccination in humans. No wildlife reservoir has ever been identified in this country even though the disease is confirmed in many wild animals.

Asia

Canine rabies is endemic in Asia. Wildlife rabies plays a very minor role but exists in some species including bats (Wilde *et al.*, 2005). Human mortality from endemic canine rabies was estimated to be 55 000 deaths per year (90% CI: 24 500–90 800) with 56% of the deaths estimated to occur in Asia and followed by Africa (44%) (WHO, 2005)

Europe

Canine rabies disappeared from the countries of western and central Europe during the first half of the 20th century. Most of them are now free of rabies in terrestrial mammals. Nevertheless, canine rabies is still reported in south-eastern Europe, the Middle East and North Africa, and there is limited information concerning countries bordering the EU towards the east. Raccoon dogs have been found infected in Poland and other central-eastern countries. In the EU, several imported cases of rabies infected pets from enzootic areas have been reported even in canine rabies free countries (ECDC, 2009).

Wild rabies poses the risk of human infection and is still endemic in some European countries. The reservoir is bats, in which rabies is common but often not recognized. The EBLV was first identified in 1954, and transmission has been reported to sheep, cats and foxes. Since 1985, only three human deaths from EBLVs have been confirmed. In Europe, rabies in foxes spread south-west ward from the 1930s to the 1980s. Oral vaccination of foxes with different strains started in 1978 and now covers more than twelve countries in Europe. It was administered by hand, helicopter or plane, and has been a very effective measure for controlling the disease. Still, in November 2008 rabies was reported in two foxes in northern Italy, where the previously reported case dated back to 1995. The virus was found to be phylogenetically connected with the strain reported in Slovenia (ECDC, 2009).

USA

Enzootic canine transmission remained the primary source of rabies in the United States until the mid 20th century, resulting in much public anxiety and fear. After World War II, following advancements in vaccine production and potency determination, large-scale control programs focusing on canine vaccination were initiated to prevent human rabies. Animal rabies was added as a nationally notifiable disease in 1938. The earliest example of a successful mass canine vaccination campaign is in 1948. Such control efforts laid the framework for elimination of canine rabies virus variants and dog to dog rabies transmission from the United States in the late 1970s. However, as canine rabies was controlled, rabies in the United States shifted to primary

circulation and maintenance in wildlife. Wildlife have accounted for > 80% of reported rabid animals in the United States since 1975. The primary reservoir species responsible for maintaining rabies are raccoons, bats, skunks, foxes, and mongooses (Blanton *et al.*, 2010).

Canada

Human rabies in Canada is rare. However, there is significant number of animal rabies cases. In Canada the animals that most often transmit rabies are foxes, skunks, bats, and raccoons. Bats are increasingly implicated as important wildlife reservoirs for rabies (CFIA, 2011).

Central and South America

Domestic dog rabies is the main reservoir in Mexico and some areas of Central and South America. Vampire bat (*Desmodus spp.*) rabies is known to cause bovine paralytic rabies from Mexico south to the northern parts of Argentina and Chile. This form of rabies has serious economic effects on farmers. The bats feed on the blood of large mammals, particularly cattle, transmitting, in the process, a form of paralytic bovine rabies. This disease causes an estimated loss of 50,000 head of cattle each year in Brazil. Outbreaks have been reported from Ecuador. Bat rabies in the Americas is all due to genotype 1 virus, whereas bats in the rest of the world harbor only the rabies related *lyssaviruses* (Warrell, 2004).

Australia

Prior to May 1996, Australia had been considered free of rabies and rabies-like viruses. This status was challenged, however, when a virus with a high degree of antigenic and genetic similarity to rabies virus (RABV), subsequently named Australian bat *lyssavirus* (ABL), was isolated from a juvenile black flying fox. Since that time, viral antigen has been detected in CNS of the major species of fruit bats and in insectivorous bat. Disease associated with infection has been reported in bats and humans (Fraser *et al.*, 1996).

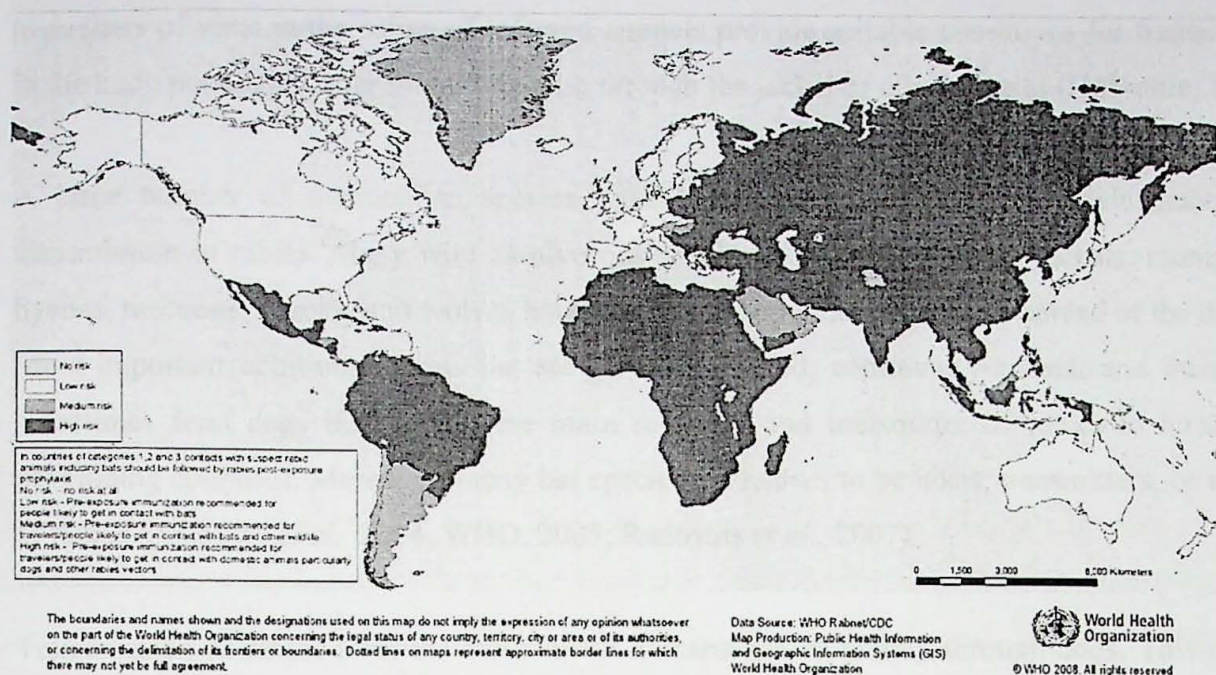


Figure 1. Rabies, countries or areas at risk (Source: WHO, 2011b)

2.3.4. Transmission

Rabies is the most widely recognized example of salivary transmission of virus. Inoculation of infected saliva through the bite of a rabid animal appears to be the predominant mode of rabies viral entry although contamination of skin wounds by fresh saliva may result in infection. Not all bites from rabid animals result in infection because the virus is not always present in the saliva and may not gain entrance to the wound if the saliva is wiped from the teeth by clothing or the coat of the animal. The virus may appear in the milk of affected animals, but spread by this means is unlikely as infection (Radostits *et al.*, 2007). There also are reports of rabies transmission resulting from transplantation of Cornea, Liver, kidney, Iliac Artery, Lung and Kidney-Pancreas (Bronnert *et al.*, 2007). Under special conditions infection through aerosols has occurred, for example in caves inhabited by bats and by exposure in the laboratory. Human-to-human transmission is extremely rare (Haaheim *et al.*, 2002; Carter and Saunders, 2007). In kudus it was found that transmission of rabies is associated with their social behavior and increased population density. The social behavior of the kudu, i.e., group browsing on acacia trees, whose thorns cause lesions in the kudu's oral cavity, as well as the excretion of relatively

high titers of virus in the saliva of infected animals provide suitable conditions for transmission in the kudu population after initial infection through the jackal or other species (Hübschle, 1988).

A large number of mammalian species have been known worldwide in maintenance and transmission of rabies. Many wild carnivorous species like coyotes, foxes, jackals, mongooses, hyenas, raccoons, skunks, and wolves have all been playing their part in the spread of the disease. Most important additions to this list are privately owned, community-owned, and ownerless, sometimes feral dogs that remain the main reservoir and transmitter of rabies to humans in developing countries. Moreover, many bat species are known to be hosts, transmitters, or victims of rabies (Andrews *et al.*, 2004; WHO, 2005; Radostits *et al.*, 2007).

Transmission of rabies from the reservoir to humans occurs mainly through dogs. This is most efficient at the onset of the rabid period and for the subsequent 7–10 days. Domestic livestock like cattle are rarely a source of infection, although chance of transmission to humans may occur if the mouth of a rabid animal is manipulated during treatment or examination in which virus in saliva is inoculated into scratches caused by teeth (Andrews *et al.*, 2004). Bats represent a serious threat of spread of rabies because of their migratory habits. Most spread is within the species, but the threat to humans and animal species by bats cannot be disregarded. Mammals infected with rabies virus often become aggressive; this change in behavior increases the likelihood of the host biting another individual and transmitting virus in the saliva (Carter and Saunders, 2007; Radostits *et al.*, 2007).

2.4. Clinical signs

The incubation period for rabies is highly variable and can be as long as six months. It is influenced by various factors including host species, virus strain, the amount of inoculum and the site of introduction of the virus. Large amounts of virus introduced into deep bite wounds in the head region are usually associated with short incubation periods. Clinical signs involve the CNS but vary somewhat depending on the host species (Zee, 1999).

2.4.1. Dogs

In the dog, the incubation period is commonly two to eight weeks, although there is a longer incubation period recorded. It is likely that the prodromal period seen in humans occurs in dogs but is recognized by few owners, after which the dog passes into the classic 'furious' phase, showing nervousness, restlessness and exaggerated response to stimuli. Self infliction of injury at the site of bite is not uncommon. They may attack anything; human, animal or inanimate objects and some will eat anything, including stones and lumps of wood. There is no forgetting the look in the dog's eyes: a fixed stare or a far-away look which is difficult to describe. They may howl between the convulsive seizures and they often die during a seizure, or paralysis ensues, the animal passes into a coma and dies. Dogs that develop the dumb form of the disease may show periods of furious activity. The muscles of the jaw are often the first to be affected and so the dog lies with its mouth open. The dog is unable to eat and many owners suspect that the dog has a bone in its throat. The clinical signs of rabies in cats are similar to those in dogs. Rabid cats have a greater tendency to hide in secluded places and are often more vicious (Zee, 1999; Andrews *et al.*, 2004).

2.4.2. Cattle

Incubation period is between two and twelve weeks. Where the disease is transmitted by jackals it is not uncommon to find several animals affected at one time. The affected animal separates itself from the herd, shows anorexia and docility. Usually there is a drop in milk production and bulls become more sexually active. After a few days the aggressive phase may begin with the animal attacking anything. A fixed stare may be seen and the animal grinds its teeth. Inability to swallow in many African animals results in saliva running from the mouth. Salivation occurs in less than half the bovine cases in the USA. As the disease continues, paralysis of the tail is seen, posterior paresis and a swaying gait are noted and the animal bellows. Loss of condition ensues and the animals go off their legs, develop paralysis and between bouts of convulsions go into a coma and die. In North West Argentina a completely dumb form of the disease is seen in cattle, with the animal showing progressive paralysis. Other domestic animals show variations on the pictures described above (Andrews *et al.*, 2004).

2.4.3. Sheep

In sheep, rabies often occurs in a number of animals at one time due to the ease with which a number of sheep can be bitten by a dog or fox. Clinically, the picture is similar to that seen in cattle. The minority, show sexual excitement, attacking humans or each other, and vigorous wool pulling. Sudden falling after violent exertion, muscle tremor, and salivation are characteristic. Excessive bleating does not occur. Most sheep are quiet and anorectic. Goats are commonly aggressive and continuous bleating is common (Radostits *et al.*, 2007).

2.4.4. Horses

Clinical signs on presentation vary and range from lameness to sudden death. Hyperesthesia, ataxia, behavior change, anorexia, paralysis or paresis, and colic have been reported as initial clinical signs. One rarely finds a bite wound and the horse may or may not be febrile. The site of inoculation and its proximity to the CNS influence what clinical signs one observes. The neurologic signs exhibited in rabies-infected horses can be classified into three forms depending on the neuroanatomic location in the CNS infected by the virus. First, in the cerebral or furious form, one may see aggressive behavior, photophobia, hydrophobia, hyperesthesia, straining, muscular tremors, and convulsions. Second, in the brainstem or dumb form, one commonly sees depression, anorexia, head tilt, circling, ataxia, dementia, excess salivation, facial and pharyngeal paralysis, blindness, flaccid tail and anus, urinary incontinence, and self mutilation. Finally, in the paralytic or spinal form, one sees progressing ascending paralysis, ataxia, or shifting lameness with hyperesthesia, and self mutilation of an extremity. Most affected animals with the paralytic form become recumbent in 3 to 5 days, with normal eating and drinking often remaining. The neurologic signs may vary as the virus spreads to other portions of the CNS. Thus, horses may have clinical signs of two or all forms of rabies. Disease progresses rapidly, and death is usually inevitable regardless of the clinical manifestation. Anti-inflammatory therapy can delay virus progression, but death usually occurs within 5 to 10 days after onset of clinical signs (Sommandahl, 2004).

2.4.5. Pigs

Pigs manifest excitement and a tendency to attack, or dullness and incoordination. Affected sows show; twitching of the nose, rapid chewing movements, excessive salivation and clonic convulsions. They may walk backward. Terminally, there is paralysis and death occurs 12 to 48 hours after the onset of signs. The clinical findings in pigs are extremely variable, and individual cases may present in a variety of ways and only one or two of the classical findings may occur (Radostits *et al.*, 2007).

2.4.6. Wild animals

The classic sign of rabies in wild animals is the loss of fear of humans. This was very dramatically seen in the kudu outbreak in Namibia in which the animals would walk into houses. Jackals become very friendly but have sudden mood swings. Wild cats become more aggressive with rabies, although even some of these animals become docile. Most humans bitten by rabid mongoose are bitten while handling these animals, thinking that they are tame (Andrews *et al.*, 2004).

2.5. Diagnosis

Ante-mortem diagnosis of rabies is difficult. Even with symptoms quite characteristic for rabies, such as changes in behaviour or difficulties in swallowing the clinical examination cannot rule out rabies nor confirm the diagnosis. Intra-vitam diagnosis is based on detecting virus or viral RNA in saliva, neck skin biopsy or epithelial cells of the cornea. Mostly the results are not sufficient and post-mortem diagnosis is the only reliable confirmation of rabies infection (WHO, 2011b).

2.5.1. Post-mortem lesions

There are no characteristic gross lesions. The stomach may contain various abnormal objects, such as sticks and stones. The typical histological signs, found in the central nervous system, are multifocal, mild, polioencephalo-myelitis and craniospinal ganglionitis with mononuclear

perivascular infiltrates, diffuse glial proliferation, regressive changes in neuronal cells, and glial nodules. Negri bodies can be seen in some but not all cases (CFSPH, 2009).

2.5.2. Laboratory diagnosis

Searching for inclusion bodies in the hippocampus for rabies diagnosis was first described by Negri at the turn of the 20th century using what later became known as Sellers' staining method (SS). It has become known as an unreliable method and can no longer be recommended because they have very low sensitivity and should be abandoned (OIE, 2008). Since then several laboratory techniques have been developed for rabies diagnosis including FAT, MIT, RTCIT, RIDT, Immunohistochemistry, RT-PCR, ELISAs and Electron Microscopy.

Fluorescent antibody test

The most widely used test for rabies diagnosis is the FAT, which is recommended by both WHO and OIE. This test may be used directly on a smear, and can also be used to confirm the presence of rabies antigen in cell culture or in brain tissue of mice that have been inoculated for diagnosis (OIE, 2008). Fluorescent antibody test provides a reliable diagnosis in 98–100% of cases for all serotypes if a potent conjugate is used (Robles and Miranda, 1992; OIE, 2008). The sensitivity of the FAT depends on the specimen, the degree of autolysis and how comprehensively the brain is sampled, on the type of *lyssavirus* and on the proficiency of the diagnostic staff (Heaton *et al*, 1997; David *et al.*, 2002). Sensitivity may be lower in samples from vaccinated animals due to localization of antigen, which is confined to the brainstem. For direct rabies diagnosis, smears prepared from a composite sample of brain tissue that includes the brain stem, are fixed in high-grade cold acetone and then stained with a drop of specific conjugate. Anti-rabies fluorescent conjugates may be prepared in the laboratory. Those available commercially are either polyclonal conjugates specific to the entire virus or specific to the rabies nucleocapsid protein, or they may be prepared from a mix of different MAbs. In the FAT, the specific aggregates of nucleocapsid protein are identified by their fluorescence. The specificity and sensitivity of these anti-rabies fluorescent conjugates for locally predominant virus variants should be checked before use. The FAT may be applied to glycerol-preserved specimens. If the specimen has been preserved in a

formalin solution, the FAT may be used only after the specimen has been treated with a proteolytic enzyme. However, the FAT on formalin-fixed and digested samples is always less reliable and more cumbersome than when performed on fresh tissue (OIE, 2008).

Cell culture or mouse inoculation tests

As a single negative test on fresh material does not rule out the possibility of infection, cell culture or mouse inoculation tests should be carried out simultaneously. A monolayer culture of susceptible cells is inoculated with a pool of several CNS tissues, including the brain stem. Fluorescent antibody test carried out after appropriate incubation will demonstrate the presence or absence of viral antigen. Alternatively, newborn or 3–4-week-old mice may be inoculated intracerebrally with similar pool tissues and then kept under observation for 28 days. For any mouse that dies between 5 and 28 days post inoculation, the cause of death should be confirmed by FAT. This *in vivo* test is time-consuming, expensive and involves the use of animals. Wherever possible, virus isolation in cell culture should replace mouse inoculation tests. One advantage of the above *in vivo* and *in vitro* assays includes the amplification of the virus isolate for future typing and analysis (OIE, 2008).

Rapid immunodiagnostic test

Another method for the detection of rabies virus antigen from postmortem samples is a recently developed rapid immunodiagnostic test (RIDT) based on the principles of immunochromatography. The immunochromatographic lateral flow strip test is a one-step test that facilitates low-cost, rapid identification of various analytes including viruses. Briefly, suspect material is subjected to a test device similar to a lateral flow device. Conjugated detector antibodies attached to two different zones on a membrane indicate the presence of viral antigen. Preliminary validation studies with a limited number of samples showed that the RIDT might have great potential as a useful method for rabies diagnosis without the need for laboratory equipment. The rapid immunodiagnostic test kit is a useful method for quick detection of rabies virus under various conditions, including laboratories, office or field (Fooks *et al.*, 2009).

The study conducted by kang *et al.* (2007) using 51 clinical samples and 4 isolates of rabies virus indicated that the rapid immunodiagnostic test was able to detect rabies virus in clinical samples, including brain tissue and saliva, in addition to $10^{3.2}$ 50% lethal dose (LD50)/mL cell-adapted rabies virus. The assay was not cross-reactive with non-rabies virus microbes. When the performance of the rapid immunodiagnostic test was compared to a fluorescent antibody test, the rapid immunodiagnostic test had a sensitivity of 91.7% and specificity of 100% (95.8% CI). Markotter *et al.* (2009) evaluated the ability of the RIDT to identify genotypes of lyssaviruses occurring in Africa (genotype 1–4) using 21 brain samples. With his study, 100% sensitivity and specificity was observed.

Direct immuno-histochemical test

The test is based on a direct immuno-histochemical detection of rabies virus in brain tissue (dRIT) visible by normal light microscopy. The dRIT showed a sensitivity and specificity equivalent to those of the FAT (Lembo *et al.*, 2006; Dürr *et al.*, 2008). The dRIT uses highly concentrated and purified biotinylated anti-nucleocapsid monoclonal antibodies to rabies virus. After incubation with a streptavidin-peroxidase complex, the antibody reagent is made visible with 3-amino-9-ethylcarbazole. The result can be read after less than one hour. The dRIT recognizes all representative *lyssaviruses* examined. The dRIT is a simple diagnostic method. However, the protocol has to be followed strictly and includes more steps than the FAT. As critical points for field use, the cold storage of the kit, and the use of various chemical reagents, have to be considered (Dürr *et al.*, 2008).

Enzyme-linked immunosorbent assay

An enzyme-linked immunosorbent assay (ELISA) that detects rabies antigen is useful for large epidemiological surveys. It should only be used after validation against numerous samples in different laboratories. The specificity and sensitivity of these anti-rabies enzyme conjugates for locally predominant virus variants should be checked before use. This test should be used in combination with confirmatory tests by FAT or virus isolation (OIE, 2008; CFSPH, 2009).

Reverse transcriptase polymerase chain reaction

The use of the reverse transcriptase polymerase chain reaction (RT-PCR) in laboratories with strict quality control procedures in place and demonstrable experience and expertise, have been successfully applied for confirmatory diagnosis and epidemiological surveys. Rabies virus RNA can be detected in a range of biological fluids and samples (e.g. saliva, cerebrospinal fluid, tears, skin biopsies and urine) (Fooks *et al.*, 2009). RT-PCR is particularly found important in detection of rabies virus in decomposed naturally infected brains, especially in cases when the sample is not suitable for other laboratory assays. Furthermore, PCR products may subsequently be exploited for sequencing and phylogenetic analyses, which enable a highly accurate identification of the virus isolate to be obtained (David *et al.*, 2002; Panning *et al.*, 2010)

Serology

Serological tests are rarely used in epidemiological surveys, due to late seroconversion and the low percentage of animals surviving the disease and therefore having post-infection antibodies. The main application of serology is to determine responses to vaccination, either in domestic animals prior to international travel, or in wildlife populations following oral immunization of rabies reservoirs. For follow-up investigations in oral vaccination campaigns, virus neutralization (VN) tests in cell culture are preferred. However, if poor quality sera are submitted, the VN tests in cell culture are sensitive to cytotoxicity, which could lead to false-positive results. For such samples, the use of an indirect ELISA with rabies glycoprotein-coated plates has been shown to be as sensitive and specific as the VN test on cells (OIE 2008; CFSPH, 2009).

2.6. Control and prevention

Canine rabies can be eliminated, as has been demonstrated in North America, Western Europe, Japan and many areas in South America (Brochier *et al.*, 1995; WHO, 2005). The study in Tanzania by Cleaveland *et al.* (2003) and Lembo *et al.* (2010) also has indicated control of dog rabies in rural African communities can be achieved through implementation of dog vaccination strategies. Canine rabies control programs should incorporate three basic elements, with priorities

varying according to the prevailing social, cultural and economic factors. The basic elements are: (a) epidemiological surveillance; (b) mass vaccination; and (c) dog population control. They require community participation, managerial skills and legislation (WHO, 2005). In areas where rabies is controlled in domestic dogs wild life is of other concern.

2.6.1. Dog population management and mass immunization

Humans become infected with rabies by the bite of an infected animal, mostly a rabid dog. Therefore, it is necessary to control rabid dogs by mass vaccination of dogs in endemic areas for elimination of rabies. Effective control of rabies requires a sound and practical dog population management relating to domestic, community and ownerless dog populations. Countries should develop effective integrated dog population management and immunization programs. There is evidence that sustained and effective immunization of 70% of dogs in a given area can result in breaking the transmission of rabies. Endemic countries should launch mass vaccination programs reaching an appropriate number of animals each year and maintain that level of herd immunity over time until elimination is achieved. Three practical methods of dog population management are recognized: movement restriction, habitat control and reproduction control (WHO, 2005)

Between 1993 and 2002, the number of human and canine rabies cases in the Americas Region fell by approximately 80%. This sharp reduction is attributable mainly to the control measures implemented by the countries of the Region, such as the mass vaccination of dogs and prophylactic treatment for people who have been exposed (Belotto *et al.*, 2005). A rapid decline in the incidence of rabies by 70% after the first campaign and by 97% after the second campaign was indicated in Tanzania with 60-70% vaccination coverage (Cleaveland *et al.*, 2003).

The situation in Africa and Asia is striking. In these areas the incidence of dog rabies and human rabies deaths continue to escalate and new outbreaks have been occurring in areas previously free of the disease (e.g. the islands of Flores and Bali in Indonesia) (CDC, 2011). Many factors have been identified for the lack of effective domestic dog rabies control in these areas. These include: low prioritization, epidemiological constraints, operational constraints and lack of resources (Lembo *et al.*, 2010). However, there are no insurmountable problems to canine rabies control in

most of Africa and Asia; that elimination of canine rabies is epidemiologically and practically feasible through mass vaccination of domestic dogs; and that domestic dog vaccination provides a cost-effective approach to the prevention and elimination of human deaths due to rabies (Cleaveland *et al.*, 2003; Lembo *et al.*, 2010).

Vaccination of owned dogs on request by the owners and poisoning of non restricted dogs in the form of campaign are measures applied to control rabies in Ethiopia. Vaccination of dogs against rabies is generally provided by government veterinary services and private sectors. Government veterinary clinics use both Fermi (limited) and cell culture vaccines for dog vaccination. Fermi type vaccine is produced in the country and is relatively less costly compared to cell culture vaccine. However, it is not produced at individual level and hence reduces its suitability for use. Private sectors use cell culture vaccines and house to house vaccination is a more common practice than the government veterinary services particularly in Addis Ababa (Kasahun, personal communication). However, it is known that the vaccination coverage is very limited to interrupt the transmission of rabies in the country. Thus, rabies is continued to be highly prevalent disease in the country.

2.6.2. Community awareness

Community awareness of all aspects of rabies is generally lacking or limited particularly in developing countries, be it first aid or management of animal bites, pre and post-exposure prophylaxis, responsible pet dog ownership, dog population management, laboratory diagnosis, etc. Regarding the immediate measures to be carried out after a bite exposure, there is inadequate knowledge of the crucial need to wash wounds with soap and running water and apply antiseptics. Many people who are exposed to rabies do not seek PEP, because they are not even aware of the risk of contracting this deadly disease, or because they live in rural areas, too far away from rabies prevention centers which are generally located in big cities. Instead, referral to traditional healers is frequent and various types of wound care are applied. In addition, the full course of vaccine may not be taken because of financial constraints or other reasons (WHO, 2005; AfroREB, 2008).

2.6.3. Wildlife immunization

Rabies control programs among wildlife species are only feasible when rabies among the surrounding domestic dog population is also being controlled (SEARG, 2011). Oral vaccination is an important and efficient method of wildlife rabies control programs. Since 1978, oral rabies vaccines have been used to control wildlife rabies (Brochier *et al.*, 1995). So far several countries such as Finland, The Netherlands (1991), Italy (1997), Switzerland (1998), France (2000), Belgium, Luxembourg (2001) and the Czech Republic (2004) have been declared as being officially free of terrestrial rabies (WHO, 2011b). In Ethiopia, little efforts have been done to control rabies in wild life. Some efforts have been made to control rabies in rare and endangered species, the Ethiopian wolves, in the Bale mountain national park at least from 1996 - 2004. In view of the fact that domestic dogs are the reservoir of rabies, attempts were made to vaccinate 70% of domestic dogs in core wolf areas within the national park. Some more dogs of the surrounding communities were also included in the vaccination campaign. However, an outbreak was occurred in 2003 and 2004 (Randall *et al.*, 2004).

No practical vaccination methods have been designed for bats. In Latin America vampire bat rabies is a major cause of death in cattle, with disastrous economic consequences. Specific control methods include vaccination of cattle or treatment with anticoagulants, to which bats, but not cattle, are highly sensitive (Warrell, 2004). Anticoagulants such as diphenadione and warfarin were shown to be effective in reducing vampire bat population and to decrease the risk of transmission of rabies in enzootic areas (Arellano-Sota, 1988).

2.6.4. Human vaccination

The first rabies vaccine was developed by Louis Pasteur in the late 19th century. Since that time vaccines with improved efficacy and less reactogenicity have been developed for use in humans and animals. Improvements in Pasteur's vaccine were achieved by Semple and Fermi who killed the virus rather than attenuated it, and by Fuenzalida and Palacios who developed a suckling mouse brain vaccine which carried a lower risk of neuromuscular complications. Growth of rabies virus in tissue culture was achieved in the 1930s, leading to the development, by Wiktor and his colleagues, of the first tissue culture vaccine for human use. This human diploid cell vaccine

(HDCV) was licensed in 1974. Since then other substrates including chick embryo and vero cells have been used to facilitate production and reduce the cost (Kammer and Ertl, 2002). In Ethiopia, Fermi type vaccine prepared from sheep brain tissue has been produced and widely used. The vaccine is produced both for humans and animals use. It is distributed on demand bases to various parts of the country (Deressa *et al.*, 2010). World health organization strongly recommends that nerve tissue vaccines should be discontinued, because of its reactogenic effects and low immunogenicity (WHO, 2005). This Fermi type vaccine is used in humans following exposure to rabid or rabies suspected animals. It is administered daily for 14 consecutive days and then 3 injections are given at 10 days interval. Even though this vaccine is produced in the country, it is available in limited public hospitals and health centers. This was reflected by many people traveling a long distance to get vaccine from EHRI. The reason for this would be its short shelf life which is six months and sometimes lack of experienced personnel for administration of this vaccine.

Development of fatal disease in humans bitten by infected animals can be prevented by post-exposure vaccination. No rabies deaths has been reported in those who were given pre-exposure prophylaxis with post-exposure boosting (100% effective) in preventing this fatal infection (Murphy *et al.*, 1999; Warrell, 2004). However, rabies remains an important yet neglected disease in Asia and Africa despite the fact that safe and effective modern vaccines exist for both human and veterinary use. This is attributed to a lack of information, limited resources, and the poor accessibility of rabies prevention center (Sugiyama and Ito, 2007; AfroREB, 2008). Furthermore, this situation is exacerbated by lack of regular surveillance and monitoring of the disease.

Pre-exposure prophylaxis

Pre-exposure vaccination is an important part of rabies control. Pre-exposure prophylaxis or preventive vaccination is recommended by WHO for anyone at increased risk of exposure to the rabies virus. It eliminates the need for RIG (Rabies immunoglobulin) and reduces the number of vaccine doses needed for PEP in the event of a subsequent exposure to the rabies virus. It should

be particularly recommended in Africa, where RIG is not available (WHO, 2005; AfroREB, 2008).

Post-exposure prophylaxis

Development of fatal disease in humans bitten by rabies infected animals can be prevented by post-exposure vaccination. These indicate that rabies is a vaccine-preventable disease. Post-exposure treatment is in great demand, especially where dog rabies is endemic (WHO, 2005). Modern post-exposure treatment is highly successful if done adequately. Wound care with passive and active rabies immunization is essential especially after severe exposure. Post-exposure treatment is assumed to neutralize or inactivate virus while it is still in the wounds, before it gains access to the nervous system where it is protected from the immune system. Therefore, treatment after exposure to rabies virus is very urgent, even if the patient was bitten months before. Thorough washing of rabies-infected wounds with soap and water can increase survival by 50%. However, this inexpensive, readily available treatment is omitted in most cases (Radostits *et al.*, 2007).

3. MATERIALS AND METHODS

3.1. Study areas

The study was conducted mainly in and around Addis Ababa (about 100 km radius), which is the capital city of Ethiopia (Figure 2). Samples that were submitted from other areas of the country were also included. The city covers an area of 530 km² and is divided in to 10 sub-cities. Based on the 2007 census conducted by the Central Statistical Agency of Ethiopia (CSA), Addis Ababa has a total population of about 2.7 million of which 1.3 million are men and 1.4 million are women. The estimated livestock population of the city is 92,700 cattle, 48,500 sheep and goats and 22,500 equines. The dog population is estimated to be 250,000 to 350,000 (UAECF, 2011). Laboratory work was conducted at Ethiopian Health and Nutrition Research Institute rabies diagnostic laboratory, which is located in Addis Ababa.

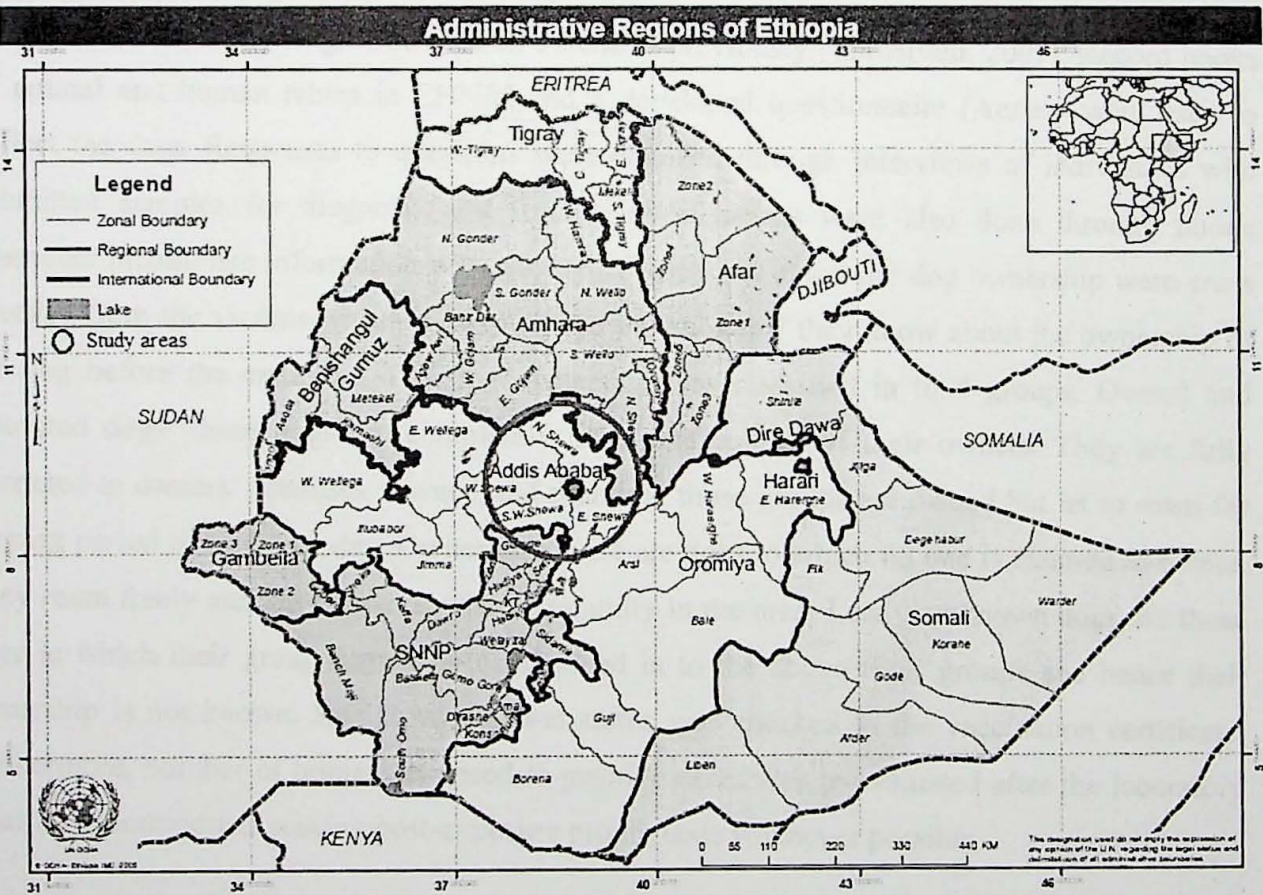


Figure 2. Administrative regions of Ethiopia (Source: UN OCHA, 2011).

3.1. Sampling

Convenience sampling technique was used for sample collection in which all samples submitted to the laboratory were included (Greiner and Gardner, 2000). Mainly heads of killed or dead animals suspected of rabies were submitted to the institute from different parts of the country. Sometimes, live rabies suspected animals were also submitted. Postmortem brain specimens were collected by opening the skull. After removing from the skull, the whole brain was put in sterilized Petri dish labeled with sample identification number and the date the sample was received. A cross section of the brain stem and either cerebellum or hippocampus was used for preparation of impression smears and brain homogenates. These tissue samples were stored and persevered at -20°C in sterilized vials for further study.

3.2. Study design

A retrospective study design was used as described previously (Thrusfield, 2007). Record books of animal and human rabies in EHNRI and a developed questionnaire (Annex) were used to collect the data. Responses to questions were obtained through interviews of individuals who submitted samples for diagnosis and further investigations were also done through phone whenever incomplete information was obtained. Responses about the dog ownership were cross checked from the victims who were exposed to the animal if they know about the ownership of the dog before the exposure. The dog ownership was classified in to 4 groups. Owned and restricted dogs: those dogs are completely under the control of their owners. They are fully restricted to owners' premises. Owned and roaming: those groups are owned but let to roam for varying period of time in a day. Ownerless: those are dogs to which no one is claimed as owner. They roam freely and are known by the community in the area. Lastly, unknown dogs are those dogs in which their group cannot be categorized in to the above three groups and hence their ownership is not known. Rabies vaccination status was checked in the vaccination certificate. Furthermore, number of humans exposed to positive cases was re-evaluated after the laboratory result from individuals seeking post-exposure prophylaxis whenever possible.

One hundred fifteen clinical brain samples that were acquired from rabies suspected animals during the study period were also examined for the purpose of comparison of the diagnostic tests. The Anigen Rapid Rabies Ag Test Kit and the FAT were used in parallel for detection of rabies virus antigen in brain samples by different personnel. The diagnostic performance of Anigen Rapid Rabies Ag Test Kit was evaluated by determining its sensitivity and specificity compared to FAT, a reference test which is supposed to determine the true disease state of the animals (OIE, 2008). Further four brain samples were used to analyze differences in the diagnostic sensitivity and intensity of color formation using different procedures.

3.3. Laboratory tests

3.3.1. Fluorescent antibody test

The FAT test is based on the principle that an animal infected by rabies virus will have rabies virus protein (antigen) present in its tissue. Because rabies is present in nervous tissue (and not blood like many other viruses) the ideal tissue to test for the presence of rabies antigen is brain. The most important part of a FAT is fluorescent-labeled anti-rabies virus antibody. When labeled antibodies are added to rabies-suspect brain tissue, it will specifically bind to rabies antigen if it is present. Unbound antibody can be washed away and the areas where the antigen has bound antibody will appear as a bright fluorescent apple green color (Figure 3a) when viewed with a fluorescence microscope. If rabies virus is absent, there will be no staining as shown in Figure 3b (OIE, 2008).

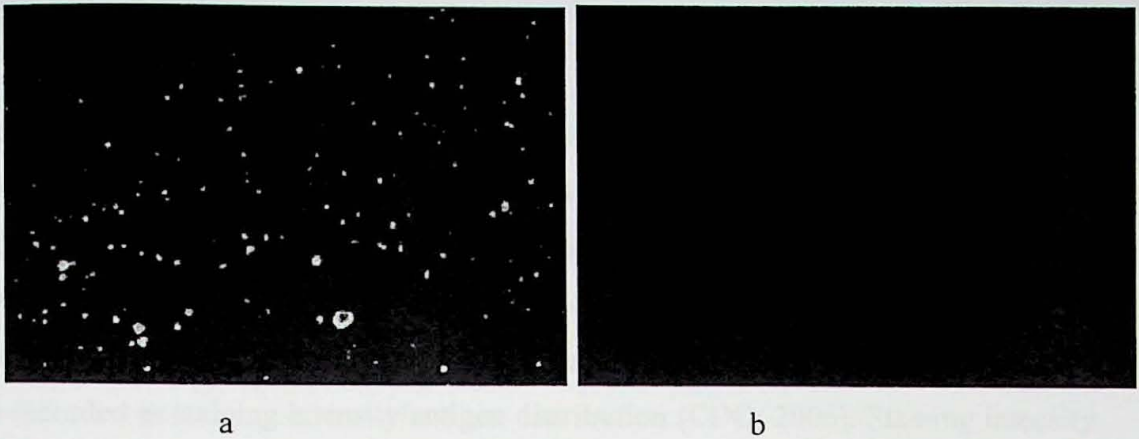


Figure 3. FAT results: a=positive, b=Negative (Source: CDC, 2006).

The protocol for postmortem diagnosis of rabies by FAT is given below (EHNRI, 2010).

Procedures

1. Impression smears were made from the brain tissue on slides.
2. Impressions or smears were air dried completely at room temperature prior to fixation. Drying took 15-20 minutes.
3. The smears were fixed in cold acetone for 1 hour to overnight.
4. After the fixations were air dried at room temperature, the smears were covered with sufficient quantity (0.45 μ l) of conjugate.
5. Then the slides were put in a humidified staining chamber and incubated for 30 minutes at 37°C.
6. After excess conjugate was drained off from the slides or wicked onto absorbent paper, the slides were rinsed in
 - a. 1st tank containing PBS for 5 minutes
 - b. 2nd tank containing PBS for 5 minutes
 - c. then rinsed with distilled water
7. The slides were carefully blotted to remove excess liquid and air dried
8. A few drops of mounting medium (50% buffered glycerol) was applied
9. Finally, it was covered with cover slide and examined under 40X objective lens.

Expected result

Rabies virus in the brains of infected animals produces intracytoplasmic inclusions of various shapes. A single microscopic field may contain dust-like particles of <1 µm in diameter and large, round to oval masses and strings 2 to 10 µm in diameter. When specifically stained with Fluorescien isothiocyanate (FITC) labeled antibody, these inclusions appear smooth, with very bright margins, and a somewhat less intensely stained central area. Observations made for each test slide were recorded as staining intensity/antigen distribution (CDC; 2006). Staining intensity was graded from +4 to +1. Positive control slides in all tests that always contain staining of + 4 intensity was used. Slightly diminished staining intensity was graded as + 3 and may occur in test samples positive for rabies when sample handling has not been optimal. Noticeably dull stain was graded +2 to +1 and was not considered as diagnostic for a rabies infection without confirmation of specificity. Even though diminished staining intensity may be the result of denaturation of rabies virus antigen, diminished staining may also result from non specific binding of antibody to components of inflamed tissue or artifacts of tissue decomposition. For each area of the brain examined, staining was graded by the amount of antigen present as presented in the following Table (Table 1).

Table 1. Grading of FAT staining

Grade	Description
+4	Massive infiltration of large and small inclusions of varying shape in almost every area of the impression.
+3	Inclusions of varying size and shape are found in almost every microscopic field, the number of inclusions per field varies, but inclusions are numerous in most fields.
+2	Inclusions of varying size and shape are present in 10%to 50%of the microscopic fields and most fields contain only a few inclusions.
+1	Inclusions of varying size and shape are present in <10%of the microscope fields and only a few inclusions are found per field (usually only one or two inclusions per field).

Result interpretation

When the tissue sample submitted for testing was adequate and suitable for rabies diagnosis, results for a test animal were reported as positive or negative for rabies (test complete) or non-diagnostic (test incomplete) based on observed patterns of staining in test and control slides.

- a. Test complete/ Reportable result: Test results were reported to the submitter as complete (then samples of brain material were stored for reference), the carcass and other materials were discarded, when the following observations were made:

Test controls: Both large and small antigen accumulations in positive control slide stain with +4 intensity and +3 to +4 antigen distribution. No staining was present on negative control slide.

Test samples: Required brain areas were present and no tissue deterioration or alteration was noted when slides were prepared. Samples were clearly negative (no specific staining in test slides) or clearly positive (at least +3 to +4 intensity and +2 to +4 distribution of antigen in slides made from brain stem and cerebellum or hippocampus).

- b. Test incomplete /results not reported until test was repeated: Sparse or weakly staining inclusions may indicate an unusual clinical course, the presence of an unusual variant of rabies virus, or non-specific or undesired binding of FITC-labeled antibody. The following observations warrant repeat testing for confirmation of staining specificity:

- Inclusions typical of rabies virus infection, but present in no more than 10% of examined fields or present in >10% of fields but with an extremely sparse distribution (i.e., 1 to 2 inclusions per field).
- Inclusions typical of rabies virus infection, but staining with uncharacteristically weak intensity (<+3).
- Inclusion morphology not typical of rabies infection, but staining with + 4 intensity (e.g., regularly sized uniform textured structures).
- Fluorescing bacteria that might mask small amounts of rabies specific staining.

- Particulate or free fluorescein that might mask small amounts of rabies-specific staining.
- Discordant results for observations made with two reagents or between two readers.

Samples were not reported as provisionally positive or negative. The direct fluorescent FAT test was repeated from the preserved original brain sample and re-smearing slides from brain tissue reserved at necropsy. If for any reason the test cannot be repeated, the test report should state that the condition of the sample is such that tests cannot rule out the presence of rabies in the specimen.

3.3.2. Rapid immunodiagnostic test

The Anigen Rapid Rabies Ag Test Kit is a chromatographic immunoassay for the qualitative detection of rabies virus antigen. This test kit has a letter of "T" and "C" as test line and control line on the surface of the device. Both the test line and control line in result window were not visible before applying any samples. The control line was used for procedural control. The control line always appeared when the test procedure was performed properly. An additional purple test line was visible in the result window when there were enough rabies virus antigens in the specimen.

Rabies virus antibodies were used in test band as both capture and detector materials. These enable the Anigen Rapid Rabies Ag Test Kit to identify rabies virus antigen.

The procedure of the test used in this study is described as follows.

1. 0.08 (0.05-0.11) grams of brain sample was taken using the supplied swab.
2. The swab was inserted into the specimen tube containing 1ml of assay diluent.
3. The swab samples were mixed with assay diluent to extract well for one minute.
4. The test device was removed from the foil pouch, and placed on a flat and dry surface.
5. Using the disposable dropper, the samples were taken from extracted and mixed specimens in the tube.
6. Four drops were added slowly into the sample hole using the disposable dropper.

7. As the test began to work, purple color moving across the result window in the center of the test device was observed.
8. Test results were interpreted within 5 - 10 minutes. Decisions were not made after 10 minutes.

Interpretation of the test

The presence of only one band within the result window indicates a negative result while the presence of two color bands ("T" and "C") within the result window indicates a positive result, no matter which band appears first (Figure 4).

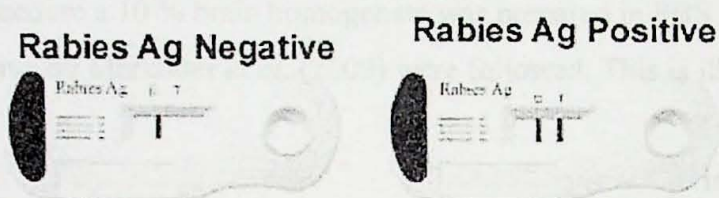


Figure 4. Results of the RIDT

Four samples were used to analyze differences in the diagnostic sensitivity and intensity of color formation using different procedures of the RIDT. These include the procedures described by Kang *et al.* (2007) and Markotter *et al.* (2009). The remaining two were based on the instructions prescribed on the manufacturers leaflet, Anigen leaflet 2004 and Anigen leaflet 2010. The details of the procedures are described as follows

Test procedure with 20% brain homogenate and dilution of the homogenate with equal volume of the diluent (Kang *et al.*, 2007).

1. 20 % brain homogenate was prepared in PBS.
2. 1 ml of the homogenate was added in to the diluent (1 ml)
3. Using the disposable dropper, the mixed specimens was taken and four drops were added into the sample hole of the kit
4. The test results were read within 5 - 10 minutes

Test procedure with a 20% brain homogenate specimen (Markotter *et al.*, 2009)

1. A 20 % brain suspension was prepared in PBS.
2. A swab supplied with the test kit was dipped into the brain homogenate
3. Then the swab was transferred to the assay diluent for extraction.
4. After 1 minute, four drops of the sample were added to the sample well of the test kit using the supplied dropper.
5. Finally, results were read within 5 to 10 min after application of the sample

Test procedure with a 10% brain homogenate specimen (Anigen leaflet 2010)

With this procedure a 10 % brain homogenate was prepared in PBS and then the remaining steps described above by Markotter *et al.* (2009) were followed. This is illustrated in Figure 5.

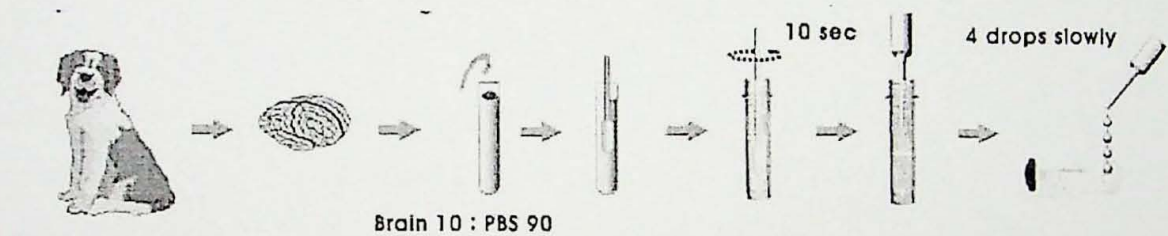


Figure 5. Schematic presentation of procedures described by Anigen leaflet 2010

3.4. Data collection and analysis

Data on sex, species, age, vaccination status, spatial and temporal distribution of confirmed cases were collected from the laboratory record book at EHNRI. The data collected covered the period from September 15th, 2008 to September 15th, 2011. Retrospective data of one year (September 15th, 2010 to September 15th, 2011) about PEP in humans were collected from daily human rabies records of the institute. Data on dog ownership, number of humans exposed to a rabid animal and clinical symptoms of dogs were recorded during the study period (March to September 2011). Furthermore, annual reports of rabies control in dogs were taken from urban agricultural extension core process (UAECP), Addis Ababa, Ethiopia. For analysis data was filled in excel spread sheet and imported to SPSS 18 statistical software program. Epidemiological data was

analyzed using descriptive statistics and chi-square test. Two by two contingency table was used to determine the diagnostic performance of the RIDT.

3.1 Rabies diagnosis

A total of 537 brain samples were examined during 2008-2011. Of this total samples, 326 (60.7%) were positive for rabies. Rabbits were more frequently diagnosed as carnivores (94.5%) as in other animal species. The proportion of rabid dogs was 87.2% from total samples followed positive for rabies, that followed by cats, 5.1%. The frequencies of occurrence of rabid in other species were very limited (Table 2).

Table 2. Frequency of rabies cases by species diagnosed in RIDT, samples 2008-2011 (n=537)

Species	Number positive	percent
Dogs	285	87.2
Cats	17	5.1
Deerkeys	16	2.3
Birds	12	2.1
Foxes	11	1.3
Mink dogs	6	0.8
Hyenas	4	0.6
Hares	2	0.3
Guinea	1	0.1
Crocod	1	0.1

3.1. Spatial and temporal distribution of rabies

The distribution of samples and species for rabies diagnosis and rabies localised cases were defined according to administrative regions. Of 537 samples submitted, in which each region was followed during 2008-2011, 386 (71.8%) were from Acre State, 19.2% (24-109) were from Roraima and 1.9% (2-17) from Southern Nations, Nationalities and People (SNNPR). Eight samples were originating from Amhara, Tigray and Benishangul. None were submitted from

4. RESULTS

4.1. Rabies in animals

A total of 935 brain samples were examined during 2008-2011. Of this total samples, 726 (77.6%) were positive for rabies. Rabies was more frequently diagnosed in carnivores (94.5%) than in other animal species. The proportion of rabid dogs was 87.2% from total samples confirmed positive for rabies virus followed by cats, 5.1%. The frequencies of occurrence of rabies in other species were very limited (Table 2).

Table 2. Frequency of rabies cases by species diagnosed at EHNRI, Ethiopia, 2008-2011 (n=726)

Species	Number positive	percent
Dogs	633	87.2
Cats	37	5.1
Donkeys	16	2.2
Bovines	15	2.1
Foxes	11	1.5
Monkeys	6	0.8
Hyenas	4	0.6
Horses	2	0.3
Goats	1	0.1
Civets	1	0.1

4.1.1. Spatial and temporal distribution of rabies

The distribution of sample submission for rabies diagnosis and rabies confirmed cases were assessed according to administrative regions. Of 881 samples submitted in which their region was registered during 2008-2011, 78% (N=687) were from Addis Ababa, 19.2% (N=169) were from Oromiya and 1.9% (N=17) from Southern Nations, Nationalities and People (SNNP). Eight samples were originated from Amhara, Tigray and Somali (Table 3). None were submitted from

the remaining administrative regions including Dire Dawa city. In Addis Ababa, lower numbers of samples were submitted from Addis Ketema (36), Akaki Kality (42), Arada (45), Kirkos (42) and Lideta (34) sub-cities. The numbers of samples submitted from other sub-cities of Addis Ababa (Bole (73), Gullelle (98), Kolfe Karanyo (140), Nefas Silk Lafto (88) and Yeka (84)) were higher (Figure 6). Samples submitted from Oromiya special zone around Addis Ababa city was the highest compared to other zones out of Addis Ababa (Figure 7)

Table 3. Sample submission by region (n=881)

Region	Number tested	percent
Addis Ababa	687	78
Oromiya	169	19.2
SNNP	17	1.9
Amhara	6	0.7
Somali	1	0.1
Tigray	1	0.1

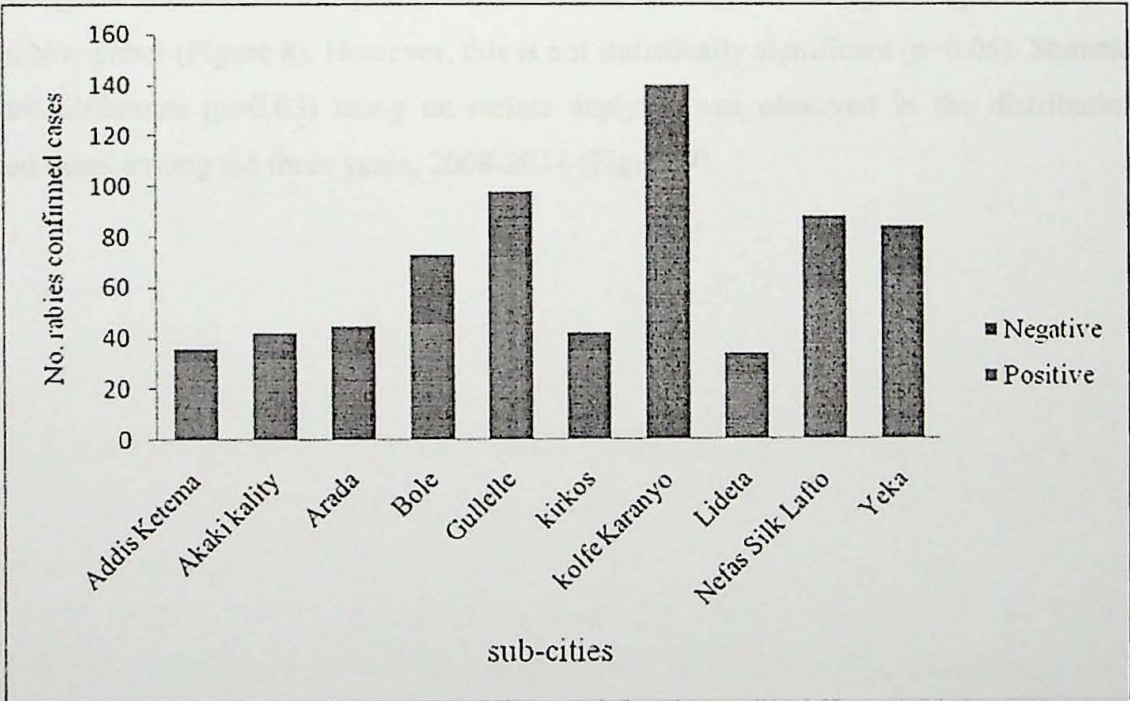


Figure 6. Rabies confirmed cases in Addis Ababa sub-cities, Ethiopia, 2008-2011

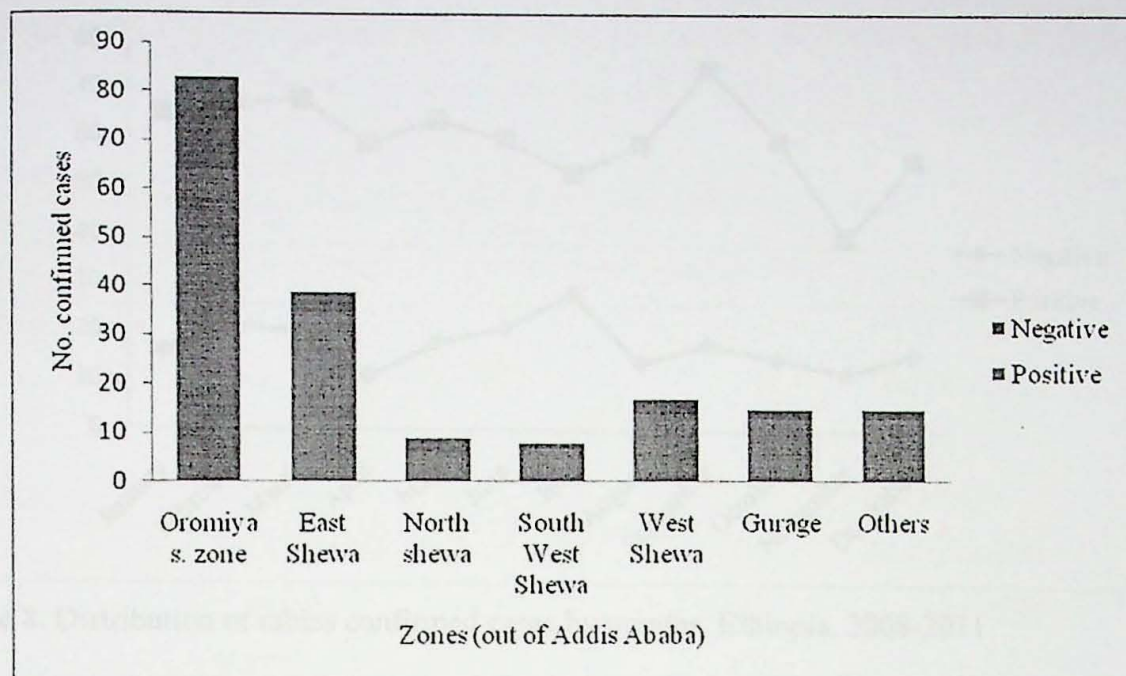


Figure 7. Rabies confirmed cases out of Addis Ababa, Ethiopia, 2008-2011

Rabies cases were reported throughout the year. Slight variations in the distribution of positive cases among the months of the year were observed, the highest being in September and the lowest in November (Figure 8). However, this is not statistically significant ($p=0.05$). Statistically significant difference ($p=0.03$) using univariate analysis was observed in the distribution of confirmed cases among the three years, 2008-2011 (Figure 9).

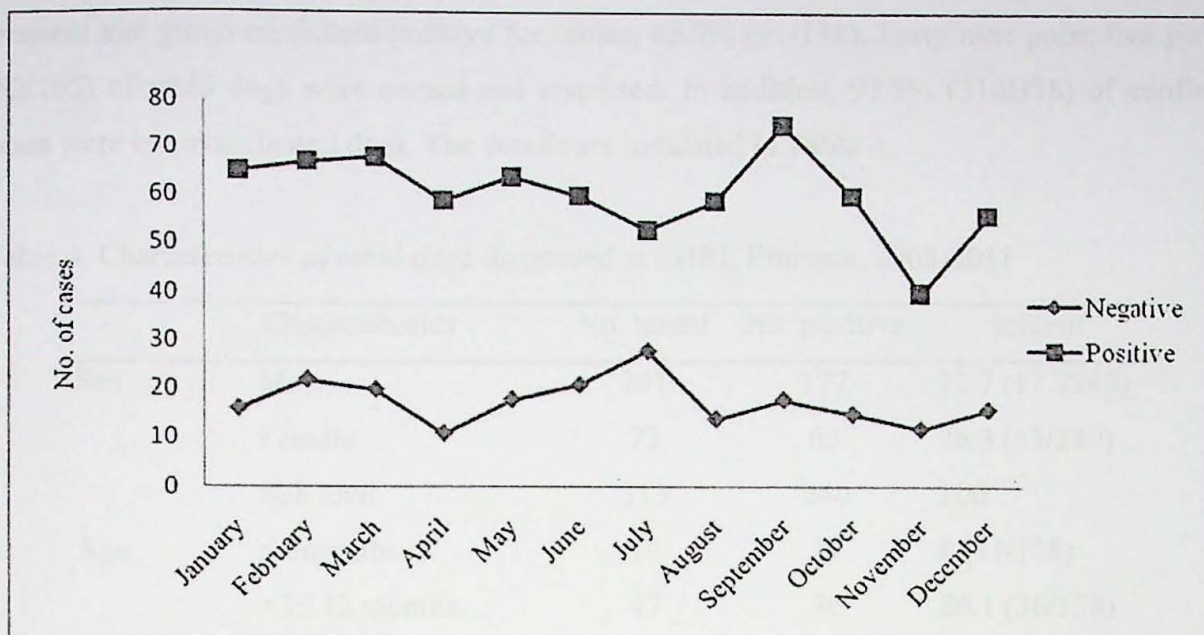


Figure 8. Distribution of rabies confirmed cases by months, Ethiopia, 2008-2011

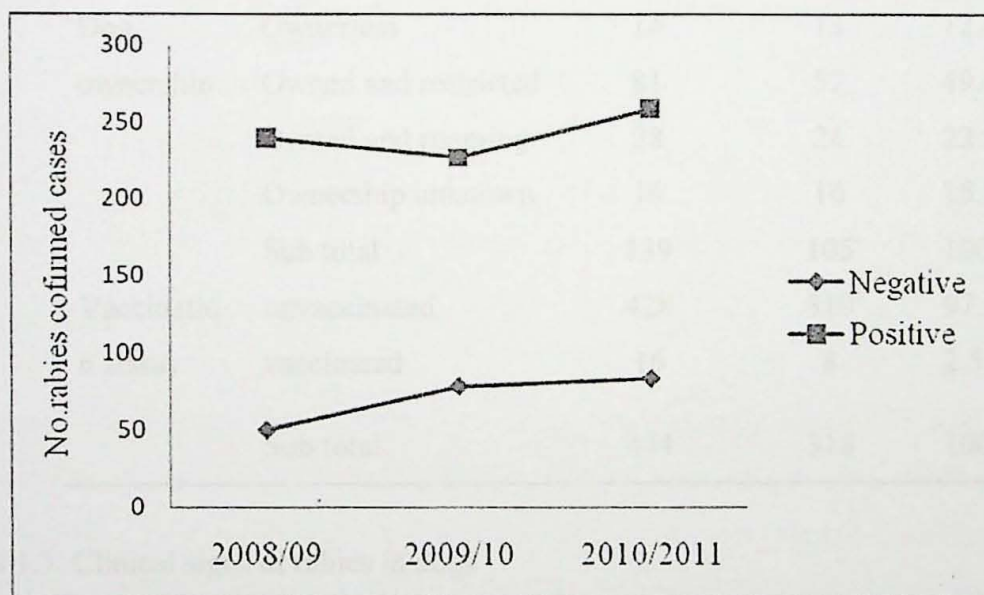


Figure 9. Distribution of rabies confirmed cases by years, Ethiopia, 2008-2011

4.1.2. Characteristics of rabid dogs

Since dogs are the most important animal species diagnosed with rabies, further analysis of characteristics of rabid dogs i.e. sex, age, ownership and vaccination status were made. The proportion of rabid male dogs was 73.7% (177/240). Adult dogs above 1 year were the most

frequent age group confirmed positive for rabies, 65.9% (91/138). Forty nine point five percent (52/105) of rabid dogs were owned and restricted. In addition, 97.5% (310/318) of confirmed cases were in unvaccinated dogs. The details are indicated in Table 4.

Table 4. Characteristics of rabid dogs diagnosed at EHRI, Ethiopia, 2008-2011

	Characteristics	No. tested	No. positive	percent
Sex	Male	241	177	73.7 (177/240)
	Female	72	63	26.3 (63/240)
	Sub total	313	240	100
Age	≤ 3months	19	11	8 (11/138)
	>3≤ 12 months	47	36	26.1 (36/138)
	>12 months	126	91	65.9 (91/138)
	Sub total	192	138	100
Dog ownership	Ownerless	14	13	12.4 (13/105)
	Owned and restricted	81	52	49.5 (52/105)
	Owned and roaming	28	24	22.9 (24/105)
	Ownership unknown	16	16	15.2 (16/105)
	Sub total	139	105	100
Vaccination status	unvaccinated	428	310	97.5 (310/318)
	vaccinated	16	8	2.5 (8/318)
	Sub total	444	318	100

4.1.3. Clinical signs of rabies in dogs

Incubation periods of 12, 16 and 19 days were recorded in dogs bitten on head and neck regions by other rabid dogs. In two cases, incubation periods of 24 and 29 days were recorded with the site of bite being not identified. In cattle, incubation periods of 24, 25 and 31 days were recorded. In the first two cases the biting sites were on the head region and the latter on the leg. Courses of illness ranged from 1 to 8 days in dogs (N=27), with a mean of 3.85 days. The furious type of rabies with clinical signs of unusual behavior, restlessness and excitability was the most common

form of dog rabies reported during the study period, whereas the paralytic form was less frequently reported (Table 5).

Table 5. Clinical signs of rabies in dogs (n=60)

Clinical signs	Number	Percent
Unusual behavior	48	80.0
Restlessness and excitability	30	50.0
Loss of appetite	27	45.0
Salivation	11	18.3
Wandering from home	8	13.3
Drooping of jaw	6	10.0
Hydrophobia	5	8.3
Paralysis of hind limb	5	8.3
Self inflicting	4	6.7
Difficulty in swallowing	2	3.3
Chocking	2	3.3

4.1.4. Dog rabies control in Addis Ababa

Well organized strategy in the control of rabies are generally lacking in Ethiopia. Low dog vaccination coverage, free movement of dogs, lack of rules and regulations for dog ownership and uncontrolled food disposal are some of the reasons for wide spread of rabies in the country. Generally vaccination, poisoning of unconfined dogs, animal birth control and public awareness using different mass media like television, radio, and pamphlet were used in attempt to control rabies in Addis Ababa during 2008-2011(Table 6). Vaccination of dogs against rabies is provided by government veterinary services and private sectors. The vaccination coverage by private sector is not included in this study as there is no reporting system for veterinary private practices. Animal birth control was conducted by collaboration between the government and non-government organizations.

Table 6. Dog rabies control in Addis Ababa, 2008-2011 (Dog population estimate=250,000-350,000)

Years	No. dogs vaccinated	No. dogs poisoned
2008/09	4468	6684
2009/10	6809	9544
2010/11	4048	13807

4.2. Post-exposure prophylaxis in humans

Post-exposure prophylaxis for humans is recommended from EHNRI, in confirmed rabies cases, exposure to unknown/unidentified animals and killed/dead animals of non confirmed cases. It is also prescribed during the 10 days observation of dogs suspected of rabies. The majority of the PEP was given to humans bitten by animals with an unknown rabies status. Only 28% (633/2286) of the PEP was associated with confirmed rabies cases (Figure 10). A total of 2345 people received post-exposure prophylaxis during the year 2010/2011 against rabies after being exposed to different species of animals and humans suspected or confirmed to be rabid in and around Addis Ababa (Table 7). The most important animal species responsible for PEP was dogs. Post-exposure prophylaxis due to exposure to suspected humans was considerable. The contributions of other domestic and wild animals were minimal.

Nerve tissue vaccine (NTV) produced from rabies virus infected brain of sheep was the most common 81% (1887/2337) vaccine type used for PEP (Figure 11). This vaccine is locally produced at EHNRI. The use of cell culture vaccine for PEP was limited (19%). No immunoglobulin has been available for use in treating rabies in the country to our knowledge.

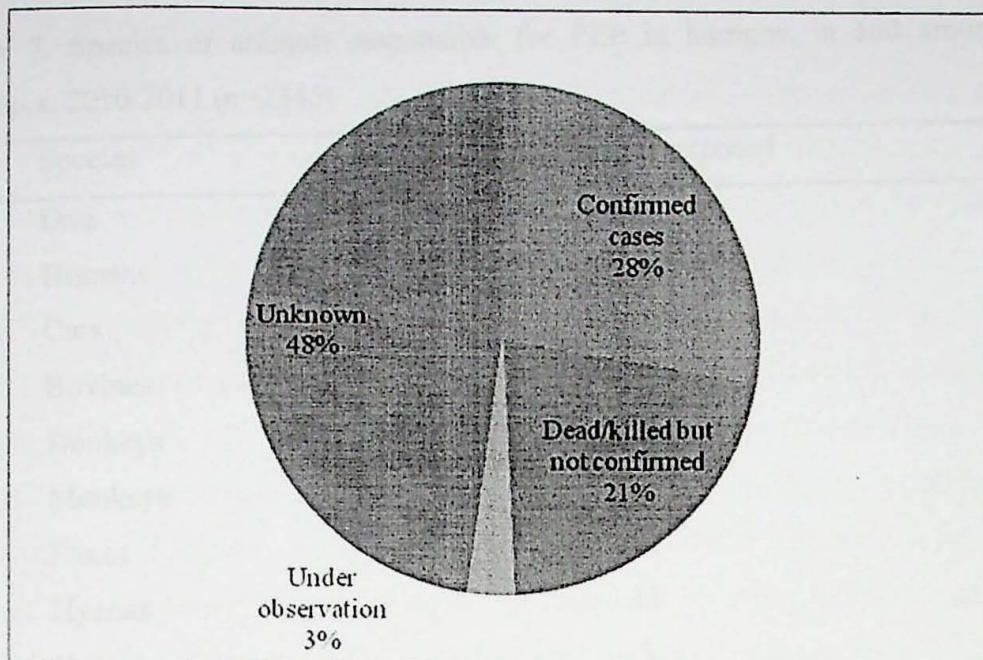


Figure 10. PEP in relation to confirmed and non confirmed cases, in and around Addis Ababa, Ethiopia, 2010/2011

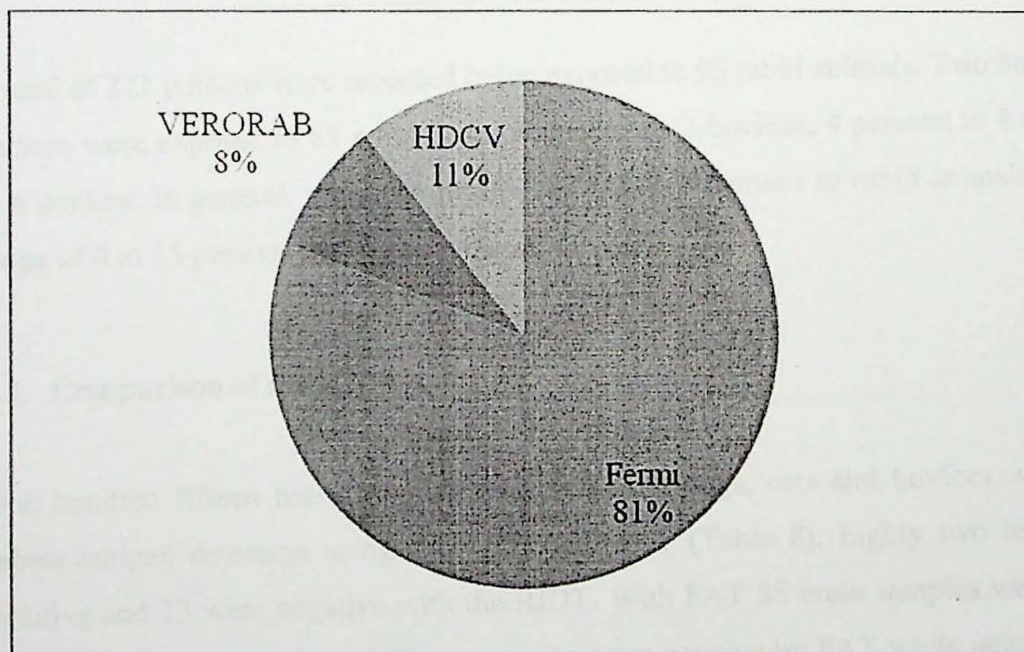


Figure 11. Types of vaccines used for PEP, in and around Addis Ababa, Ethiopia, 2010/2011

Table 7. Species of animals responsible for PEP in humans, in and around Addis Ababa, Ethiopia, 2010/2011 (n=2345)

Species	No. of humans exposed	percent
Dog	2054	87.6
Humans	93	4
Cats	61	2.6
Bovines	55	2.3
Donkeys	35	1.5
Monkeys	15	0.6
Foxes	12	0.5
Hyenas	11	0.5
Horses	3	0.1
Goats	2	0.1
Sheep	2	0.1
Cheetah	2	0.1

A total of 271 persons were recorded being exposed to 95 rabid animals. Two hundred fifty seven persons were exposed to 88 rabid dogs, 9 persons to 2 bovines, 4 persons to 4 cats and 1 person to a donkey. In general, the mean number of exposed humans to rabid animals was 2.85 with a range of 0 to 15 persons per a rabid animal.

4.3. Comparison of the RIDT and FAT

One hundred fifteen brain samples collected from dogs, cats and bovines were examined for rabies antigen detection using the RIDT and FAT (Table 8). Eighty two brain samples were positive and 33 were negative with the RIDT. With FAT 85 brain samples were positive and 30 brain samples were negative. Three samples were positive by FAT while negative by the RIDT. The sensitivity and specificity of the RIDT were 96.5 (95% CI: 90.0 – 99.3%) and 100% (95% CI: 88.4 – 100%), respectively compared to FAT (Table 9). Seventy samples were examined immediately after collection and 45 were examined after storage in -20°C for 1 to 112 days. The frozen samples were found easy in preparation of homogenates in the diluents as they liquefy

after storage. We observed differences in the intensities of color formation in the test zone from slightly visible to deep purple. Lines in the control zone always appeared deep purple irrespective of the test zone. Two samples were analyzed twice by the RIDT, immediately after collection and after being preserved in deep freeze (-20°C). Both samples were positive by the FAT. In the first test, immediately after collection, one sample was negative and the other one was positive with the RIDT. However, the intensity of color formation was weak in the positive one. After the samples were being kept in -20°C , the negative one in the previous test remained negative while the one with weak color formation gave a strong purple color. In one sample, we found absence of diffusion in the plate for three minutes and addition of a drop of phosphate buffer solution facilitated the diffusion. We also found no change in the results over extended period of time beyond 10 minutes recommended by the manufacturer for at list 24 hours. Rather the intensity of color formation relatively became higher and the results became clearer.

Table 8. Animal species tested for comparison of the RIDT and FAT

Species	Number tested	Results	RIDT	FAT
Dogs	107	Positive	80	82
		Negative	27	25
Cats	6	Positive	1	2
		Negative	5	4
Cattle	2	Positive	1	1
		Negative	1	1

Table 9. Two by two contingency table for comparison of the RIDT and FAT

		FAT		
		positive	Negative	
RIDT	positive	82	0	82
	Negative	3	30	33
		85	30	115
Sensitivity= 82/85=96.5%				
Specificity=30/30=100%				

Four brain samples were tested using FAT and the procedures indicated in Anigen leaflet 2004 and Anigen leaflet 2010 of the RIDT. Two samples which were positive by FAT were found also positive by the procedure indicated in Anigen leaflet 2004. However, these samples were negative using the procedure indicated by Anigen leaflet 2010 within the recommended 10 minutes. One of these negative samples indicated positivity after extended time (within 24 hours). From the remaining two samples, one was positive and the other one was negative by FAT and the same results were obtained by the two procedures of the RIDT. However, a very weak indication of positivity was observed in the procedure indicated by Anigen leaflet 2010. These results are summarized in Table 10. Another four samples were examined using procedures mentioned by Kang *et al.* (2007), Markotter *et al.* (2009), Anigen leaflet 2004 and the FAT. The results obtained using these three procedures were in total agreement with results obtained by FAT. The entire four samples positive by the FAT were also positive by the three procedures. However, the intensity of color formation was found more deep purple in the procedure indicated by Kang *et al.* (2007) and Anigen leaflet 2004 compared to Markotter *et al* (2009). The procedure in Anigen leaflet 2004 (a method used in this study) was relatively short by avoiding the preparation of brain homogenate in PBS. It also reduces time and materials to be used in this process. The intensity of color formation was also good. No any ambiguity was observed in decision making on the results.

Table 10. Comparison of FAT and procedures by Anigen leaflets

		FAT	
		Positive	Negative
Anigen Leaflet 2004	Positive	3	0
	Negative	0	1
Anigen Leaflet 2010	Positive	1	0
	Negative	2	1

5. DISCUSSION

Rabies is endemic in Africa and Ethiopia and represents a serious veterinary and public health burden. In the present study, we showed that canines remain to be important source of rabies to humans and other animals in Ethiopia. The vast majority of confirmed rabies cases (87.2%) were in dogs. This result is consistent with other studies from Africa, in which 80 to 95% of rabies cases were found in dogs (Edelsten, 1995; Kitala *et al.*, 2000; Tefera *et al.*, 2002; WHO, 2005). The results of our study demonstrate the principal role of dogs in the maintenance and transmission of rabies to other animals and humans. The occurrences of rabies in wildlife species like hyena, monkeys, foxes and civet which could be found close to residential areas are a high risk both for domestic animals and humans. The lower values obtained in this study is due to limited number of wild animals available for diagnosis compared to domestic ones. Generally, however, dog mediated rabies is more important in Ethiopia than the wildlife rabies as is elsewhere in Africa.

From the total suspected cases submitted, the proportion of positive cases was 77.6%. This result is higher than reports from Chad (72%), Kenya (51%), South Africa (49%) and Swaziland (50%) (Kretzmann, 1993; Dlamini, 1995; Kitala and McDermott, 1997; Kayali *et al.*, 2003). However it is lower than other report in 2008 from Chad (89%). The high proportion of rabies positive cases among the suspected cases shows that the occurrence of higher incidence of the disease in the study area. It is also due to the submission of samples to the laboratory with typical clinical signs of rabies, mainly furious form.

The majority of the samples (78%) submitted for rabies diagnosis was from Addis Ababa city. This would be mainly because of the availability of rabies diagnostic laboratory in the city. This is further implicated by high number of samples submitted from Oromiya special zone which is in the immediate vicinity of Addis Ababa city. Regions in distant locations from the laboratory, Amhara, Tigray and Somali, were with very limited (0.9%) number of samples submitted to the laboratory. Variations in the number of samples submitted from Addis Ababa sub-cities were also observed. Lower percentage (29.2%) of samples submitted was from Addis Ketema, Akaki Kality, Arada, Kirkos and Lideta sub-cities. Seventy point eight percent were from the remaining sub-cities. Sub-cities with the lower proportions except Akaki Kality have certain common

characteristics which could affect incidence of rabies and subsequently sample submission. They are found in the central parts of the city. These sub-cities are densely populated (26,900 to 43,200/ km²) (www.addisababacity.gov.et/index) and are mainly business areas. Therefore, the dog populations in these sub-cities are probably lower and the movement of dogs may also be restricted since there is limited space to keep dogs. Akaki Kality is in the opposite end of the city from the rabies diagnostic laboratory which is found in Gullele. In this sub-city, the low number of samples is probably due to its far distance from the laboratory. The remaining sub-cities with higher percentage of sample submission are less densely populated (1,400 to 10,500/km²) (www.addisababacity.gov.et/index), mainly residential areas and have wider boundaries with the surrounding rural areas. Therefore, the dog population in the area is probably high and there is also high probability for migration of dogs from the surrounding rural areas in to the sub-cities. Generally, distance affected the number of samples submitted to the laboratory. This indicates the need for rabies diagnosis at various parts of the country.

Slight fluctuations in the occurrence of rabies among the months of the year were observed. The peak was in September and the lowest was in November. This peak is probably following peak breeding season of dogs which is from June to September. The decline in November is probably due to the mass destruction of unconfined dogs in response to increased movement of dogs during mating and increased rabies cases (personal communication). However, this was not statistically significant ($p=0.05$).

The proportion of male dogs (77%) submitted for rabies diagnosis was higher than the female dogs (23%). Similarly, more male (73.7%) than female (26.3%) rabid dogs were found. The predominance of male rabid dogs was also reported from other parts of the world (Brooks, 1990; Eng *et al.*, 1993; Kayali *et al.*, 2003, Du'rr *et al.*, 2008). This is probably due to a preference in keeping male dogs than females. Attraction of male dogs by female dogs during estrus, large puppies at birth, high cost of spaying and the belief that males are better in guarding the owner's house probably leads to the preference of male dogs than females.

The majority (65.9%) of the confirmed rabid dogs were more than one year of age. Age group over three months up to 12 months accounted for 26.1%. Only 8% of the confirmed cases were 3

months or younger. Higher rabies cases in older dogs were also reported by different authors elsewhere in Africa. Foggin (1988), in Zimbabwe, reported 76% of confirmed rabies cases in dogs that were over 12 months of age and 4 % in dogs younger than 3 months of age. Brooks (1990), in Zimbabwe, also noted a large proportion of older dogs with rabies (66%) even though the prevalence in younger ones was higher than our finding. He observed 20% rabies cases in dogs 3 months of age or younger. Similarly, Kayali *et al.*, (2003), in Chad, noted that large proportions (59%) of rabid dogs were older than one year and 3% were in dogs less than three months of age. We expected younger dogs to have a lower incidence of rabies, since they have less tendency to fight with other dogs and are less mobile. Thus, they have fewer contacts with rabid dogs.

The majority of rabies cases were observed in non-vaccinated dogs as vaccination has a protective effect. However, out of the 16 vaccinated dogs submitted, 8 were positive for rabies. This is probably because of vaccination failure associated with vaccine handling and storage. The existence of Lagos bat virus in dogs (Mebatsion *et al.*, 1992) could be another reason for the occurrence of rabies in vaccinated dogs as vaccination is not protective against this genotype.

Rabies confirmed cases were highest in owned and restricted dogs (49.5%) followed by owned and roaming dogs (22.9%). In total, the proportion of owned dogs among tested ones was 72.4%. The proportions of ownerless and those in which their ownership could not be determined were 12.4% and 15.2%, respectively. In support of our finding Du`rr *et al.* (2008) reported that 71% of confirmed rabid cases were in owned dogs in Chad. Similarly, Kayali *et al.* (2003) reported much higher proportion (85%) of owned rabid dogs than ownerless ones in the same country. The highest proportion of rabies in owned and restricted dogs in this study was probably due to the accessibility of this group of dogs for laboratory diagnosis. The concern of dogs' ownership is likely to be higher in owned and restricted dogs in submission of samples to the laboratory. However, Foggin (1988) reported a much higher proportion (66%) of ownerless rabid dogs in Zimbabwe. Kitale and McDermott (1997) reported 46% of rabid dogs were in those in which the owners could not be identified.

The incubation period recorded in this study (12 to 29 days in dogs and 24 to 31 in cattle) is in a range previously described for dogs (10 days to 6 months) and cattle (25days to more than 5 months) (CFSPH, 2009). The course of illness (1-8days) is also consistent with the known course of illness elucidated so far (2-12 days) (Foggin, 1988; Lyles and Rupprecht, 2007). In this study, the most common signs of rabies recorded from 60 rabies confirmed dogs were suggestive of the furious form of the disease. Unusual behavior (aggression, exaggerated response to stimuli, attacking of anything; human, animal or inanimate objects and abnormal barking) accounted for 80% in rabid dogs. This shows that detection of these signs can be used as an alarm by the community to suspect the occurrence of rabies in dogs. In 50% of the cases, restlessness and excitability were recorded. However, clinical signs suggestive of the paralytic form of the disease were fewer. Salivation, drooping of jaw and paralysis of hind limb accounted for 18.3%, 10% and 8.3% respectively. In consent with our observation, Foggin (1988), reported 75% of furious form in Zimbabwe. Sixty one point eight percent and 62% of aggressiveness in rabid dogs were reported in Chad and Kenya respectively (Kitala *et al.*, 2000; Kayali *et al.*, 2003). The high proportion of furious form of rabies in this study could be because of the actual high proportion of this form of rabies in the area. Furious form of rabies is probably well realized by the community than the paralytic form. In addition, people are more interested in submission of rabid dogs of furious form as this is more related to human exposure through bite than the paralytic form.

The occurrence of confirmed rabies cases was inversely related to the vaccination coverage during the three years period. A lower number of rabies cases were observed in 2009/10 compared to the two years. In the same year increased vaccination coverage was observed. This indicates that vaccination of dogs can play a role in the reduction of number of rabies cases.

The majority of PEP (72%) in humans was administered without having a confirmed rabies case. Only 28% of PEP was against exposure to confirmed rabies cases. The low number of PEP against confirmed animal cases is probably associated with lack of awareness by the community in sample submission to the laboratory. Majority of the 21% of dead/killed animals responsible for PEP could be accessible for laboratory diagnosis. It also indicates low effort to identify and follow the animal, particularly dogs, by the victims for the recommended period of quarantine

following exposure. Besides, this retrospective study indicates the high possibility of unnecessary administration of PEP in non-confirmed rabies cases. This is particularly unfortunate where nerve tissue derived rabies vaccines with their associated risks and discomforts are still in common use. Moreover, unnecessary cost of vaccination is a big burden to the country in general, and individuals in particular.

Rabid or rabies suspected dogs were found to be the main (87.6%) responsible animal for PEP in humans followed by exposure to rabies suspected humans (4%) in and around Addis Ababa. Previous report from Ethiopia also indicated that PEP in humans (92%) was due to exposure to rabid or rabies suspected dogs (Yimer *et al.*, 2002). Higher proportion of human cases (97%) due to dog bites was reported from Kenya (Kitala and McDermott, 1997). Other report from India indicated that 90.5% of the positive human cases were due to dog bites (Sudarshan *et al.*, 2006). As majority of confirmed rabid animals were dogs, it is expected that dogs are the main cause for transmission of the disease to humans. Even though, human-to-human transmission is extremely rare (Haaheim *et al.*, 2002; Carter and Saunders, 2007), individuals in close contact with rabies suspected humans received PEP in fear of acquiring the disease.

Nerve tissue vaccine (Fermi type vaccine) was widely (81%) used for PEP in humans in and around Addis Ababa during 2010/2011. In 1973, the WHO recommended that Fermi vaccines, crude rabies vaccines prepared from a variety of animals and partially inactivated with phenol, be discontinued because they contain residual, live, fixed rabies virus (WHO, 1973). Despite its serious side effects, the Fermi type vaccine is still widely used in and around Addis Ababa due to its low cost and availability. We believe that the situation in the rural area of the country might be worse than the proportion observed for Addis Ababa and its surroundings. Cell culture vaccines are available only in a few private pharmacies and NGOs in the capital city and it is less likely for the availability of cell culture vaccine in the wider rural areas of the country.

In the present study, it was recorded that a mean of 2.85 with a range of 0-15 persons were exposed to a rabid animal. This finding is consistent with the report from Chad in which 2.3 persons per rabies case were reported (Kayali *et al.*, 2003) and a range of 0-13 persons of rabies dog's bite in Mexico (Eng *et al.*, 1993).

In this study, the RIDT kit had 96.5% sensitivity and 100% specificity compared to FAT. Three samples which were positive by FAT were negative by the RIDT, showing 3.5% false negativity. Previous study by Kang *et al.* (2007) showed a slightly lower sensitivity (91%) and the same specificity (100%). Markotter *et al.* (2009) reported higher sensitivity and specificity (100%). However, all of test characteristics were obtained using different procedures. The 3.5% false negativity observed in this study shows the need for further analysis of samples using FAT in case of negative results. Difference in sensitivity and intensity of color formation using different procedures were observed in this study. With the procedure in leaflet 2010, 2 out of 4 samples were false negative when compared to FAT. The intensity of color formation in the procedure indicated by Markotter *et al.* (2009) was less than the two procedures Kang *et al.* (2007) and Anigen leaflet 2004 even though, the results obtained were comparable. This variation in the sensitivity and intensity of color formation could be due to the concentration of the virus applied on the kit as different dilution and amount of samples were used by the different procedures. Furthermore, the more deep purple color formation observed after keeping the sample in -20°C could be due to better extraction of the antigens in the diluent. Samples liquefy after freezing and hence make better homogenate in the diluent. A standardized protocol needs to be followed for the RIDT, as with different available procedures a varying level of sensitivity and intensity of color formation was observed

From our experience, laboratory results are strong to convince that individuals exposed to rabies suspected animal should take PEP or not. Sometimes, it is difficult to convince the dog owners, particularly for owned and restricted dogs and vaccinated dogs, that their dogs are died of rabies even after confirmation with FAT. For the owned and restricted dogs, they believe that their dogs have no contact with any other dogs and thus cannot acquire rabies. In the same manner, in vaccinated dogs, they do not expect any rabies case. So in areas where there is no laboratory for rabies diagnosis this test can help as screening test and in positive results PEP can be initiated. Post-exposure prophylaxis must not be avoided in negative test results by RIDT. Further confirmation with FAT is mandatory as negative result with this test is unreliable.

A major advantage of the RIDT is that it requires no additional equipment like microscope, incubator and refrigerator as the case in FAT. The test kit is complete with necessary materials for the diagnosis. It can be stored at room temperature, simple to perform and rapid technique in

6. CONCLUSION AND RECOMMENDATIONS

Rabies is widely distributed in Addis Ababa and its surrounding. It was diagnosed in various species of domestic and wild animals. Since this study was based on passive surveillance the actual number and species involved could be higher than this. Domestic dogs were the most frequently affected species with rabies and they were the most responsible species for post-exposure prophylaxis in humans. Rabies occurs throughout the year with slight variation among the months of the year in the study area. The number of samples submitted to the laboratory and the subsequent rabies cases was highly influenced by the distance of the locations from laboratory. The distant areas submit low number of samples. Many rabies cases were observed in male, owned, dogs above 3 months of age and that were non-vaccinated. These factors are generally important in designing rabies control in dogs. Majority of people were receiving post-exposure prophylaxis (PEP) against exposure to animals where there rabies status is not known. Fermi type of vaccine is widely used as PEP in and around Addis Ababa in spite of its side effects and multiple injections required due to its low cost and availability. The use of this vaccine should be discontinued. Rabies diagnostic laboratory is limited to EHNRI in Ethiopia. Since the RIDT is feasible to use in field condition it could be an option for rabies surveillance and targeted post-exposure prophylaxis in areas where there is no rabies diagnostic laboratory.

Hence the following points are forwarded as recommendations:

- At present targeting the main reservoir and transmitter species, the domestic dog, in reduction of rabies transmission is vital in Ethiopia.
- Rabies diagnosis should be established in various parts of the country to have better understanding of rabies epidemiology and for targeted post-exposure vaccination in humans.
- Enhancing people awareness to submit rabies suspected samples to rabies diagnostic laboratory and making them aware of in identifying and following animals after being exposed is very essential in reduction of unnecessary administration of PEP.
- Dogs above 3 months of age should be considered in rabies control strategy as many of the rabies cases were found in this age group.

- The widely used Fermi type vaccine should be replaced by cell culture vaccine. Production of cell culture vaccine in the country or its importation should be encouraged to have consistent supply. The supply of this vaccine should also be at affordable price to the low income individuals.
- Since there is variation in sensitivity and intensity of color formation to indicate positivity using different procedures, the RIDT procedures should be standardized.
- Samples used in this study should be further tested using different techniques including RTCIT, monoclonal typing, RT-PCR and sequencing. This will help in understanding of the molecular epidemiology (phylogeny) of rabies virus in Ethiopia.

7. REFERENCES

- AfroREB (2008): Fighting rabies in Africa. *Vaccine*, **26**: 6295-98.
- Ali, A., Mengistu, F., Hussen, K., Getahun, G., Deressa, A., Yimar, E. and Tafese, K. (2010): Overview of rabies in and around Addis Ababa, in animals examined in EHNRI zoonoses laboratory between, 2003 and 2009. *Ethiop. Vet. J.*, **14**: 91-101.
- Andrews, A. H., Blowey, R. W., Boyd, H. and Eddy, R. G. (2004): Bovine medicine, diseases and husbandry of cattle, 2nd edition. Blackwell Science Ltd, UK, pp. 1164-1171.
- Arellano-Sota, C. (1988): Biology, ecology and control of vampire Bat. *Reviews of infectious diseases*, **10**: 1988.
- Baer, G. M. (2007): The History of Rabies. In: Jackson, A. C. and Wunner, W. H. (ed): rabies, 2nd edition. Elsevier inc, San Diego, CA, pp. 1-22.
- Belotto, A., Leanes, L. F, Schneider, M. C., Tamayo, H., Correa, E. (2005): Overview of rabies in the Americas. *Virus Res*, **111**(1): 5-12.
- Bjorvatn, B. and Haukenes, G. (2002): Rabies virus. In: Haaheim, *et al* (ed): A practical guide to clinical virology, 2nd edition. John Wiley & Sons, Ltd., England, pp. 245-249.
- Blanton, J. D., Palmer, D. and Rupprecht, C. E. (2010): Rabies surveillance in the United States during 2009. *J Am Vet Med Assoc*, **237**(6): 646-657.
- Brochier, B., Costy, F. and Pastoret, P. (1995): Elimination of fox rabies from Belgium using a recombinant vaccinia-rabies vaccine. *Veterinary Microbiology*, **46**:269-279.
- Bronnert, J., Wilde, H., Tepsumethanon, V., Lumlertdacha, B. and Hemachudha, T. (2007): Organ Transplantations and rabies transmission. *Journal of Travel Medicine*, **14**: 177-180.
- Brooks, R. (1990): Survey of the dog population of Zimbabwe and its level of rabies vaccination. *Veterinary Record*, **127**: 592-596.
- Carter, J. B. and Saunders, V.A. (2007): Virology: principles and applications. John Wiley & Sons Ltd, England, pp. 175-184.
- CDC (2006): Protocol for postmortem diagnosis of rabies in animals by Direct Fluorescent Antibody Testing: A minimum standard for rabies diagnosis in the United States. http://www.cdc.gov/ncidod/dvrd/rabies/Professional/publications/DFA_diagnosis/DFA_protocol-b.htm Accessed on 10 January 2010.

- CDC (2011): CDC. Rabies in Bali, Indonesia. <http://wwwn.cdc.gov/travel/content>. updated: March 01, 2011. Accessed on 12 Marc 2011.
- CFIA (2011): Canadian Food Inspection Agency. www.inspection.gc.ca. Accessed on 11 March 2011.
- CFSPH (2009): Center for Food Security and Public Health. www.cfsph.iastate.edu. Accessed on 12 March 2011.
- Cleaveland, S., Kaare, M., Tiringa, P., Mlengeya, T. and Barrat, J. (2003): A dog rabies vaccination campaign in rural Africa: impact on the incidence of dog rabies and human dog-bite injuries. *Vaccine*, **21**: 1965-73.
- Courtin, F., Carpenter, T. E., Paskin, R. D. and Chomel, B. B. (2000): Temporal patterns of domestic and wildlife rabies in central Namibia stock-ranching area, 1986-1996. *Preventive Veterinary Medicine*, **43**: 13-28.
- CSA (2007): Central Statistical Agency. Addis Ababa, Ethiopia.
- David, D., Yakobson, B., Rotenberg, D., Dveres, N., Davidson, I. and Stram, Y. (2002): Rabies virus detection by RT-PCR in decomposed naturally infected brains. *Vet Microbiol.*, **87**(2):111-8.
- Deressa, A., Ali, A., Beyene, M., Newayesilassie, B., Yimer, E., Hussen, K. (2010): The status of rabies in Ethiopia: A retrospective record review. *Ethiop. J. Health Dev.*, **24**:127-132.
- Dlamini, R. X. (1995): Rabies in Swaziland. In: Proceedings of the Third International Conference of the Southern and Eastern African Rabies Group, Harare, Zimbabwe, 7-9 March, 1995. pp. 58-60.
- Dürr, S., Naïssengar, S., Mindekem, R., Diguimbye, C., Niezgoda, M., *et al.* (2008): Rabies diagnosis for developing countries. *PLoS Negl Trop Dis*, **2**(3): 1371-1378.
- ECDC (2009): European Center for Disease prevention and Control. Expert consultation on rabies post-exposure prophylaxis, Meeting report Stockholm, 15 January 2009.
- Edelsten, R. M. (1995): Epidemiology and control of rabies in Malawi. *Trop Anim Health Prod*, **27**(3):155-63.
- EHNRI (2010): Ethiopian Health and Nutrition Research Institute, standard operational procedure. Addis Ababa, Ethiopia.

- Eng, T. R., Fishbein, D. B., Talamante, H. E., Hall, D. B., Chavez, G. F. *et al.* (1993): Urban epizootic of rabies in Mexico: epidemiology and impact of animal bite injuries. *Bulletin of the World Health Organization*, **71** (5): 615-624.
- Ernst, S. N. and Fabrega, F. (1989): A time series analysis of the rabies control program in Chile. *Epidemiology and Infection*, **103**: 651-657.
- Ezeokoli, C. D. and Umoh, J. U. (1987): Epidemiology of rabies in northern Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**: 268-272.
- Fekadu, M. (1982): Rabies in Ethiopia. *American Journal of Epidemiology*, **115** (2): 266-273.
- Fishbein, D. B., Miranda, N. J., Merrill, P., Camba, R. A., Meltzer, M., *et al.* (1991): Rabies control in the Republic of the Philippines: benefits and costs of elimination. *Vaccine*, **9**: 581-588.
- Foggin, C.M. (1988): Rabies and rabies-related viruses in Zimbabwe: historical, virological and ecological aspects. Ph. D. thesis, University of Zimbabwe.
- Fooks, A. R., Johnson, N., Freuling, C. M., Wakeley, P. R., Banyard, A. C. Carlos, *et al.* (2009): Emerging technologies for the detection of rabies virus: challenges and hopes in the 21st century. *PLoS Negl Trop Dis.*, **3**(9): 530.
- Fraser, G. C., Hooper, P. T., Lunt, R. A. *et al.*, (1996). Encephalitis caused by a *Lyssavirus* in fruit bats in Australia. *Emerg Infect Dis*, **2**: 327-331.
- Fu, Z. F. (1997): Rabies and rabies research: past, present and future. *Vaccine*, **15**:24-28.
- Greiner, M. and Gardner, I. A. (2000): Epidemiologic issues in the validation of veterinary diagnostic tests. *Preventive Veterinary Medicine*, **45**: 3-22.
- Haaheim, L. R., Pattison, J. R. and Whitley, R. J. (2002): A practical guide to clinical virology. John Wiley & Sons Ltd, England, pp. 245-249.
- Hanlon, C. A., Niezgoda, M. and Rupprecht, C. E. (2007): Rabies in terrestrial animals. In: Jackson, A. C. and Wunner, W. H. (ed): Rabies, 2nd edition. Elsevier inc, San Diego, CA, pp 201-258.
- Heaton, P. R., Johnstone, P., Mcelhinney, M. L., Cowley, R., O'sullivan, E. and Whitby, J. (1997): Heminested PCR assay for detection of six genotypes of rabies and rabies-related viruses. *Journal of Clinical Microbiology*, **35**(11): 2762-2766.
- Helmick, C.G. (1983): The epidemiology of human rabies post-exposure prophylaxis, 1980-1981. *J Am Vet Med Assoc*, **250** (15), 1990-1996.

- Hübschle, O. J. (1988): Rabies in the Kudu Antelope (*Tragelaphus strepsiceros*). *Rev Infect Dis*, **4**: 629-633.
- <http://www.addisababacity.gov.et/index>. Accessed on 20 Sep, 2011.
- ICTV (2011): Master species list. <http://talk.ictvonline.org/files/ictvdocuments/m/msl/1231.aspx>. Accessed on 1 March 2011.
- Kammer, A. R. and Ertl, H. C. (2002): Rabies Vaccines: From the Past to the 21st century. *Hybridoma and Hybridomics*, **21**(2):2002.
- Kang, B., Oh, J., Lee, C., Park, B. K., Park, Y., *et al.* (2007): Evaluation of a rapid immunodiagnostic test kit for rabies virus. *J Virol Methods*, **145**: 30–36.
- Kayali, U., Mindekem, R., Yémadji, N., Oussiguéré, A., Na'issengar, S. *et al.* (2003): Incidence of canine rabies in N'Djaména, Chad. *Preventive Veterinary Medicine*, **61**:227–233.
- Kitala, P. M., and McDermott, J. J. (1997): The epidemiology of rabies in Machakos district, Kenya. In: Kitala, P. M., Perry, B., King, A. A. (ed.), Proceedings of the Southern and Eastern African Rabies Group (SEARG) meeting, Nairobi, Kenya, pp. 145–152.
- Kitala, P. M., McDermott, J. J., Kyule, M. N., and Gathuma J. M. (2000): Community-based active surveillance for rabies in Machakos District, Kenya. *Preventive Veterinary Medicine*, **44**:73-85.
- Knobel, D. L., Cleaveland, S., Coleman, Fèvre, E. M., Meltzer, M. I., *et al* (2005): Re-evaluating the burden of rabies in Africa and Asia. *Bull WHO*, **83**(5):360–8.
- Kretzmann, P.M. (1993): Problems associated with rabies diagnosis in a busy private practice. In: Proceedings of the Southern and Eastern African Rabies Group International Symposium, Pietermaritzburg, South Africa, 29-30 April, 1993, pp. 49-56.
- Kuzmin, I. V., Niezgoda, M., Franka, R. Agwanda, B., Markotter, W., *et al.* (2008): Lagos bat virus in Kenya. *J. Clin. Microbiol*, **46**: 1451–1461.
- Kuzmin, I. V., Mayer, A. E, Niezgoda, M., Markotter, W., Agwanda, B., Breiman, R. F., and Rupprecht, C. E. (2010): Shimon bat virus, a new representative of the Lyssavirus genus. *Biological Sciences*, **149**: 197-210.
- Lembo, T., Niezgoda, M., Velasco-Villa, A., Cleaveland, S., Ernest, E., Rupprecht, C. E. (2006): Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. *Emerging Infect Dis*, **12**: 310–313.

- Lembo, T., Hampson, K., Kaare, M. T., Ernest, E., Knobel, D., *et al.* (2010): The Feasibility of Canine Rabies Elimination in Africa: Dispelling doubts with data. *PLoS Negl Trop Dis.*, **4**: 626-635.
- Lyles, D. S. and Rupprecht, C. E. (2007): Rhabdoviridae. In: Knipe, D.M. and Howley, P.M. (ed). *Fields virology*, 5th edition. Lippincott Williams and Wilkins, pp. 1364-1408.
- Markotter, W., York, D., Sabeta, C.T., Shumba, W., Zulu, G., Le Roux, K., and Nel, L. H. (2009): Evaluation of a rapid immunodiagnostic test kit for detection of African lyssaviruses from brain material. *Journal of Veterinary Research*, **76**:257-262.
- McElhinney, L. M., Fooks, A. R. and Radford, A. D. (2008): Diagnostic tools for the detection of rabies virus. *EJCAP*, **18**(3): 224 - 229.
- Mebatsion, T., Cox, J. H. and Frost, J. W. (1992): Isolation and characterization of 115 street rabies virus isolates from Ethiopia by using monoclonal antibodies: identification of 2 isolates as Mokola and lagos Bat Rabies. *J Inf Diss.*, **166**: 972-977.
- Murphy, F. A., Gibbs, E. P. J., Horzinek, M. C. and Studdert, M. J. (1999): *Veterinary Virology*, 3rd edition. California academic press, USA, pp. 429-444.
- Newayesilassie, B., Yimer, E., Paulos, A., Bekele, A., Zewdie, B. and Beyene, M. (2004): Occurrence of rabies in humans, animals in Addis Ababa. *Ethiop. J. Biol. Sci.*, **3**: 59-67.
- OIE (2008): *Manual of diagnostic tests and vaccines for terrestrial animals*. Pp. 304-23.
- Pankhurst, R (1970): The history and traditional treatment of rabies in Ethiopia. *Med Hist*, **14**(4): 378-389.
- Panning, M., Baumgarte, S., Pfefferle, S. Meier, T., Martens, A., and Drosten, C. (2010): Comparative analysis of rabies virus RT-PCR and virus isolation in rabies patient samples. *J. Clin. Microbiol*, **10**:1128.
- Radostits, O. M., Gay, C. C., Hinchcliff, K.W. and Constable, P.D. (2007): *Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats*, 10th edition. Saunders, London, pp. 1384 -1394.
- Randall, D. A., Williams, S. D., Kuzmin, I. V., Rupprecht, C. E., Tallents, L. A. *et al.* (2004): Rabies in endangered Ethiopian wolves. *Emerging Infectious Diseases*, **10**: 2214-2217.
- Robles, C. G. and Miranda, L .J. (1992): Comparative Evaluation of the Rabies Fluorescent Antibody Test and Direct Microscopic Examination. *Phil J Microbiol Infect Dis*, **21**(2):69-72.

- SEARG (2011): Southern and Eastern African rabies group. www.searg.info/doku.php?id=aboutrabies. Accessed on 11 March 2011.
- Seifert, H. H. (1996): Tropical Animal Health. Kluwer academic publisher, London, pp. 260-270.
- Sommardahl, C. S. (2004): Rabies. In: Reed, S. M., Bayly, W. M. and Sellon, D. C (ed): Equine internal medicine, 2nd edition. Elsevier, USA, pp. 644-646.
- Sudarshan, M. K., Mahendra, B. J., Madhusudana, S. N., Ashwoath Narayana, D. H., Rahman, A. *et al.* (2006): An Epidemiological Study Of Animal Bites In India: Results Of A Who Sponsored National Multi-Centric Rabies Survey. *J Commun Dis.*, 38(1):32-
- Sugiyama, M. and Ito, N. (2007): Control of rabies: Epidemiology of rabies in Asia and development of new-generation vaccines for rabies. *Comparative Immunology, Microbiology & Infectious Diseases*, 30: 273-286.
- Tefera, G., Yimer, E. and Geyid, A. (2002): Endemic existence of rabies in Ethiopia. *Ethiop Med J.*, 40: 163-170.
- Thrusfield, M. (2007): Veterinary epidemiology, 3rd edition. Blackwell Science Ltd, UK, pp. 266-318.
- UAECP (2011): Urban agriculture extension Core process. Addis Ababa, Ethiopia.
- UN OCHA (2011): United Nations Office for the Coordination of Humanitarian affairs. www.ochaonline.un.org/OchaLinkClick.aspx?link=ocha&docid. Accessed on 12 March 2011.
- Warrell, M. J. (2004): Rabies and other *Lyssavirus* infections. In: Zuckerman *et al.* (ed): Principles and practice of clinical virology, 6th edition. John Wiley and Sons Ltd., UK, pp. 777-800.
- WHO (1973): Human rabies vaccine. In: Jackson, A. C. and Wunner, W. H. (ed): Rabies, 2nd edition. Elsevier Inc, San Diego, CA. pp 505-515
- WHO (2005): WHO expert consultation on rabies first report. WHO technical report series 931. Geneva, Switzerland, pp. 1-121.
- WHO (2011a): Rabies - bulletin – Europe. Rabies information system of the WHO collaboration centre for rabies surveillance and research. www.who-rabies-bulletin.org. Accessed on 20 January 2011.
- WHO (2011b): Rabies. www.who.int/mediacenter/factsheets/fs099/en/. Accessed on 9 March 2011.

- Widdowson, M. A., Morales, G. J., Chaves, S. and McGrane, J. (2002). Epidemiology of urban canine rabies, Santa Cruz, Bolivia, 1972-1997. *Emerging Infectious Diseases*, **8**: 458-461.
- Wilde, H., Khawplod, P., Khamoltham, T., Hemachudha, T., Tepsumethanon, V. *et al.* (2005): Rabies control in South and Southeast Asia. *Vaccine*, **23**: 2284-2289.
- Wunner, W. H. (2007): Rabies virus. In: Jackson, A. C. and Wunner, W. H. (ed): Rabies, 2nd edition. Elsevier inc, San Diego, CA. pp 23-68.
- Yimer, E., Newayeselassie, B., Teferra, G., Mekonnen, Y., Bogale, Y. *et al.* (2002): Situation of rabies in Ethiopia: A retrospective study 1990 -2000. *Ethiop. J. Health Dev.*, **16**(1):105-112.
- Zee, Y. C. (1999): *Rhabdoviridae*. In: Hirsh, D. C. and Zee, Y. C (ed): Veterinary microbiology. Blackwell science, Inc., USA. pp. 412-416.
- Zhang, G, Guo, J. and Wang, X. (2009): Immunochromatographic lateral flow strip tests. *Methods Mol Biol.*, **504**: 169-183.

8. ANNEX

Specimen information

Date collected: _____ Sex _____

Type of animal submitted: dog / / cat / / Other / / (specify) _____

Animal classification: Pet / / Wild / / owned and restricted dog / / owned and roaming dog / /
Ownerless dog / / unknown / /

Date of onset of illness _____ Date of death _____ Cause of death _____

Indicate whether the animal exhibited any of the following symptoms.

Difficulty in swallowing

Unusual behavior

Choking

Salivation

Drooping Jaw

Loss of Appetite

Straining

wandering from Home

Restlessness & excitability

Paralysis in hind legs

Other signs _____

Exposure to rabies suspected animal:

Human exposure / / Number exposed _____

Domestic animal exposure / / Number and species exposed _____

Other (wild animal) _____

Exposure type: Bite / / Scratch / / Contact Saliva / / Other _____

Exposure Date: _____

Address, where was animal found?

Region _____ Zone _____ District _____ kebele _____

Animal vaccination status (if domestic) / / Vaccinated / / None / / Unknown

If the answer is 'yes', please provide the date of the last vaccination: Date: _____

Veterinarian who observed animal

Name _____ Phone number _____

Has animal recently fought with a suspected rabid animal? Unknown / / No / / Yes / /
date ____ / ____ / ____

Submitter: Name _____ Phone no. _____

FAT result

RIDT result.....

9. CURRICULUM VITAE

Personal data

Name: Reta Tesfaye Digafe

Sex: Male

Place of birth: Kofele, South-West Arsi zone, Oromiya region, Ethiopia.

Date of birth: July 07, 1979

Nationality: Ethiopian

Marital Status: Married

Language skill: Afan Oromo, Amaharic, English

Address: University of Gondar, Faculty of Vet. Medicine, Po box 196, Ethiopia.

Email: tesfaye_reta@yahoo.com Tele. + 251-9119207

Profession: Veterinarian

Educational background

Years of study	Award	Field of study	Institution
2010-2011	Msc	Transboundary Animal Disease Management	Addis Ababa University and Freie Universität, Berlin
I			
2002-2008	DVM	Veterinary medicine	Addis Ababa University, Faculty of veterinary medicine
1993-1995	Diploma	Animal health	Addis Ababa University, Faculty of veterinary medicine
1990-1993	Ethiopian School Leaving Certificate	Secondary School	Kofele Secondary School
R			

Research out put

DVM thesis: Sero-prevalence of infectious bursal disease in non-vaccinated local chickens in selected areas of East Shoa, Ethiopia.

Msc thesis: Epidemiology of rabies in Ethiopia and evaluation of the rapid immunodiagnostic test kit for rabies diagnosis.

Other trainings

Basic computer skill

Experience sharing and training workshop in modern Beekeeping

Basic GIS training

Work experience

Years	Position
2008/2009	Lecturer in University of Gondar, Faculty of Veterinary Medicine
2009	Department Head of Basic Science in University of Gondar, Faculty of Veterinary Medicine
1996- 2002	Team leader and field expert

Scholarship honor

Belgium in country scholarship, 2006/2007 academic year.

DAAD scholarship, 2010 – 2011.

Referees

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10. SIGNED STATEMENT OF DECLARATION SHEET

“This thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.”

Name	Signature	Date
Reta Tesfaye Digafe	_____	_____

Approval by University advisors

Name	Signature	Date
1. Dr. Teshale Sori	_____	_____
2. Dr. Conrad Freuling	_____	_____