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# ESTIMATING THE HEALTH BURDEN AND STATUS OF RABIES IN ANIMALS AND HUMANS FROM 2014-2019 IN ADDIS ABABA, ETHIOPIA

**MVSc Thesis**

BY

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**JULY 2020**

**Bishoftu, Ethiopia**

# ESTIMATING THE HEALTH BURDEN AND STATUS OF RABIES IN ANIMALS AND HUMANS FROM 2014-2019 IN ADDIS ABABA, ETHIOPIA

A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Public Health

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As MVSc research advisors, we here by certify that we have read and evaluated this thesis prepared under our guidance by Elsa Zerabiruk Desalegn

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**ESTIMATING THE HEALTH BURDEN AND STATUS OF RABIES IN ANIMALS AND HUMANS FROM 2014-2019 IN ADDIS ABABA, ETHIOPIA**

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**LIST OF ABBREVIATIONS**

| | |
|---|---|
| ABLV | Australian Bat Lyssa Virus |
| ARAV | Aravan Virus |
| BBB | Blood Brain Barrier |
| CCEEV | Cell Culture Embryonated Egg Based Rabies Vaccine |
| CCIT | Cell Culture Inoculation Test |
| CDC | Center of Disease Control |
| CDNA | Complementary Deoxyribo Nucleic Acid |
| CNS | Central Nervous System |
| CT | C Terminal |
| DALYs | Disability Adjusted Life Years |
| DNA | Deoxy Ribonucleic Acid |
| DUVV | Duvenhage Virus |
| EBLV | European Bat Lyssa Virus |
| EHNRI | Ethiopian Health and Nutrition Research Institute |
| ELISA | Enzyme Linked Immuno Sorbent Assay |
| EPHI | Ethiopian Public Health Institute |
| ERA | Evelyn Rokitniki Abelseth |
| ETB | Ethiopian Birr |
| FAT | Fluorescent Antibody Test |
| FAVN | Fluorescent Antibody Virus Neutralization |
| GDP | Gross Domestic Product |
| IHCT | Immune Histochemical Test |
| IRKV | Irkut Virus |
| KAP | Knowledge, Attitude and Practices |
| KHUV | Khujand Virus |
| LAT | Latex Agglutination Test |

**LIST OF ABBREVIATIONS (Continued**

| | |
|---|---|
| LBV | Lagos Bat Virus |
| MIT | Mouse Inoculation Test |
| MOKV | Mokola Virus |
| MTCV | Modern Tissue Culture Vaccine |
| NCAM | Neural Cell Adhesion Molecule |
| NP | Nucleo Protein |
| NTV | Nerve Tissue Vaccine |
| PCR | Polymerase Chain Reaction |
| PEP | Post-exposure prophylaxis |
| PET | Post-exposure Treatment |
| RABV | Rabies Virus |
| RdRp | RNA dependent RNA polymerase |
| RFFIT | Rapid Fluorescent Focus Inhibition Testing |
| RIG | Rabies Immunoglobulin |
| RNA | Ribonucleic acid |
| RNP | Ribo Nucleoprotein Complex |
| RT-PCR | Reverse Transcriptase Polymerase Chain Reaction |
| RVG | Rabies Virus Glycoprotein |
| SAD | Street Alabama Dufferin |
| SAG | Street Alabama Guifn |
| SPSS | Statistical Package for Social Sciences |
| SNNPRS | Southern Nations, Nationalities, and Peoples ' Regional States |
| VNA | Viral Neutralization Antibody |
| VRG | Vaccina Rabies Glycoprotein |
| WHO | World Health Organization |
| YLD | Year of Life with Disabilities |
| YLL | Year of Life Lost |

## ABSTRACT

Rabies is a notoriously underreported and neglected disease of low-income countries like Ethiopia where financial resources are limited and numerous interests compete, there is a need for updated quantitative data on the public awareness, health burden and costs of diseases to support prioritization. The aim of this study was to determine the level of community knowledge, Attitude and Practices (KAP) regarding rabies and to estimate the health burden and post-exposure treatment (PET) costs of rabies in Addis Ababa. Hospital based cross-sectional study was conducted from February 2020 to April 2020 to assess the KAP towards rabies. Two hundred and sixty-seven people who were bitten by rabies suspected animals were purposively interviewed for this study and retrospective study was conducted to assess the burden of rabies over the period of six years (2014 to 2019). Based on the collected data PET costs were evaluated and the health burden was estimated in Disability-Adjusted Life Years (DALYs). The KAP study showed that 62.8% of the respondents had good level of knowledge and had medium (51.1%) level of attitude and practices towards rabies. In this study the good scores were higher in males (62.87%) than females. There was strong association between knowledge scores and type of employment (P= 0.003), Attitude and Practice scores and gender (P=0.001). There was significant positive correlation between Knowledge and Attitude and practice (P=0.001). The retrospective data indicated that a total of 8613 victims were registered and received post exposure vaccine in Addis Ababa over the period of six years (2014 to 2019). From this most important animal species responsible for PEP was dogs (93.8%). The annual suspected rabid dog exposures were estimated, and higher exposure was recorded in 2019 which was 54.5 per 100,000 populations. An annual estimate of approximately 10 human deaths and 57 disabilities resulting 430-955 DALYS per 100,000 populations per year due to administration of nerves tissue vaccine (NTV). Costs per completed (PET) estimated around 9.2 ±22.8 USD for children and 11.8±27 USD for adults in Addis Ababa.



**Key Words:** *Addis Ababa, Attitude, Community, DALYS, Knowledge, Practice, Rabies*

## 1. INTRODUCTION

Rabies is a deadly viral zoonosis, which causes encephalitis in warm-blooded animals and humans (Zulu *et al*., 2009) caused by rabies virus. Rabies virus belongs to the order *Mononegavirales*, family *Rhabdoviridae*, and genus *lyssavirus*. The virus is transmitted to humans from rabid animals through bite and sometimes scratches. Regardless of the invention and application of the first rabies vaccine by Louis Pasteur in 1885, human rabies, is still deadly disease worldwide (Hanlon and Childs, 2013).

In the world, it has been anticipated that about 60,000 people die every year, of which the highest death is in Asia and Africa due to the existence of endemic canine rabies, dogs (almost 99%) remain the major animal reservoirs in such areas (Undurraga *et al*., 2017). Rabies was first reported in Ethiopia (Addis Ababa) in 1903 and reportable disease (WHO, 1987). In Ethiopia, when we saw the last two decades, the retrospective data showed that during the years, 1990–2000, an average of about 2200 people received post exposure anti rabies treatments annually (96.2 %) of which were bitten by dogs. During the same period 322 fatal human rabies cases were recorded and 95% of these were acquired from dogs (Yimer *et al*., 2002). During the next decade (2001-2009) the number of fatal cases increased to 386 ranging from 35 to 58 per year. The magnitude of post exposure vaccination escalated to 17,204 mostly from Addis Ababa. During this period a total 3,460 brains were examined from animals involved in human bite and 75% of them were positive (The amount of vaccine used was 130,673 doses for humans 85,055 doses for animals) (Deressa *et al*., 2010).

Between the years 2009 and 2011, a total of 1,088 dogs and cats were examined for rabies, of which 73.62% of dogs and 5.1% of cats were found positive. The highest proportion of rabid dogs was whose ownership was not known (Reta *et al*., 2014). Approximately 10,000 people were estimated to die of rabies annually in Ethiopia which makes it to be one of the worst affected countries in the world (Fekadu, 1997). The highest number was reported during June to September (Admasu and Mokonen, 2014). The highest incidence was registered from Tigray (11.4 cases per 100,000), followed by Oromia (3.5 cases per 100,000), Benshangul (3.3 cases per 100,000), Amhara (1.5 cases per 100,000), SNNPR (1.2 cases per 100,000) and Addis Ababa (0.8 cases per 100,000)

Making a national average of 2.6 cases per 100,000 people (EHNRI, 2012). In addition to human diseases rabies also threatened the endangered Ethiopian wolves in the Bale Mountains. For example 13 out 15 brain samples of wolves examined after human exposure were found positive for rabies virus (Randill *et al*., 2004). All these clearly show that rabies remains of primary public health concern in the country and needs to be given due attention.

Rabies is an urban human problem in developing countries characterized by the incidence of disease in domestic animals such as pet dogs and cats (Shite *et al.,* 2015). Several studies indicated that rabies had been well established and become endemic in Addis Ababa (Tefera *et al*., 2002). It has been supported by high number of animal rabies confirmed cases in Addis Ababa during the past two decades and majority of rabies cases were confirmed in dogs. (Yimer *et al*., 2002; Ali *et al*., 2010; Deressa *et al*., 2010). It is highly associated with the presence of large population of stray dogs (Yimer et al., 2002). Poor public awareness towards rabies considered as one of the bottle necks for the prevention and control of the disease in Ethiopia particularly in canine endemic cities like Addis Ababa. Understanding communities' perceptions of cause, mode of transmission and possible intervention measures of rabies is an important step towards developing strategies aimed at controlling the disease and determining the level of implementation of planned activities in the future.

Beyond these the human and economic costs of rabies are poorly known. A major challenge to estimating the burden of rabies is the absence of reliable surveillance data for countries where the disease is most prevalent (Hampson *et al*., 2015). The revised 2010 estimate of the rabies burden in Africa by the probability decision-tree approach of about 23 800 deaths (95% CI, 21 000–28 000) And 609 000 DALYs (95% CI, 522 000–707 000) (Knobel *et al*., 2005). Rabies in exposed humans is preventable when an effective post-exposure treatment (PET) is applied immediately after exposure which includes post-exposure prophylaxis (PEP), wound washing, antibiotic and tetanus antitoxin administration.

With respect to the applied PEP vaccine WHO recommends using inactivated rabies vaccines produced in cell culture or embryonated eggs (CCEEV) (WHO, 2013). In Ethiopia, the PEP vaccine used to treat most of the rabies exposures is produced from nervous tissue composed of rabies virus-

Infected sheep or goat Brain inactivated with phenol and called Nervous Tissue Vaccine (NTV). This type of vaccine is less immunogenic than the CCEEV and known for its fatality rate and disability rate due to the occurrence of severe and sometimes fatal allergic encephalomyelitic reactions. Use of nerve tissue vaccine remains widespread in Ethiopia, contributing about 1000 DALYs per annum (WHO, 2013). According to Beyene *et al* approximately 3,000 human deaths resulting in about 194,000 DALYs per year and 97,000 exposed persons requiring on average 2 million USD Treatment costs per year country wide (Beyene *et al*., 2018). However, the actual numbers are expected to be higher as many cases are not report.

The health burden of rabies, especially in canine endemic cities like Addis Ababa has not been, therefore, sufficiently investigated in Ethiopia but supposed to include fatality and disability rate as a consequence of neurological complications, following NTV administration (Knobel *et al*., 2005). Thus, the magnitude of cases of rabies in humans and animals in Ethiopia signals the need for rigorous epidemiological studies to have up-to-date and reliable information about the disease. Moreover, quantification of the health burden enhances the understanding of its long-term effects and of the comparative advantages of different levels of treatment and prevention.

Therefore, the objectives of this study were:

- To assess the level of Knowledge, Attitude and Practice of community in Addis Ababa, and
- To estimate the status of rabies in Addis Ababa by assessing its health burden and associated PET costs using data obtained from health center recordings.

## 2. LITERATURE REVIEW

Rabies is a well-known viral infection which is affecting the central nervous system of all animals which are warm-blooded besides to humans (Moges, 2015; Richard *et al*., 2015). The Disease is mainly transmitted from rabies affected animal to human through close contact with infected saliva through bites or scratches (Deressa *et al*., 2011). The infection is characterized through the appearance of rigorous nervous symptoms that results in paralysis following the death of the patient (Abera *et al*., 2015). Rabies originated about 3000 B.C from the word 'rabha' meaning violence. Rabies is one of the most typical zoonosis that has been well known since ages and has been identified for more than 4300 years (Takayama, 2008).

### 2.1 Classification and Taxonomy of Rabies Virus

The rabies virus genome consists of a single stranded, non-segmented, enveloped, negative sense RNA of around 12kb (Tordo *et al*., 1986). It is under *Lyssavirus* genus (Wunner, 2002), in the famil y of *Rhabdoviridae*, order *Mononegavirale* which is transmissible to all mammals (WHO, 1996). Eleven different species can be distinguished within the genus *Lyssavirus* by cross-protection tests and molecular biological analysis (Baer, 1991; Bourhy *et al*., 1993), namely the classical rabies virus itself (RABV, genotype 1, serotype 1), Lagos bat virus (LBV, genotype 2, serotype 2), Mokola virus (MOKV, genotype 3, serotype 3), and Duvenhage virus (DUVV, genotype 4, serotype 4). The European bat lyssa viruses (EBLV), subdivided into two biotypes (EBLV1, genotype 5 and EBLV2, genotype 6) and the Australian bat lyssa virus (ABLV, genotype 7), isolated in Australia (Hooper *et al*., 1997), are also members of the *Lyssa virus* genus, but are not yet classified into serotypes. Also four putative viruses (Aravan, Khujand, Irkut and West Caucasian Bat Virus, Shimoni bat virus isolated in 2009) as well as Bokeloh bat lyssavirus (Freuling *et al.*, 2011) and the Ikoma *lyssavrus* were discovered in 2011 and 2012, respectively. Viruses of serotypes 2–4, EBLV and ABLV are known as rabies-related viruses (Marston *et al*., 2012).

The *lyssaviruses* have been categorized into two phylogroups with different pathogenicity and immunogenicity (Badrane *et al.*, 2001). For RABV, DUVV, EBLV and ABLV, conserved antigenic sites on the surface glycol-proteins permit cross-neutralization and cross-protective immunity to be elicited by rabies vaccination. A reduced defense with pre-exposure vaccination and with conventional rabies post-exposure prophylaxis was observed against IRKV, ARAV, and KHUV (Hanlon *et al*., 2005) and all of the above-mentioned *lyssavirus* species were categorized to phylogroup 1. Slight or no cross-protection against infection with the members of phylogroup 2 (MOKV and LBV) is elicited by rabies vaccination and most anti-rabies virus anti-sera do not neutralize these *lyssaviruses* (Badrane *et al.*, 2001).

### 2.2 Morphology

Rabies viruses have approximately 180 nm long and 75 nm wide (Drew, 2004) and its genetic information is structured in the form of a helical ribonucleoprotein complex (RNP), in which the linear RNA is tightly connected with the viral nucleoprotein (Figure 1). The genome of RABV encodes for only five proteins in the order: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and the large protein (L, also termed RNA-dependent RNA polymerase, RdRp) (Albertini *et al.*, 2011). The N plays a vital role in viral transcription and replication. The G forms around 400 trimeric spikes, which are compactly arranged on the surface of the virions. The G is a key determinant for RABV neuropathogenicity by binding specific receptor(s), entering the nervous system via the endosomal transport pathway by means of a low-pH-induced membrane fusion process (Gaudin, 2000). All transcription and replication events occur in the cytoplasm inside a specialized “virus factory“, the Negri body (Lahaye *et al*., 2009).

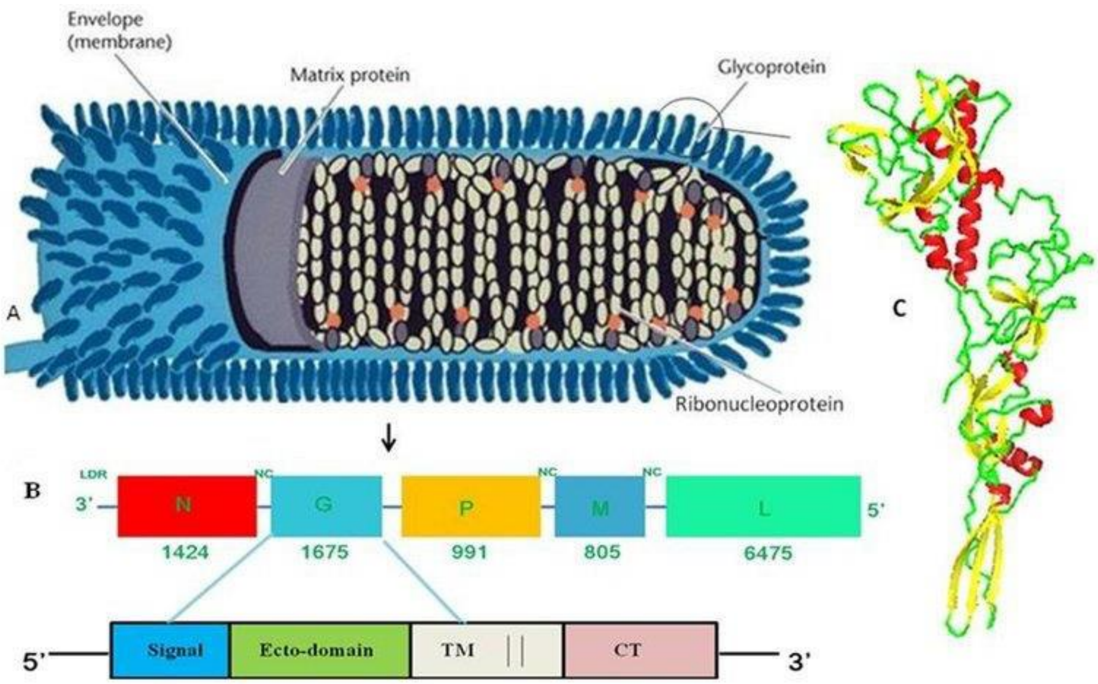


**Figure 1:** A) Diagrammatic representation showing bullet shaped structure of rabies virus. B) Different protein structures encoding the genome of rabies virus with the lengths of it; Glycoprotein (G) is shown with its signal, ectodomain, transmembrane and C terminal (CT) C) Three-dimensional structure of Rabies Virus Glycoprotein (RVG)

Source: http://viralzone.expasy.org/all_by_species/2.html. Accessed on 18 October 2019

### 2.3 Epidemiology

Even though advances in laboratory methods and enhanced vaccination, Rabies still remains a deadly infection in humans and animals (Pal, 2007). There are 60,000 people dying from rabies globally in each year and over 3 billion people continue to be at risk of rabies virus infection in over 100 countries in the 21st century with 95% of cases occurring in Africa and Asia (WHO, 2017). Except in some countries where there is strict quarantine system, rigorous eradication program or natural barriers i.e. mountains and rivers (Rupprecht and Tumphey, 2007) rabies remains a key public health problem in resource-limited countries, particularly in Asia and Africa. 99% of rabies

Cases are dog-mediated, and the burden of disease is disproportionally borne by rural poor populations, with around half of cases attributable to children under fifteen (WHO, 2013). The three main global areas of rabies include countries with enzootic canine rabies (all of Asia, Latin America, and Africa); countries in which canine rabies has been brought under control and wildlife rabies predominates (Western Europe, Canada, and the United States); and rabies-free countries (mostly islands, including England, Australia, and Japan. Based on the species of animals that plays role as a major vector, three rabies cycles have been distinguished (Radostits *et al*., 2000). The primary is the urban rabies cycle, in which dogs are the main reservoir host. This cycle dominated in areas of Africa, Asia, and Central and South America where the proportion of unvaccinated and semi-owned or stray dogs is high. It has been almost eliminated in North America and Europe; although sporadic cases occur in dogs infected by wild animals, where the urban cycle is not perpetuated in the canine population (Rupprecht and Tumphey. 2007).

The second is the sylvatic (wildlife) cycle of rabies in which wild carnivores for instance jackals, foxes; skunks, mongooses, wolves etc. do play a vital role as vector. This rabies cycle frequently reverts to urban cycle due to frequent contact among rabid wild carnivores and stray dogs. The majority common victims are dogs, cattle and man (Quinn *et al*., 2002). The sylvatic cycle is the major cycle in Europe and North America. It is also present simultaneously with the urban cycle in some parts of the world. The epidemiology of this cycle is complex; factors affecting it include the virus strain, the behavior of the host species, ecology and environmental factors. The third cycle is vampire rabies (paralysa), this type of rabies is predominantly important in Latin America and is transmitted by bite of bats. These bats frequently transmit the bovine paralytic rabies and maintain the cycle in endemic areas while cattle and human are victims. Spread of the disease is often seasonal, with high prevalence in late summer and autumn because of large scale movement of wild animals at the mating time and in search for food (Shite *et al*., 2015).

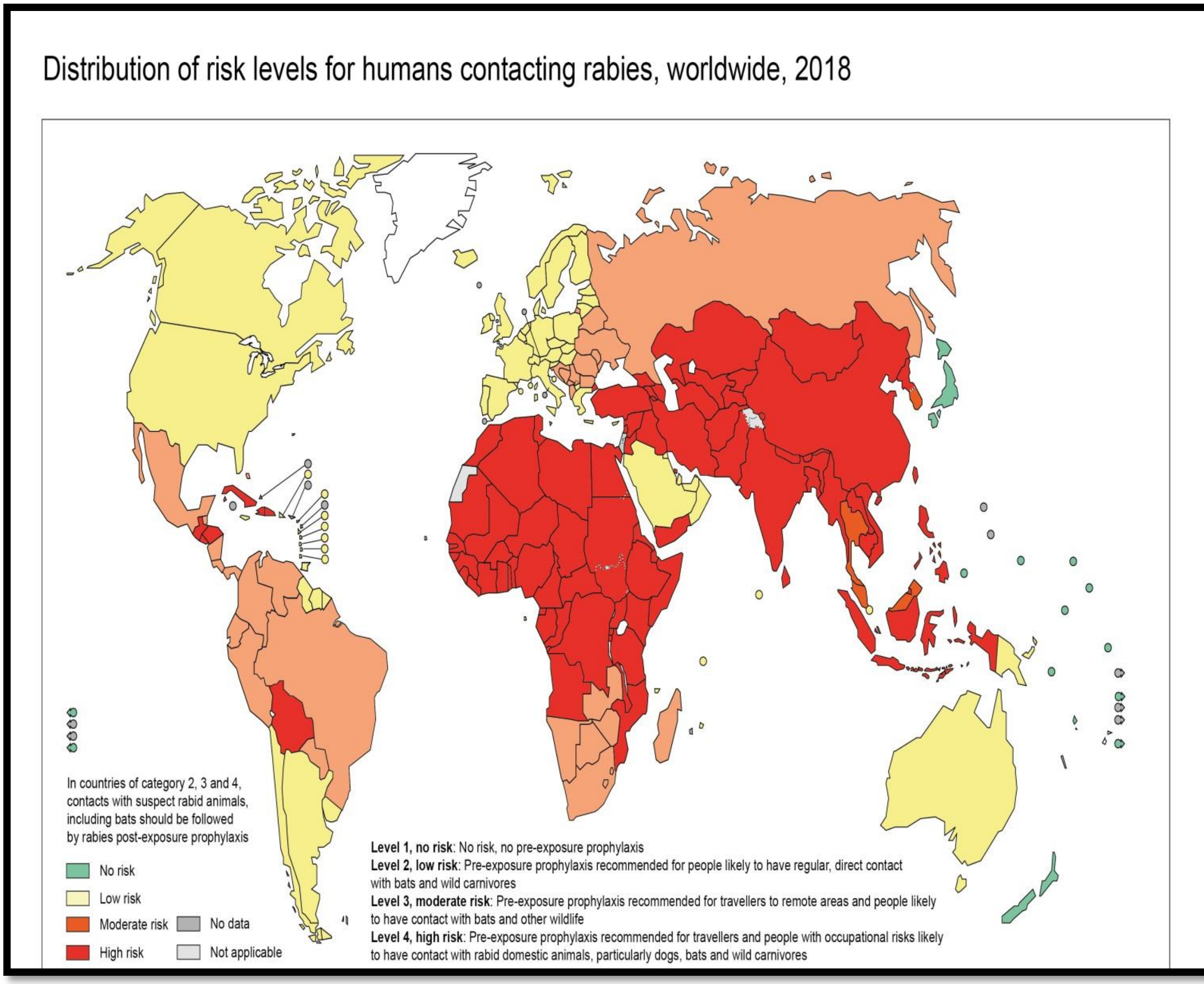


**Figure 2**: Distribution of rabies in the world, based on data of 2018

Source: (WHO, 2018)

### 2.4 Mode of Transmission

The *lyassavirus* infection is transmitted by all animals that considered as warm-blooded, though the *lyssavirus* can also grow up in cells of cold-blooded animals (Mustafa *et al.*, 2015). The transmission of this disease requires entrance of virus via the saliva of an infected animal due to biting, wounds or unwrap cuts in fur or mucous membranes (Langley, 2009). Rabies disease is not a true zoonotic disease as the infected animal dies due to severe infection (Sikes, 1962). A study of

Infected dogs within the USA discovered that all rabid dogs died within just 8 days of becoming infected. Almost the entire transmissions are by means of bites. Since the virus is secreted in saliva, the disease can infrequently arise through scrape contaminated by saliva; while the disease rate is 50 times lower (Fishbein *et al*., 1993). In many cases, the infected animal is unusually aggressive, may attack without provocation, and exhibits otherwise uncharacteristic behaviour (Turton, 2000).

The spread of the virus from individual to individual is very rare; however, few cases were recorded as a result of transplant surgery (Srinivasan *et al*., 2005). After a typical human infection by bite, the virus enters the peripheral nervous system. It then moves along the afferent nerves toward the central nervous system (Jackson and Wunner, 2002). During this phase, the virus cannot be simply detected within the host, and vaccination may still give cell-mediated immunity to prevent symptomatic rabies. When the virus reaches the brain, it quickly causes encephalitis, the prodromal phase, which is the beginning of the symptoms. Once the patient becomes symptomatic, treatment is almost never successful, and mortality is over 99%. Rabies may also inflame the spinal cord, producing transverse myelitis (Lynn *et al.*, 2012).

### 2.5 Rabies Pathogenesis

The *lyssavirus* enters the body through abrasions or by direct in touch with mucosal membranes. The rabies virus multiplies inside the bitten muscle tissue and then it achieves entry towards the central nervous system (Ugolini, 2007). The virions are passed in carrying vesicles (Klingen *et al*., 2008) and travel to the central nervous system (CNS) completely via rapid retrograde movement beside motor axons, by means of no uptake via sensory or sympathetic endings (Hemachudha *et al*., 2013). The entrance of virus inside tissues of the brain leads to death, frequently through respiratory dysfunction and secondary metabolic and circulatory defects (Bishop *et al*., 2003, Shite *et al*., 2015).

The overall effect of an exposure to RABV depends in part upon the rabies genotype (different strains and mutants) or variant involved, its pathogenicity (apoptogenicity, neuroinvasiveness), the dose of virus inoculated (severity of exposure), the route as well as the host species and its susceptibility to the particular pathogen jointly with innate and adaptive immune responses of the

Host. Nevertheless, various studies in animal models indicate that the pathogenic wild-type/street RABV and the fixed (laboratory-adapted) RABV markedly behave in a different way in each step of their life cycle in the host (Franka *et al*., 2009).

RABV G is the only surface protein of the virion and proficient for inducing virus neutralizing antibodies (V NA) (Benmansour *et al*., 1991). The G protein plays a vital role in rabies pathogenesis (Ito *et al*., 2001) by bi nding to neural receptor for instance acetylcholine receptor (Lentz *et al*., 1982) and neural cell adhesion mole cules (NCAM) (Thoulouze *et al*., 1998) contributing to the exclusive neurotropism and neuroinvasiveness of RABV (Thoulouze *et al*., 2000). Virus may enter muscles and duplicate at the site of inoculation or enter directly into peripheral nerves exclusive of prior replication in non-neural tissues (Shankar *et al*., 19916). It is believed that once virus particles enter the peripheral nervous system and begin to spread to the CNS, a fatal outcome of the disease is unavoidable, while there are some reports of rabies survivors. RABV enters motor and/or sensory axons of peripheral nervous system and spreads to the CNS by retrograde fast axonal move at a rate of approximately 50–200 mm/day (Tsiang *et al*., 1991).

The pathogenic RABV have evolved specific mechanisms to flight early immune system detection in the periphery through limited replication, minimized G expression (Wang *et al*., 2005). Alternatively, fixed RABV induces extensive inflammation by activating innate immune responses (Wang *et al*., 2005), induces apoptosis (Sarmento *et al.,* 2006), replicates to higher levels and express high levels of the G protein (Zhang *et al*., 2013). However, the method adopted by the fixed RABVs to elicit immune responses and the wild-type RABVs to evade immune system is still not completely understandable. It has been shown that the innate immune responses and inflammation in the CNS is associated with BBB permeability improvement (Fabis *et al*., 2008) in mice infected with fixed RABV but not in those infected with street RABV (Kuang *et al*., 2009).

### 2.6 Clinical Manifestation

It is investigated that as the disease becomes advanced, the animal shows strange behavior (Chernet and Nejash, 2016). The main clinical signs are often non-specific and consist of anxiety, restiveness, anorexia or unimproved appetite, nausea, diarrheal, a minor fever, dilation of the pupils, hyperactivity to any stimuli in addition to extreme salivation. The clinical course may be divided into three phases namely prodromal, excitative (furious) and paralytic or end stage (Banyard *et al*., 2013).

**Prodromal Stage**

Following a specific incubation phase, the beginning of clinical symptoms starts. During this first stage which typically ends within 1-3 days, minor behavioral change may occur, *i.e.* anger in domestic animals, daytime tricks in nocturnal animals, no fear of humans in the wild animals or else irregularities in the appetite (WHO, 2013).

**Excitement (Furious) Phase**

The furious type is expressed via agitation, wandering, weeping, polypnea, drooling and attacks upon other animals, community or unresponsive objects. Infected animals often ingest foreign items such as firewood and gravels. The wild animals frequently drop their fright of humans and may harass humans or another surrounding animal that they would regularly avoid (*e.g.*, porcupines). On the other hand, the nocturnal animals may be observable all over the day. In cattle, odd attentiveness can be an indication of this phase (Banyard *et al*., 2013).

**Paralytic (Dumb) Phase**

The "dumb" type of rabies is frequently characterized by the progressive paralysis. In this phase, the gullet and masseter muscles become paralyzed; the animal might be unable of swallowing and salivating abundantly. There may be an alter in voice of infected animal because of laryngeal paralysis, with atypical bellowing in cattle and barking in dogs. Besides that, there might be facial paralysis along with dropping of the lower jaw. Ruminants may become isolated from the herd (Yang *et al*., 2012). Moreover, this stage is also characterized by dropping of foamy salivary secretion and paralysis of hind limbs finally leading complete paralysis followed by death (WHO, 2013).

**Hydrophobia**

The term hydrophobia stands for the fear of water is the historic synonym of rabies (Smallman-Raynor *et al*., 2004). This situation refers to a collection of warning signs during the advanced phases of an infection in which the patients have obscurity in swallowing and taking water. Any mammal infected by the virus may reveal hydrophobia. In this situation, there is over production of saliva, and animal struggles to drink and could suffer from painful spasms of the muscular tissues within the throat as well as in vocal cord (Mustafa *et al*., 2015).

## 2.7 Public Health Significance of Rabies

### *2.7.1 General overview of rabies in humans*

Rabies infection in humans is still a main public health problem all over the world. About 98% of the human rabies cases take place in developing countries that possess large number of dogs, many of which are strays (WHO, 2004). This condition occurs because dog rabies is endemic with dog-to-dog transmission of the infection, which is related with an ongoing threat to humans due to dog bites. Unfortunately, children share strangely high burden of the disease. Estimates of human mortality due to endemic canine rabies in Asia and Africa annually exceed 30,000 and 23,000, respectively. The annual cost of rabies in Africa and Asia was estimated at 583.5 million USD most of which is due to cost of post exposure prophylaxis (PEP) (Knobel *et al*., 2005).

### *2.7.2 Pathogenesis and clinical signs*

Rabies virus enters the body via wounds or by direct contact with mucosal surfaces but cannot cross intact skin. Then rabies virus duplicates in the bitten muscle and gains access to motor end plates and motor axons to reach the central nervous system (Ugolini, 2011). Rabies virus binds to the nicotinic acetylcholine receptor at the neuromuscular junction (Lewis and Lentz, 2000). The virus quickly ascends the nervous system to the brain by entering the pre-synaptic nerve ending through hendocytosis and may be connected with synaptic vesicles. Inside peripheral nerves, the virus is carried in a retrograded irection by rapid axonal transport, centripetally to the CNS. The virus remains intra-neural throughout its passage and is hard to find by extra-neural antibodies. On reaching the CNS, there is enormous viral multiplication on membranes within neuron and trans-synaptic transmission of virus occurs from cell to cell (Venugopal *et al*., 2013) Neurons are the neural cell type primarily infected by rabies virus and there are few degenerative changes in neurons. Infected neurons may contain eosinophilic inclusions in the cytoplasm called Negri bodies. Negri bodies are most prominent in large neurons (e.g., Purkinje cells) and, ultra- structurally, they are composed of large aggregates of granulo filamentous matrix material and variable numbers of viral particles (Rossiter and Jackson, 2007). The virus then travels centrifugally from the central nervous system by slow anterogradeaxoplasmic flow in motor axons to the ventral roots and nerves and in peripheral sensory axons of the infected dorsal root ganglia, leading to infection of muscle spindles, skin, hair follicles and other tissues, such as salivary glands, heart muscle, lung and abdominal visceral organs via their sensory innervations (Ugolini, 2011).

Clinical rabies in humans can be categorized into five stages: incubation period, prodromal stage, acute neurological phase, coma and death (Bernard, 2015). The average incubation period of between 31 to 90 days has been reported, but it can be as short as 7 days. Exposures through bites on abundantly innervated areas of the body like the face, neck, hand and, especially, finger-tips lead more rapidly to clinical infection and a shorter incubation period. Bites that occur on the trunk or proximal portions of the limbs may take longer time, except such exposures are in direct proximity to major nerve trunks (Yousaf *et al*., 2012).

In humans, the prodromal period usually lasts for 24 to 48 hours, but seldom may this extend to one week or longer. During this time, patients show vague symptoms of a state of being unwell, often with considerable apprehension. Malaise, anorexia, low-grade fever, fatigue, headache, restlessness and tension are all possible symptoms (Dimaano *et al*., 2011). Classical signs of brain involvement include spasms in response to tactile, auditory, visual or olfactory stimuli (aerophobia and hydrophobia) alternating with periods of lucidity, agitation, confusion and signs of autonomic dysfunction. Spasms occur in majority rabid patients in whom excitation is prominent (WHO, 2004). Hydrophobia, literally the "fear of water," is a descriptive term applied to clinical rabies in human and stems from the severe, involuntary, and painful spasms provoked by attempts to drink, or sometimes the mere sight or sound of water (ADPH, 2010).

### *2.7.3 Source of infection and modes of transmission in humans*

Over 99% of human rabies cases are caused by dog bites. Although other mammals such as bats, foxes and raccoons can transmit rabies to humans (WHO, 2013). While the dog is moderately susceptible, it acts as a reservoir for urban rabies virus in developing countries (Qasim *et al*., 2015). Dogs have been the key reservoir of rabies to humans in Africa and are accountable for above 95%

of human rabies cases in China and India (Fitzpatrick *et al*., 2015). Bats are increasingly implicated as main wildlife reservoirs for variants of rabies virus transmitted to humans (LOPH, 2010).

The most likely means of rabies transmission is by introduction of saliva containing rabies virus into a bite wound. Beside this rabies transmission can also occur if saliva or central nervous system tissue from a rabid animal contacts a fresh wound or mucous membrane (MCDC, 2012). Different course of transmission has been documented and include contamination of mucous membranes (i.e eyes, nose, and mouth), aerosol transmission, and corneal transplantations (ADPH, 2010). Post exposure prophylaxis is suggested in rare instances of non-bite exposure, such as inhalation of aerosolized virus by spelunkers exploring caves inhabited by infected bats or by laboratory technicians homogenizing tissues infected with rabies virus without appropriate precaution (Knobel *et al*., 2005). Despite the amount of viable rabies virus that maybe shed in cows' milk, the theoretical risk for transmission of rabies from this route can be eliminated if all dairy products are pasteurized before consumption. However, because rabies virus is inactivated by temperatures below those used for cooking and pasteurization, eating cooked meat or drinking pasteurized milk from a rabid animal is not an indication for Post-Exposure Prophylaxis' (PEP) (MCDC, 2012).

## 2.8 Laboratory Diagnosis of Rabies

The diagnosis of rabies has to be fast and reliable in order to estimate the risk of infection to the exposed individual (Zimmer *et al*., 1990), and it is also essential for health authorities accountable for the surveillance and control of the epidemics and epizootics. The techniques for the diagnosis of rabies are standardized worldwide, and numerous tests are available currently. The detection of Negri bodies in brain smears and the histological test were the primary methods for diagnosing rabies, but these are not presently used widely because of their low sensitivity and due to the accessibility of highly sensitive and specific modern techniques which can be categorized into three main groups: ***Detection of viral antigen*** – fluorescent antibody test (FAT), enzyme linked immunoassay (ELISA), immune histochemical test (IHCT), and latex agglutination test (LAT);***Detection and identification of viral genome*** – reverse transcriptase polymerase chain reaction (RT-PCR) with subsequent nucleotide sequencing; ***Virus isolation*** – mouse inoculation test (MIT) and cell culture inoculation test (CCIT) (Zimmer *et al*., 1990).

### 2.8.1 Detection of viral antigen

**Fluorescent antibody test:** this method to be an efficient tool for the diagnosis of rabies, and it later on became the reference technique for the diagnosis of this infection. This method implies the preparation of smears, impressions or cryosections from brain tissues (Ammon's horn, cerebellum, cerebral cortex, and the brain stem), tissue fixation, mostly in cold acetone, and staining with fluorescein isothiocyanate-labeled polyclonal or monoclonal anti-rabies antibodies (OIE, 2004). The FAT permits specific and highly sensitive detection of the rabies virus antigens in brain smears, salivary gland sections, and infected cell cultures. It can be used for the intravitam rabies diagnosis in the skin biopsies (Warrell *et al*., 1988) and to stain rabies virus antigens in the salivary glands. It is also practicable to perform the FAT with formalin-fixed paraffin-embedded brain sections using digestion with proteases, such as pepsin or trypsin (Warrell *et al*., 1988).

**Enzyme-Linked Immunoassay Test:** it is a quick method that facilitates the evaluation of a large number of samples simultaneously, which is performed on micro plates previously sensitized with anti-rabies immunoglobulin. Suspensions of homogenized material are placed on the wells of micro plate for specific binding which can be discovered using a peroxidase conjugate. Furthermore, quantitative ELISA (N-ELISA) for rabies virus detection based on the quantization of nucleoprotein (N) in rabies virions captured by rabies-virus-specific polyclonal antibodies on an ELISA plate can be used for the quantitative detection of both infective and defective interfering particles of rabies virus (Katayama *et al*., 1999).

**Immunohistochemical Testing:** it is primarily used for research purposes and permits the perfect identification and localization of rabies virus antigens, and is ideal for retrospective diagnosis (Fekadu *et al*., 1982). This technique is usually used to stain histological paraffin sections after deparaffinization, rehydration, and digestion with a proteolytic enzyme (proteinase K etc.) (Warrell *et al*., 1988).

**Latex Agglutination Test:** (LAT) is a simple and quick technique, which may be used more commonly in the laboratory diagnosis of rabies in the future. It has been used to detect rabies virus antigens in the saliva of dogs with 99% specificity and 95% sensitivity. The core of the LAT is inducing agglutination on a glass slide using polystyrene latex beads coated with anti-rabies IgG (Kasempimolporn *et al*., 2000).

### *2.8.2 Detection of viral genome*

The reverse transcriptase polymerase chain reaction (RT-PCR) with consequent nucleotide sequencing allows the diagnosis of rabies, typing, and molecular epidemiological studies. Since the rabies genome is RNA, the amplification procedure consists of the reverse transcription of the target RNA strain into complimentary DNA (cDNA), followed by the amplification of the cDNA by PCR (Tordo *et al*., 1996). A rapid RT PCR technique was developed for the detection of the classical rabies virus (genotype 1) and the rabies related EBLVs (genotypes 5 and 6), and also to differentiate between the six established rabies and rabies-related virus genotypes (Black *et al*., 2002). The PCR can also be useful to detect the rabies virus genome in formalin-fixed paraffin embedded brain tissue and for the intravitam diagnosis of rabies in humans by testing saliva and cerebrospinal fluid (Kulonen *et al*., 1999). The Real-time PCR is a quantitative technique which permits the detection of an increase in the quantity of DNA (cDNA) during amplification. It is presently used for the ante- and post-mortem diagnosis of rabies and the discrimination of the *Lyssa*virus genotypes (Wakeley *et al*., 2005).

### *2.8.3 Virus isolation*

The mouse inoculation test (MIT) was one of the first diagnostic tests for rabies. Laboratory mice are inoculated intra cerebrally or subcutaneously by the supernatant of the sample suspension. The inoculated mice must be observed for up to 30 days after inoculation. Death during the first 48 hours after inoculation must be taken as nonspecific; all the animals dead after this period must be dissected and brain samples must be tested for rabies by the FAT. This method can be used for testing the brain and salivary gland suspensions, as well as the saliva samples, for the presence of live rabies virus (Delpietro *et al*., 2001).The cell culture inoculation test has already substituted the MIT in many countries and implies the isolation of rabies virus in a cell culture monolayer with visualization by the FAT (Clark, 1980).

### *2.8.4 Detection of virus neutralizing antibodies*

The detection of anti-rabies virus neutralizing antibodies (VNA) is commonly used to evaluate the potency of anti-rabies vaccines because the minimal level of the VNA required to protect animals against rabies has been determined as ≥ 0.5 IU/ml (OIE, 2004). Virus neutralization assay has also been found functional for the monitoring of *Lyssaviruses* among bats. The determination of the VNA was formerly conducted by virus neutralization in mice and subsequently substituted by the fluorescent antibody virus neutralization (FAVN) or rapid fluorescent focus inhibition testing (RFFIT) (Cliquet *et al*., 2000).

This technique enables the determination of antibody levels by the neutralization of a known dose of the rabies virus (commonly the CVS strain). Serum samples are tested and compared with the neutralization of reference standard serum with an antibody level of 0.5 or 1.0 IU/ml. This test can be carried out on micro plates and the results viewed with fluorescence microscopy. The registration of the results can be automated by various ways: by using an inverted fluorescence microscope joined with a video camera and color image analysis software computer system; a peroxidase conjugate can be used instead of the fluorescent conjugate, and in this case an automatic multi-channel spectrophotometer can be used for the registration of the results (Hostnik, 2000); flow cytometry technique for calculating anti-rabies VNA has also automated results registration (Bordignon *et al*., 2002).

## 2.9 Control and Prophylaxis of Rabies

### *2.9.1 Rabies vaccines*

The vaccine against rabies was invented in 1885 by Louis Pasteur beside with Emile Roux, prior to which almost all human cases of rabies were fatal. A critical component of rabies prevention and control in human includes, making responsible pet ownership, routine veterinary care and vaccination, and professionals should provide public education. The majority animal and human exposures to rabies can be prevented by raising awareness regarding rabies transmission routes, the importance of avoiding contact with wildlife, and the need for appropriate veterinary care (Petersen *et al*., 2011).

In human: Since their progress more than four decades ago, concentrated, purified cell culture and embryonated egg-based rabies vaccines (jointly referred to as CCEEVs) have proved to be secure and effective in preventing rabies. These vaccines are deliberate for both pre- and post-exposure prophylaxis and have been administered to millions of people worldwide (WHO, 2010). On time administration of CCEEVs after exposure combined with appropriate wound management and simultaneous administration of rabies immune-globulins is almost invariably successful in preventing rabies, even after high-risk exposure. Nerve tissue vaccines induce more severe adverse reactions and are less immunogenic than CCEEVs. Since 1984, WHO has recommended discontinuation of the production and use of nerve tissue vaccines and proclaimed their substitution by CCEEVs (WHO, 2010).

Sheep brain derived Fermi type of rabies vaccine is still being manufactured and consumed since 1944 for the majority of exposed patients in Ethiopian health and nutrition research institute; currently it is known as Ethiopian public health institute (EPHI) even though this vaccine has been discouraged by the WHO (EHNRI, 2012). The high costs of tissue culture vaccine have been the main obstacle to the substitution of Fermi type vaccine. Currently Ethiopian Public Health Institute (EPHI) is working to improve anti-rabies vaccine production by changing the nervous tissue vaccine (Fermi type) to cell culture-based vaccine. The vaccine is based on the *Evelyn Rokitniki Abelseth* (ERA) fixed rabies virus seed strain obtained from CDC donation and propagated on Vero cell line. It is inactivated with 5% formalin and it is in liquid form (Hurisa *et al*., 2013).

In animals: Inactivated rabies vaccines are presently used for regular vaccination of pet animals like dogs and cats, however, multiple immunizations have to be carried out to provide adequate immunity throughout the life of the animals (Wu *et al*., 2011). Oral rabies vaccines (live attenuated or live-recombinant vaccines) have been effectively developed for wildlife and two of them are commercially available (Flamand *et al*., 1993). Vaccine virus expressing RABV G (VRG) is found to be successful oral immunogen for raccoons and foxes under laboratory settings and in the field (Kieny *et al*., 1984). Even if VRG is safe and successful in vaccinated animals, its exposure to humans can cause skin inflammation and systemic vaccine infections. SAG-2, derived from an attenuated Street Alabama Dufferin (SAD) strain, has been effectively used in Europe for oral immunization of foxes (SAG-2 has also been shown to be safe, immunogenic and successful in dogs in field trails (MacInnes *et al*., 2001). However, the immunogenicity of SAG-2 is low and only low levels of VNA titers are detected in the immunized dogs (Hanlon *et al*., 2002).

### *2.9.2 Challenges encountered in rabies vaccine development*

The greatest tackle in the industry of anti-rabies vaccine production for both humans and animals, especially in some developing countries, is little or non-availability of modern technology to transfer from production of nerve tissue vaccine (NTV) to the tissue culture (MTCV)or sub-unit vaccines. For example, due to existence of high content of myelin of the adult brain tissue in NTV there is a high prevalence of neuroparalysis after usage of the NTV (Ghosh, 2005). NTV is not only paralyticogenic, it is less suitable, less immunogenic, more reactogenic, less tolerable and less acceptable. Besides, a greater number of doses is needed and the administration which compromise comparatively a pain full procedure MTCV on the other hand more antigenic, acceptable, well tolerated, and convenient with less reactogenicity (Ghoh, 2005).

Cost of vaccines is also another trouble, as new vaccines require not less than $300-$800 million to develop, and the companies doing the research and development must first recover this cost (Plotkin, 2005). To resolve this problem therefore, support from governments and donor agencies to

Buy the vaccines for developing countries is necessary. Another main challenge to be contended with in anti-rabies vaccine development and production in developing countries is lack of enforcement of registration and compulsory vaccination of dogs, free or subsidized vaccine/vaccination cost, regular mass vaccination campaigns and creation of awareness on rabies among the population (WHO, 2010). In general, there is a growing demand for vaccine safety worldwide. One of the advantages of the newer molecular technology is enhanced safety, even though zero risk is not possible (Plotkin, 2005).

### *2.9.3 Treatment*

Treatment is not recommended after signs are evident. In animals, instantly after exposure irrigation of wound with *20% soft soap* or *zephiran solution* prevent establishment of infection. Immediate and through washing of all wounds and scratches with soap and water is the most important action for preventing rabies in veterinarians. Post-exposure vaccination is unlikely to be of value in animals, as death frequently occurs before appreciable immunity has had time to develop (Gautret *et al*., 2014). Euthanasia of suspect animals must be avoided, mainly if human exposure has occurred, since the development of the disease in the animals is necessary to establish a diagnosis. In humans once, rabies warning signs have become visible, the treatment is generally supportive. The patients are sedated to handle their fear and pain. The basis of treatment is serious care support, counting paralysis, sedation, as well as ventilation. The ketamine is frequently recommended as a suitable mediator for these circumstances (Jackson *et al*., 2003). *Lyssa* virus is simply inactivated by the sunshine, soap, in addition to aeration. The wound concern is essential for the hindrance of rabies infectivity. The injured area should be rinsed carefully with antiseptic soap and water. After that povidone-iodine or alcohol should be applied in order to reduce the virus further (Gautret *et al*., 2014).

### 2.10 The Status of Rabies in Ethiopia

The treatments suggested for people bitten by rabid animals mainly dogs have been recorded in Many Ethiopian medical books since the early 17th century (WHO, 1995). The early 19th century travelers including Edward Ruppel, Rochetd'Hericourt, A. d'Abbadie and others reported either seeing a rabid dog or people bitten by rabid dogs and the first and only recorded rabies epidemic in Addis Ababa take place in August 1903. In addition, to this the continued existence of traditional medicine practitioners in the different parts of the country to date is an evidence for the significance of the disease in Ethiopia (WHO, 1987).

Rabies in Ethiopia is mainly a disease of dogs. Various people are at increased risk of being exposed to rabies since; human-dog contact is very common. Besides this Reports show that there is significant higher dog to human ratio, approximately 1:6 and 1:8 in urban and rural areas, respectively. Such a large figure of dogs in both urban and rural settings along with low vaccination imply the risk of rabies circulation and spread to human and other domestic animal populations (Admassu and Mekonnen, 2014). Yearly reports of the Ethiopian public health institute (EPHI) indicated a total of 488 human deaths occurred in 1964 to1975 (Deressa *et al*.,2010). On dog ownership pattern and awareness of rabies in Addis Ababa indicated that 90.7% of the dog owners manage dogs for the safeguarding of their properties from theft out of which 52% of them are without regular vaccination (EHNRI, 1997). According to (EHNRI, 2012) annual consumption of rabies vaccine in Ethiopia is above 36,000 doses for human and 12,000 doses for animals in particular for dogs (EHNRI, 2012).

The current situation in Ethiopia indicates mainly a serious threat of canine rabies virus transmission in the rapidly growing human population where a poorly controlled stray dog population is equally growing fast (Deressa *et al*., 2010). Retrospective study showed that during the years 1990–2000, several people received post exposure vaccines 96.2 % of which were bitten by dogs. During the same period 322 fatal human rabies cases were recorded and 95% of these were acquired from dogs (Yimer *et al*., 2002). During the next decade (2001-2009) the number of fatal cases increased to 386 ranging from 35 to 58 per year. The magnitude of post exposure vaccination escalated to 17,204 mostly from the capital. During this period a total 3,460 brains were examined from animals involved in human bite and 75% of them were positive (Deressa *et al*., 2010).

Rabies is an urban human problem in developing countries characterized by the incidence of disease in domestic animals such as pet dogs and cats (Shite *et al.*, 2015). Around 10,000 people were estimated to die due to rabies annually in Ethiopia which makes it to be one of worst affected country (Moges, 2015).Human mortality from endemic canine rabies was estimated to be 55, 000 deaths per year and was responsible for 1.74 million disability adjusted life years (DALYs) losses each year (Jemberu *et al.,* 2013). The magnitude of the problem is higher in big cities like Addis Ababa linked with the presence of large population of stray dogs and associated factors (Ali *et al*., 2013). In Ethiopia, approximately 76 persons per million of the population receive anti-rabies post-exposure treatments annually due to the widespread nature of rabies in the country (Reta *et al*., 2014; Ramos *et al*., 2015). According to Beyene *et al* approximately 3,000 human deaths were resulting in about 194,000 DALYs per year and 97,000 exposed persons requiring on average 2 million USD Treatment costs per year in Ethiopia (Beyene *et al*., 2018).

### *2.10.1 Rabies occurrences in Livestock and wildlife in Ethiopia*

In Ethiopia During the years (199 –2000), a total of 7749 animals were observed and tested for rabies and 1228 of them found to be positive. Dogs accounted for 95% of the total animals examined. Most of the time, hyena, jackals, mongooses and cerval cats were animals that were encountered in the incident of rabies (Yimer, 2001). Besides Reta *et al.* (2013), also account that 87.19% of the dogs examined were confirmed to be rabid. Among this 87.5% was a rabid female dog (Reta *et al*., 2013). According to the study conducted by Ali *et al* in the year 2003-2009 report showed that, out of 2517 animal brain tissue samples examined, 76.9% (1936) were positive for

Rabies virus. From total of 1936 1724 were dogs, 116 cats, 37 cattle, 13 Horses, 19 Donkeys, 13 sheep & goats, 7 Hyenas and 7 Monkeys in and around Addis Ababa (Ali *et al*., 2010).

Study done at North Gonder of Ethiopia showed an annual estimated rabies incidence of 2.33 cases per 100,000 humans; 412.83 cases per 100,000 dogs; 19.89 cases per 100,000 cattle; 67.68 cases per 100,000 equines, and 14.45 cases per 100,000 goats (Fagbami *et al*., 1981). Dog bite was the cause of infection for almost all fatal rabies cases throughout the country. The epidemiological study of Ethiopian Wolf rabies has been reported, as out of 15 brain samples of wolves submitted to rabies diagnosis center at CDC Atlanta, USA,13 were diagnosed positive. The report provided more emphasis on a rabies outbreak in a subpopulation of endangered Ethiopian wolves in the Bale Mountains between 2003 and 2004 (Randill *et al*., 2004).

#### *2.10.2 Myths and Beliefs in Rabies treatment in Ethiopia*

In Ethiopia, peoples who are exposed to rabies virus frequently see traditional healers for the diagnosis and treatment of the disease. The widespread use of traditional medicine among urban and rural population of Ethiopia could be attributed to cultural acceptability, physical accessibility and economic affordability as compared to modern medicine. Traditional practices of handling rabies cases are assumed to interfere with timely seeking of Post exposure prophylaxis (PEP) (Moges, 2015). Healing in Ethiopian traditional medicine is not only concerned with curing of diseases but also with the safeguard and support of human physical, spiritual, social, mental and material wellbeing (Deribe *et al*., 2006; Adimasu and Mekonnen, 2014).

#### *2.10.3 The effort of rabies control in Ethiopia*

There are several obstacles in the prevention and control of rabies in Ethiopia. One of these is inadequate laboratory capability and lack of diagnosis centers at different sites for effective surveillance and response. Rabies diagnosis and management is fundamentally dependent on diagnosis of rabid animals at one center, at Ethiopian Public Health Institute. Thus, there is only one laboratory that has the capability to confirm rabies in humans or animals resulting in poor management of rabies cases. Dog bites are used as a proxy for suspect rabies cases in humans for rabies surveillance and response system. Nevertheless, dog bites are under-reported in health facilities resulting in missed cases and misclassification of deaths due to rabies (Deressa *et al*., 2010). There is also inadequate sharing of surveillance data among human and animal health care sectors at both local and national levels, resulting in loss of opportunity to prevent human rabies, early detection and timely response to rabies outbreak. The national surveillance data is unpredictable, meaning that the true burden of the disease in the country or high-risk areas remains undefined; making it hard to target prevention and control measures. There is low awareness within the public, human and animal health workers have inadequate knowledge on management of dog bite wounds, and pre- and post-exposure prophylaxis (Jemberu *et al*., 2013).

Most patients who die from rabies are either do not report the case immediately or do not receive timely and appropriate post exposure treatment after exposure to the virus. Many dog bites in Children are not reported and may go completely unrecognized or be discovered by both parents and health care providers late after disease development. The other is the challenge of traditionally used medication like root and leaf of medicinal plants, and other religious approaches to treat the case as evil. The country is still producing and using Fermi type anti-rabies vaccine for post exposure treatment which is banned by WHO. Despite of its quality, there is limited supply of rabies vaccine and lack of adequate, safe and effective PET and PEP biologics in public health. Whereas high quality vaccine may be available in some private facilities, the cost is too expensive and cannot be afforded by public at large (Deressa *et al*., 2010).

# 3 Materials and Methods

## 3.1 Study Areas

This study was conducted Addis Ababa at Ethiopian Public Health Institute (EPHI), from February to September 2020 to assess the Knowledge, Attitudes and Practices (KAP) of the communities on rabies and the burden of rabies over the period of six years (2014 to 2019). EPHI is the only rabies diagnostic center in Ethiopia that produces the nerve tissue vaccine, so called Fermi type vaccine in adult sheep brain for use both in humans and animals. In addition, the laboratory provides post exposure anti rabies treatments and performs rabies diagnosis from brain samples originated from animals and humans. Samples processed at the laboratory originate from various parts of the country, the majority being from Addis Ababa and its surroundings. The city covers an area of 530 $km^2$ and is divided into ten sub-cities. The study area is depicted in Figure 3. The dog population in the city is estimated to be 250,000 to 350,000 (UAECP, 2011). According to CSA projection (2020) the total population of Addis Ababa is, 4, 794,000 of whom 2,238,798 are males and 2,555,202 are females.

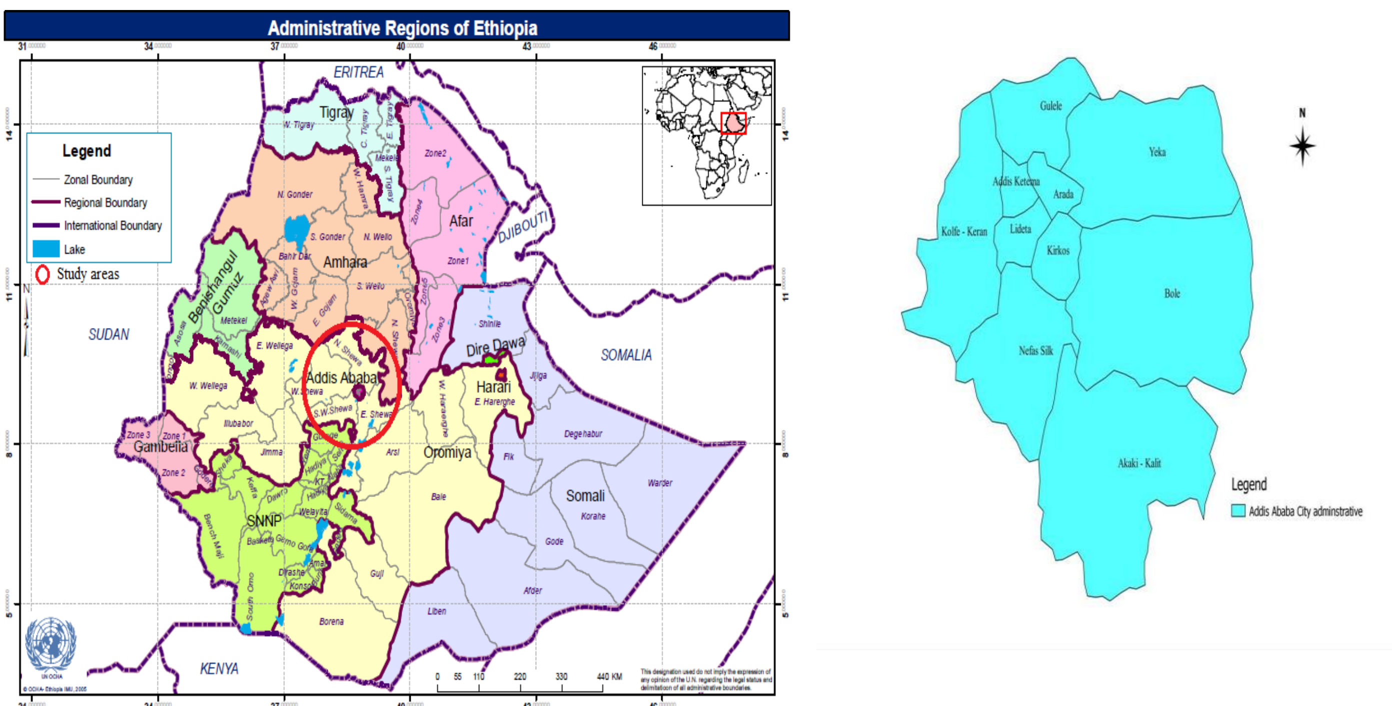


**Figure 3:** Map of ETHIOPIA and Addis Ababa City depicting the various sub-cities from which the data was collected

## 3.2 Study Design

### *3.2.1 Cross-sectional study design*

Hospital based cross-sectional study was conducted from February 2020 to April 2020 to assess the knowledge, attitude and practices of the community towards rabies. Two hundred and sixty-seven people who were bitten by rabies suspected animals were purposively interviewed for this study. The questionnaire was semi-structured with both open and closed-ended questions. The questionnaire was first prepared in English and translated to Amharic for appropriateness and clarity in approaching the study participants. Before the interview begun, they were briefed about the purpose of the study and were asked for their consent. Only voluntary participants were involved in the study. All the information obtained from the study participants was kept confidential. Knowledge of the disease, treatment and prevention practices were also included in the questionnaire. Then, the responses for which respondents give correct answer was counted and scored. This score was then pooled together, and the mean score was computed to determine the overall KAP of respondents.

### *3.2.2 Retrospective data collection*

A retrospective data was retrieved from February to September 2020 and assess for human and animal rabies exposure cases registered in the period from 2014-2019 in Addis Ababa.

**Animal cases**

The data on rabies in animals was a six-year data from 2014 -2019. For study involving rabies cases in animals, the following variables were retrieved from the record books of St. Paul hospitals and the ten sub-city health centers: Species of animals, Dog ownership, Vaccination status and Exposing animal status.

**Human cases**

The data on rabies in humans was also a six-year data recorded between 2014 -2019. For this part of the study the variables were retrieved include date, sex, age, address, type of biting animal, vaccine given and health institution where vaccine is given. The number of people that received post exposure anti rabies treatments during the same period was obtained by reviewing the records of people being bitten by rabid or suspected rabid animals that came mainly to the St. Paul hospitals and the ten sub-city health centers that provide post exposure treatment for humans.

**Estimating the burden of rabies**

The health and economic burden of rabies was estimated based on the cost classification (Jo, 2014). With the PET costs reflecting the direct costs, consisting of healthcare and non-healthcare costs expressed in monetary terms, and indirect costs in terms of DALYs as shown in Table one.

**Table 1: Classification of costs of rabies exposure to assess its Post exposure treatment and health burden.**

| Direct Cost | | Indirect Cost |
|---|---|---|
| Health care cost | Non-health care cost | |
| Post exposure vaccine | Transportation | Mortality (DALYs) |
| Wound care and disinfection | Productivity loss /opportunity costs of time while seeking treatment | Morbidity (DALYs) |
| Antibiotics | | |
| Tetanus Immunization | | |

**Estimating post-exposure treatment costs**

The costs of rabies post-exposure treatment were classified into two. Direct costs category including health care costs reflected by the expenditures for rabies vaccine, antibiotics, tetanus immunizations and disinfection and non-healthcare costs categorized as the expenses for transport, and productivity loss /opportunity costs of time while seeking PET (Table 1). Costs were estimated from the record books in St. Paul hospitals and from health centers under the auspices of the ten sub-cities on the number of PEP doses received, the number of visits to the health centers to receive PEP, from which sub-city they came. Expenditures during the medical treatment related to transportation, and the time spent by themselves and their caregivers (if applicable) to account for productivity losses while seeking treatment were also included. For patients older than 15 years, the productivity losses while seeking treatment were valued in monetary terms using the GDP per capita daily income. Related productivity losses were also captured considering Ethiopian average income per capita for each year (World Bank, 2019). We valued the daily average income for each year in terms of USD per day. All the costs collected were in Ethiopian birr (ETB) and later converted to US dollars (USD) using the average annual exchange rate for the studies period published by the National Bank of Ethiopia (NBE, 2019).

For the estimation of PET costs, we considered PET costs per sufficient treatment. A complete PEP dose of NTV consists of 17 doses of vaccine administered consecutively for the first 14 days, with the remaining 3 doses at intervals of 10 days i.e. at day 24, 34 and 44. A treatment consisting of at least 14 of the 17 PEP doses was considered to be "sufficient", while any treatment consisting of less than 14 doses was regarded to be "insufficient", based on the minimum number of required doses to produce the neutralizing antibody level (Ayele *et al*., 2001). Consequently, the average costs per sufficient treatment only accounted for the costs of patients who successfully received the minimum recommended doses (at least 14 out of the 17 Doses) of PEP including the opportunity cost of time spent while seeking treatment.

**Estimating the Health Burden of the Disease**

To calculate a DALY score for rabies the following components were considered a direct DALY score derived from mortality due to the disease and indirect DALY score considering morbidity and mortality following side effect of nerve tissue vaccine (Knobel *et al*., 2005).

$$DALY=YLL + YLD = \sum_{i=1}^{18} (\mathrm{N}deathi * \mathrm{e}i) + (Ndisi\_ * t\_ * DW)$$

YLL is calculated as the number of human deaths (*Ndeath*) within age category (*i*) multiplied by the life expectancy (*e*) of the concerned age category (*i*), cumulated over all age categories. Eighteen age groups with a 5 years age interval from 0 to 85 years and above were considered ($i$ = 1. . .18) according to the age classification of WHO life expectancy table. The expected years of life lost at death were derived from the standard life table as given by the WHO using the projected frontier life expectancy of 2050. Similarly, YLD is calculated by multiplying the total number of disability cases (*Ndis*) of the concerned age group (*i)*, with the duration of the disability (*t* in years) resulting from the NTV vaccine and its corresponding disability weight (*DW*) cumulated over the 18 age categories (Knobel *et al*., 2005; WHO, 2017).

The disability (YLD) due to the disease itself was considered insignificant as the disease is very acute, leading to death once it progresses (Hotez and Kamath, 2009). As a result, the health burden due to NTV was indirectly derived from the number of persons receiving PEP corrected for the number of deaths due to rabies and estimated likelihoods of adverse effects based on the study of Knobel *et al* (Knobel *et al*., 2005). YLL was derived from the number of patients who got at least one dose of NTV PEP, multiplied by the expected rate of neurological complications (0.4 out of 100 Patients receiving NTV) and case fatality rate (17 out of 100 cases of neurological complications). The YLD due to NTV was calculated by multiplying the number of patients who got at least one dose of NTV PEP by the expected rate of neurological complications (0.4 out of 100 patients receiving NTV) and corresponding disability weight (DW = 0.613) for a most likely duration of disability of 8 days (t = 0.022 years) (Knobel *et al*., 2005; Beyene *et al*., 2018).

### 3.3 Statistical Analysis

Descriptive statistics were used to summarize proportions of rabid suspected dog bite cases per age category, gender, health center visits and sufficient doses of PEP receiving status. The data collected were subjected to statistical package for social sciences (SPSS) version 20 for analysis. Descriptive methods such as chi-squared tests will be used to test the association of the dependent variable (occurrence/incidence of rabies in animals and humans) and the independent variables (sex, age, etc). *P-value* less than 0.05 were taken as significance level presence of association among categorical variables.

### 3.4 Ethical Considerations

Ethical clearance and approval were obtained from Addis Ababa University, College of Veterinary Medicine and Agriculture. Each participant was informed about the purpose of the study and informed consent was obtained from each respondent. Participation in the study was voluntary and respondents were free to withdraw from the study at any time. Their consent was recorded (marked) on the questionnaire paper, interview was anonymous, and data remained confidential throughout the study.

# 4 RESULTS

## 4.1 Socio-Demographic Characters of the Participants

A total of 267 people bitten by rabies suspected animals were interviewed during the study period. The age of the respondents ranged from 16 to 68 years, 82.02% of them were between the age of 16-45 years and the majority of the respondents were male (65.5 %). Among the study participants, 30.0% had completed secondary school followed by primary school (33.3%) whereas 28.1% of the participants had tertiary education. More than half of the respondents (68.2%) did not own dog. Regarding the type of employment 30.4% were engaged in running their own private businesses, 24.2% were employed by private companies, 20.6% of the respondents were unemployed, and the rest (20.2%) were government civil servants. In addition to this, 75.7% of the people were bitten by stray dogs, 94% of the cases were acquired through bite and when we saw the status of rabies suspected animals 78.7% was unknown, 11.2% were tested positive for rabies and 10.1% were under clinical observation (quarantine).

## 4.2 Knowledge, Attitude and Practice of the Community Concerning Rabies

The data showed that 62.8% of the respondents had good level of knowledge, regarding the attitude and practice scores, 51.1% of them had medium level of attitude and appropriate practices towards rabies (Table 2). In this study 98.1% of the participants had heard about rabies and fully understood that the disease affected both humans and animals and also transmitted from animals to humans and humans to humans. All most all the respondents (91%) heard about rabies informally from the community. The majority 168 (62.9%) of the respondents replied starvation and thirst as the causative agent of rabies. Moreover, 92 (34.5%) of them did not know about the cause of rabies. Only twenty two percent of the study respondents were able to identify most recognized clinical signs of the disease in animals. It was widely perceived among respondents that bite was a main mode of rabies transmission from animals to animals and from animals to humans. Most of the respondents knew that rabies can be prevented in animals (47.62%) through regular vaccination against the disease and thirty six percent agreed on both regular vaccinations and killing of stray dog. The majority 173 (64.8%) of them correctly answered that immediate medical seek is necessary. and 91% of the respondents believe taking post exposure treatment as effective preventive measures.

**Table 2: Knowledge of respondents in relation to cause, host range, clinical sign and transmiss ion of rabies in Addis Ababa, Ethiopia.**

| Variable | Frequency % |
|---|---|
| **Heard about rabies** | |
| Yes | 262(98.1%) |
| No | 5(1.9%) |
| **Cause of rabies** | |
| Virus | 7(2.6%) |
| Starvation and thirst | 168(62.9%) |
| Associated with sprit | |
| Do not know | 92(34.2%) |
| **Species affected by rabies** | |
| Animal | 5(1.9%) |
| Human | |
| Animal and human | 262(98.1%) |
| I do not know | |
| **Transmit from animal to human** | |
| Yes | 262(98.1%) |
| No | 5(1.9%) |
| **Means of transmission** | |
| Bite | 149(55.8%) |

| | |
|---|---|
| Contact with Saliva only | 2 (0.7%) |
| Bite and saliva contact with open wound | 99(37.1%) |
| Rabid animal respiration | 12(4.49%) |
| Do not know | 5(1.9%) |
| **Sign and symptom in animals** | |
| Stop eating and drinking | 7(2.6%) |
| Biting and change in behaviour | 50(18.7%) |
| Salivation and Paralysis | 114(42.7%) |
| All | 58(21.7%) |
| I don't know | 5(1.9%) |

Association between independent variables and knowledge scores on rabies was calculated using Pearson's Chi square. There was strong association between knowledge scores and type of employment (P=0.003), those respondents who run their own private businesses had good knowledge than the other respondents. In this study the good scores were higher in males (62.87%) than females.

### 4.3 Attitude and Practice Concerning Rabies

From 267 people 85 (31.8%) of them had dog among whom 57 (67%) vaccinated their dogs this year (2019/2020) and 76 (89.4%) own dog for the purpose of guarding. More than half of the dog owners (58.8%) secured their pets by keeping them in cage. The majority (95.5%) of the bitten victims were annoyed by the presence of stray dogs. Nearly 60% of the respondents practiced killing of rabid animals. In the current study, 54.7% of the respondents reported that they washed their wound using soap and water as a first aid. Over half (55.1%) of the victims knew that rabies is fatal;

However, 70 % of bitten people believe that it is easily treatable after onset of clinical signs. In addition to this, 108 (40.4%) of them knew that suspected pet head should be submitted for diagnosis. This study found statistically significant difference between attitude and practice scores and gender (P= 0.001). The good scores were higher in males (51.3%) than females (Table 3).

### 4.4 **KAP Correlation Analysis**

Linear correlation analysis was done based on individual scores to determine the relationship between Knowledge, Attitude and Practice. There was significant positive correlation between Knowledge and Attitude and practice (P=0.001). People with good knowledge score had good score for attitude and practice than others.

**Table 3: Community attitudes and practices regarding rabies**

| **Variable** | **Frequency%** |
|---|---|
| **Is rabies fatal** | |
| Yes | 147(55.1%) |
| No | 76(28.5%) |
| I don't know | 44(16.5%) |
| **Action for rabid animal** | |
| Tie | 86(32.2%) |
| Killing | 160(59.9%) |
| Nothing | 21(7.86%) |
| **Preferred action taken for bitten human** | |
| Traditional healers | 24(9%) |
| Post exposure vaccine | 243(91%) |
| **First aid for bitten human** | |
| wash with water and soap | 146(54.7%) |

| | |
|---|---|
| Tying with cloth | 45(16.9%) |
| Nothing | 76(28.5%) |
| **Anti-rabies vaccine in human after exposure** | |
| Immediate | 173(64.8%) |
| Latter | 82(30.7%) |
| I do not know | 12(4.8%) |
| **Easily treatable after onset of clinical signs** | |
| Yes | 187(70.03%) |
| No | 28(10.48%) |
| I don't know | 52(19.5%) |
| **How rabies transmissions can be prevented /controlled in animals** | |
| Vaccination | 126(47.2%) |
| Killing stray dog | 45(16.9%) |
| Both | 96(35.96%) |
| **Knows that suspected pet head to be submitted to a veterinarian** | |
| Yes | 108(40.4%) |
| No | 159(59.6%) |
| **Annoyed by the presence of stray dogs** | |
| Yes | 255(95.5%) |
| No | 12(4.5%) |

### 4.5 Status of Rabies Exposure

A total of 8613 victims were registered and received post exposure vaccine in Addis Ababa during the last six years (2014 to 2019). From this most important animal species responsible for PEP was dogs (93.8%) followed by cats (2.5%), human bite and contact (2.1%) and others (1.6%) (Cattle, Donkeys, Horses, Monkeys, Hyenas, Goats and Sheep). The overall rabid suspected animal bite exposure ratio of male to female was 0.62:0.38. There was statistically significant difference between the two gender in the frequency of exposure (P=0.001). Age wise, the exposure to rabies suspected animals in Addis Ababa was highest in individuals aged 16-30 years followed by those aged 6-15 years. Very young children and elderly people, i.e. 0-4 years of age and older than 60 years of age, were the least exposed age categories (Table 4).

More than ninety percent (91.3%) of the victims received sufficient PEP. The remaining portions ether did not show compliance because the animals were not rabid (when people bitten around face, head, upper part of the body they started the post exposure and if the animal not died after ten days they stop) or seek cell culture vaccine from private pharmacies. Among the ten sub-cities the highest post exposure treatment was given to victims from Kolfe Keraniyo (20.2%) while the lowest was given to victims from Lideta (4.6%) as shown in Table 5.

In 2018 and 2019 a total of 1455 people received post-exposure prophylaxis against rabies because of exposure to rabid or rabies suspected animals in Kolfe Keraniyo, Yeka and Bole. From this, 87% (1265/1455), of the post-exposure prophylaxis was given to humans bitten by animals with an unknown rabies status. Eleven percent of the PEP vaccines were prescribed while the dogs were under quarantine and observation for the development of clinical rabies. While 1.8% (26/1455) of the PEP was given to humans bitten by animals in which rabies was confirmed.

**Table 4: Frequency of occurrence of exposure to rabies suspected animal bites during the study period (2014 – 2019).**

| . Year | Age | | | | | | Gender | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | 1-4 year | 5-14 year | 15-29 year | 30-44 year | 45—59 year | >60 year | Female | Male | |
| 2014 | 73 (6.6%) | 344(30.9%) | 397(35.7%) | 199(17.9%) | 41(3.7%) | 58(5.2%) | 478(43%) | 634(57.0%) | 1112 |
| 2015 | 76(6.0%) | 308(24.1%) | 466(36.5%) | 234(18.3%) | 121(9.5%) | 72(5.6%) | 495(38.8%) | 782(61.2%) | 1277 |
| 2016 | 92(5.9%) | 404(25.9%) | 535(34.3%) | 303(%) | 198(12.7%) | 29(1.9%) | 654(41.9%) | 907(58.1%) | 1561 |
| 2017 | 54(4.7%) | 275(24.2%) | 394(34.7%) | 215(18.9%) | 131(11.5%) | 68(6.0%) | 403(35.4%) | 734(64.6%) | 1137 |
| 2018 | 27(2.6%) | 208(20.3%) | 434(42.4%) | 191(18.7%) | 141(13.8%) | 23(2.2%) | 364(35.5%) | 660(64.5%) | 1024 |
| 2019 | 61(2.4%) | 631(25.2%) | 960(38.4%) | 528(21.1%) | 300(12.0%) | 22(0.9%) | 964(38.5%) | 1538(61.5) | 2502 |
| Total | 383(4.5%) | 2170(25.4%) | 3186(37.4%) | 1670(19.6%) | 932(10.94%) | 272(3.2%) | 3358(39.4%) | 5255 | 8613 |

**Table 5: Distribution of post exposure treatment (NTV) for rabies in the sub city of Addis Ababa from 2014-2019.**

| Sub city | Year | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | Total |
| Addis Ketema | 131 (11.8%) | 123(9.6%) | 175(11.2%) | 167(14.7%) | 148(14.5%) | 295(11.8%) | 1039(12.06%) |
| Nifassilk/Lafto | 115(10.3%) | 125(9.8%) | 124 (7.9%) | 79(6.9%) | 72 (7%) | 120(4.8%) | 635(7.37%) |
| Gullele | 129(11.6%) | 125(9.8%) | 174(11.1%) | 142(12.5%) | 108(10.5%) | 202(8.1%) | 880(10.21%) |
| Yeka | 121(10.9%) | 153(12.0%) | 151(9.7%) | 75(6.6%) | 96(9.4%) | 239(9.6%) | 835(9.69%) |
| Bole | 125(11.2%) | 167(13.1%) | 102(6.5%) | 67(5.9%) | 59(5.8%) | 323(12.9%) | 843(9.78%) |
| Lideta | 51(4.6%) | 76(6.0%) | 94(6.0%) | 90(7.9%) | 51(5%) | 152(6.1%) | 514(5.96%) |
| Kirkos | 68(6.1%) | 81(6.3%) | 99(6.3%) | 77(6.8%) | 83(8.1%) | 174(7.0%) | 582(6.75%) |
| Arada | 73(6.6%) | 95(7.4%) | 108(6.9%) | 50(4.4%) | 152(14.8%) | 137(5.5%) | 615((7.14%) |
| Kolfe Keraniyo | 225(20.2%) | 237(18.6%) | 282(18.1%) | 208(18.3%0 | 153(14.9%) | 585(23.4%) | 1690(19.62%) |
| Akaki Kality | 74(6.7%) | 95(7.4%) | 252(16.1%) | 182(16.0%) | 102(10.4%) | 275(11.0%) | 980(11.37%) |
| TOTAL | 1112(12.9%) | 1277(4.8%) | 1561(18.12%) | 1137(13.2%) | 1024(11.88% | 2502(29.04%) | 8613 |

### 4.6 Estimated Burden of Rabies in Addis Ababa

**The Cost of Post Exposure Treatment (PET)**

The average PET costs per bite case was estimated from health care expenditures and non-health care costs of all 7862 animal bite cases, which account for NTV. Table 6 presents the results of the distribution of health care costs and non-health care costs per animal bite considered for the estimation of the burden of rabies. Generally, the non-health care costs account for the largest part in total PET costs. Healthcare costs per bite case were generally higher in the sub-city health centers than St. Paul Millennium hospital. In St. Paul Millennium hospital the post exposure vaccine and the tetanus immunization for children less than five years is free whereas in the other sub-city health centers the post exposure vaccine account from 20 to 60 ETB. Tetanus antitoxin was the highest cost in health care costs. The non-health care costs per sufficient treatment were higher in Kality, Nifas Silk /lafto, Yeka, Bole and Kolfe Keraniyo sub-cities than the others sub cities because of transportation cost as they are far from St. Paul Millennium hospitals. Except Akaki Kality, which started providing post exposure treatment since 2015, the other sub cities started providing post exposure during the last two years.

Gullele sub city provided the cell culture derived vaccine for six months in 2018 but currently stopped. In Addis Ababa there are three private pharmacies that provide the cell culture and embryonated egg-based rabies vaccines (jointly referred to as CCEEVs) costing ETB 6000-8000, which is difficult to afford by the majority of the population. Regarding productivity losses due to the time spent by victims in search of treatment accounted an average 3-6 hours per days for seventeen days per sufficient treatment.

**Table 6: Distribution of direct health costs associated with the demonstration of PET in USD in Addis Ababa during 2014 – 2019.**

| Costs | Year | Children | | Adult | |
|---|---|---|---|---|---|
| | | Minimum | Maximum | Minimum | Maximum |
| Health care | 2014 | $1 | $1.2 | $2.29 | $3.83 |
| cost | 2015 | $ 0.71 | $1.43 | $2.62 | $4.75 |
| | 2016 | $0.9 | $1.37 | $2.75 | $5.97 |

| | | | | | |
|---|---|---|---|---|---|
| | 2017 | $0.83 | $1.25 | $2.5 | $5.43 |
| | 2018 | $1 | $1.3 | $3.08 | $7.76 |
| | 2019 | $0.95 | $1.2 | $3.09 | $8.08 |
| Non-health care cost | 2014 | $10.71 | $18.4 | $10.71 | $18.4 |
| | 2015 | $10.68 | $21.1 | $10.68 | $21.1 |
| | 2016 | $9.53 | $17.2 | $9.53 | $17.2 |
| | 2017 | $13.55 | $21.55 | $13.55 | $21.55 |
| | 2018 | $8.2 | $14.1 | $8.2 | $14.1 |
| | 2019 | $9.24 | $16.2 | $9.24 | $16.2 |
| Total direct cost | 2014 | $11.7 | $19.6 | $13 | $22.23 |
| | 2015 | $11.39 | $21.03 | $15.62 | $25.85 |
| | 2016 | $10.43 | $18.57 | $12.28 | $23.17 |
| | 2017 | $14.38 | $22.8 | $16.05 | $26.98 |
| | 2018 | $9.2 | $15.4 | $11.28 | $21.86 |
| | 2019 | $10.19 | $16.6 | $12.33 | $24.28 |

The average cost of transportation decreased in 2018 and 2019 compared to the former years due to the availability of post exposure treatment in every sub city health center but the health care cost increased because the cost of post exposure treatment is not free in the sub city health centers and anti-tetanus immunization cost is high and not constant (Table 7).

**Table 7: Average cost of transportation and productivity cost in USD**

| Year | Average cost of transportation | Average productivity cost |
|---|---|---|
| 2014 | $9.5 | $5.08 |
| 2015 | $10.2 | $5.7 |
| 2016 | $7.5 | $6.4 |
| 2017 | $10.6 | $7 |
| 2018 | $4.2 | $7 |
| 2019 | $5.1 | $7.6 |

In 2014-2019 we got seventeen recorded fatal cases. Among these 9 (53%) were females 8 (47%) males their age ranging from 8-65 years; when we saw sites of bite 6 (35.3%) were on face and leg, 4 (23.5%) on hand and 1(5.9%) on thigh. Majority of the cases (seven) were from Kolfe Keraniyo three cases Nifas Silk Lafto, two from Yeka and one case each from Gullele, Akaki Kality, Lideta, Kirkos and Arada.

### 4.7 Health Burden due to the NTV Application

Based on the number of people taken nerve tissue vaccine, we estimated the indirect DALYS. The health burden due NTV was estimated to be 430-955 DALYs with average 10 human death and 57 disability per 100,000 population per year. The highest health burden due to NTV and exposure rate was observed in 2019 while the lowest health burden and exposure rate was in 2018 in Addis Ababa (Table 8).

**Table 8: Annual health burden due to adverse effects of Nervous Tissue Vaccine (NTV) in DALYs per 100,000 populations and Exposure rate.**

| | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 |
|---|---|---|---|---|---|---|
| YLL | 279 | 313 | 359 | 254 | 260 | 485 |
| YLD | 196 | 228 | 276 | 194 | 170 | 470 |
| DALYS | 475 | 541 | 635 | 448 | 430 | 955 |
| Exposure Rate | 30 | 33 | 38.6 | 26.96 | 23.3 | 54.5 |
| Death due to NTV complication | 7.6 | 8.7 | 10.6 | 7.7 | 7 | 17 |
| Disability due to NTV complication | 44.5 | 51 | 62.4 | 45.5 | 41 | 100 |

# 5 DISCUSSION

Rabies remains an important disease both in humans and animals in Addis Ababa and its surrounding. In Ethiopia, the Fermi type vaccine has been widely used to treat most of the exposures to rabies cases. This type of vaccine is less efficacious than the cell culture derived vaccines and known for its fatality and disability rate due to post vaccinal reactions. The scenario seems worse in Addis Ababa city than other areas due to the presence of large number of pets. Thus, the magnitude of cases of rabies in humans and animals calls for rigorous epidemiological studies to have up-to-date and reliable information about the disease. Quantification of the health burden enhances the understanding of its long-term effects and of the comparative advantages of different levels of treatment and prevention. To this end the KAP of the victims and the burden of rabies were investigated. The results are important for the health and veterinary authorities in their endeavor to prevent or control its occurrence.

In this study the overall knowledge, attitude and practice (KAP) revealed that majority of the respondents had good level of knowledge, and medium level of attitude and appropriate practices towards rabies. The good level of knowledge and attitude is an attribute that can be considered for the control of rabies. Since the community had good level of knowledge it would be easier to participate in the control activities. This finding was consistent with results recorded in Bahir Dar town (Guadu *et al*., 2014) and Debark District, North Gondar (Yalemebrat *et al*., 2016). In contrast this finding is higher than the study conducted in Dedo district of Jimma zone (Abdela *et al*., 2017). However, it is lower than the study conducted in Kombolcha, Southern Wollo (Gebremeskel *et al.***,** 2019). This difference could be due to the difference in sample size and lack of health education programs about rabies and level of awareness of community about rabies in the study area.

In the current study 98.1% of the respondents had heard about rabies suggesting that the community is familiar with the disease. This observation is in agreement with study conducted in Gondar Zuria District (Digafe *et al*., 2015), Debark District, North Gondar (Yalemebrat *et al*., 2016), and in Dedo district of Jimma zone (Abdela *et al*., 2017) in Ethiopia. However, the proportion of the community who reported awareness about the disease in this finding was higher when compared to the results of

Survey of knowledge, attitudes and practices about animal bite and rabies in India (Ichhupujani *et al*., 2006), and in Addis Ababa (Ali *et al*., 2013). This is mainly because of the difference in the source of information, that is, the composition of the selected population and the way by which the information is collected.

Sixty three percent of the respondents in this finding perceived that starvation and thirst is the cause of rabies. Moreover, 34.5% of them did not know about the cause of rabies. Similar to these finding previous authors also recorded lack of knowledge about rabies in significant proportion of the respondents in Debark District, North Gondar (Yalemebrat *et al*., 2016). However, this result is higher as compared to the result of study in Bahir Dar town (Guadu *et al*., 2014) in and around Dessie town (Serebe *et al*., 2016) and in Dedo district of Jimma zone (Abdela *et al*., 2017) in Ethiopia. In addition, this KAP analysis revealed that 98.1.0% respondents knew that rabies could affect human and other domestic animals in line with the study conducted in Addis Ababa (Ali *et al*., 2013). However much higher than in Debark District, North Gondar (Yalemebrat *et al*., 2016), and in Bahir Dar town (Guadu *et al*., 2014). The possible reason for this could be, level of awareness and educational status of community.

In this study low level (22%) of the respondents were able to identify most recognized clinical signs of rabies in animals suggesting the lack of knowledge of the diseases despite its widespread occurrence and frequent bite by rabid animals. This is in line with the results of study done in Debretabor (Awoke *et al*., 2015) and Bahir Dar (Tadesse *et al*., 2014) towns of northern Ethiopia. Although the level of knowledge of the respondents is low, 56% of them believed bite was main mode of transmission, which better. However, the knowledge about the mode transmission observed in this study is lower than the results of study conducted in Kombolcha, Ethiopia (Gebremeskel *et al*., 2019), Nigeria (Asabe *et al*., 2012) and Addis Ababa (Ali *et al*., 2013) but it is higher than the results of research conducted in Dedo district of Jimma zone (Abdela *et al*., 2017). Such variations could have emanated from variations in the source of information.

In the present study, 54.7% of the respondents reported that they washed their wound using soap and water as a first aid. However the study in Debark District, North Gondar (Yalemebrat *et al*., 2016), and Kombolcha, Southern Wollo (Gebremeskel *et al*., 2019) showed that higher number of respondents were aware of the fact that wound washing is an immediate post exposure (after dog bite) action but higher as compared to the study conducted in Dedo district of Jimma zone (Abdela *et al*., 2017), in Gondar zuria district (Digafe *et al*., 2015) and in a rural community of Gujarat, India (Singh and Choudhary, 2005). This difference could be associated with awareness level of the community. Washing of rabies-infected wounds with soap and water can increase survival by 50% (Radostits *et al*., 2007).

More than half (64.8 %) of the victims correctly answered that immediate medical seek is necessary and 91% of the respondents believe taking post exposure treatment as effective preventive measures, which is agreed with the study conducted in Dedo district of Jimma zone (Abdela *et al*., 2017) and in Bahir Dar town (Guadu *et al*., 2014). In contrast to this the study conducted in Dabat, Gondar, which reported that majority of respondents used traditional medicine when exposed to the disease (Jemberu *et al*., 2013). The World Health Organization (WHO) also recommends wound washing and vaccination immediately after contact with a suspected rabid animal which can prevent almost 100% of rabies deaths (WHO, 2013).

Nearly half of (47.62%) respondents in this study knew that rabies can be prevented in animals through regular vaccination against the disease and thirty six percent agreed on both regular vaccinations and killing of stray dog. This result in agreement with the study in Kombolcha, Southern Wollo (Gebremeskel *et al*., 2019). In contract to this study in Dedo district of Jimma zone (Abdela *et al*., 2017) revealed that more than half of participants do not believe in rabies prevention by vaccinating dog and of them replied that rabies cannot be prevented by eliminating stray or confining dogs. This is serious knowledge deficiency and community deserves public awareness.

The current study revealed that 55.1% of the victims knew that rabies is fatal; however, 70% of bitten people believe that it is easily treatable after onset of clinical signs. This result is higher than the study conducted in Addis Ababa (Ali *et al*., 2013). Conversely, to the study conducted in the city Kombolcha, Southern Wollo (Gebremeskel *et al*., 2019) reported that almost all of the study participants know rabies as a killer disease.

In the present study 31.8% of the respondent had dog among whom 67% vaccinated their dogs this year (2019/2020) and 89.4% own dog for guarding. More than half of the dog owners 58.8% secured their pets by keeping them in cage. The majority (95.5 %) of the bitten victims were annoyed by the presence of stray dogs. This is an indication of their willingness to vaccinate their pets and believe that vaccination program and depopulation of stray dogs are effective measures for controlling the disease. The finding of the present study was also in consistence with results in Addis Ababa (Ali *et al*., 2013) and recorded in Sir Lanka, where the majority of the participants were in favor of rabies control programs that mainly focused on stray dog population control (Gino *et al*., 2009). In contract a significant number of households in Sub-Saharan Africa were less likely to confine their dogs, whether in urban and rural areas. Only, less than half of the pet owners had vaccinated their animals against rabies (Butler and Bingham, 2000).

In this study the good scores were higher in males (62.87%) than females beside this study found statistically significant difference between attitude and practice scores and gender (P=0.001) being higher in males (51.3%) than females (49.7%). The same proportion of statistical difference on KAP score of male (53.4%) and female (10.75%) was reported in Bahir Dar town (Guadu *et al*., 2014), in Addis Ababa (male, moderate (77.09%) and good (10.55%), female, moderate (73.62%), good (5.08%) (Ali *et al*., 2013), in Debark District in males (64.6%) in females (54.5%) (Yalemebrat *et al*., 2016) and males (71.4%) than females (25.8%) in Dedo district of Jimma zone (Abdela *et al*., 2017). Statistically significant difference in KAP score between males and females might be due to increased activity of males in their daily life when compared with females and better chance of acquiring correct information about rabies.

Furthermore, the present study statistically proved the fact that, the types of occupations play significant role to KAP scores on rabies (P=0.003), which agreed with the study Kombolcha, Southern Wollo (Gebremeskel *et al*., 2019). The probable justification could be types of the works, sharing of information between different people with different experience, status and social background, which might be important factors for higher KAP score obtained. The Correlation analysis results showed that strong positive correlation; between Knowledge and Attitude and Practice. This suggests that good knowledge on rabies lead to good attitude and practice in prevention of rabies. Knowledge affects the individual’s behaviour and literatures on KAP studies revealed that healthy behaviours are enhanced by a person’s increased level of knowledge (Ellen, 2009).

The retrospective data in this study indicated that dogs are responsible in maintaining as well as dissemination of rabies in Addis Ababa. The actual number of rabid dogs in Addis Ababa is expected to be higher in comparison to the large number of stray dogs roaming around in the streets. Moreover, in the present study dogs contributed to (93.8%) of the human rabies post exposure cases that demanded post exposure anti rabies treatments. These findings are consistent with those study conducted in Ethiopia (Fekadu, 1997; Ayalew, 1985; Yimer *et al*., 2002; Deressa *et al*., 2010; Reta *et al*., 2014).

In the current study the exposure to rabies suspected animals in Addis Ababa was highest in individuals aged 16-30 years followed by those aged 6-15 years. Very young children and elderly peoples were the least exposed age categories which agreed with the study conducted in Bishoftu, Lemuna-bilbilo, Yabelo (Beyene *et al.,* 2018) ,in Tigray (Teklu *et al.,* 2018) and countries in the East African region (Hampson *et al.,* 2015). Among the ten sub-cities the highest post exposure treatment was given to victims from Kolfe Keraniyo while the lowest was given to victims from Lideta. The probable justification could be the number of stray dogs roaming in the sub city.

In the present investigation males were shown to be at higher risk of exposure to rabies than females. This could be due to the socio-cultural influence of allocating most of the outdoor activities to males while females mostly work indoor. Similar findings were observed by the different studies in Ethiopia (Beyene *et al*., 2018; Teklu *et al*., 2018; Yibrah and Damtie, 2015). Further in this study showed that 87% of the post-exposure prophylaxis was given to humans bitten by animals with an unknown rabies status this is agreed with study conducted in Addis Ababa in the period 2010-2011 (Reta *et al*., 2014).

In the current finding an average about 1435 people received post exposure anti rabies treatments annually during the year 2014-2019. This finding was lower as compared from recording in the period between 1990-2000 which was 2200 (Yimer *et al*., 2002). In contrast, it was higher than from recording in the period between 2001-2009 which was 1224 in Addis Ababa (Deressa *et al*., 2010). Currently in Addis Ababa there is no stray dog elimination/depopulation because of animal welfare issues and the Authority tried to vaccinate stray dogs but it was very difficult to handle and exhausted. In this finding lack of dog immunization, lack of community awareness of dog management and living in an urban setting where stray dogs are abundant were major risk factors associated with the spread of rabies exposure between humans and animals. Similar finding was observed by the different studies in Ethiopia (Yimer *et al*., 2002; Deressa *et al*., 2010; Yibrah and Damtie,. 2015; Teklu *et al*., 2018).

Regarding the health impact we estimated approximately 10 human deaths and 57 disabilities resulting 430-955 DALYS per 100,000 populations per year due to administration of nerves tissue vaccine (NTV). This result is higher than the research conducted in Bishoftu, Lemuna-bilbilo and Yabelo (Beyene *et al*., 2018). Additionally, in this study the highest exposure rate was recorded in 2019 (54.5/100,00) while the lowest exposure rate was in 2018 (23.3/100,000), respectively. This finding similar with reports from studies in New York (27/100,000) (Humphrey *et al*., 2010), Tanzania (58/100,000) (Blanton *et al*., 2005), but lower than Kenyan active surveillance report (234/100,000) (Kitalaa *et al*., 2000). Our suspected rabid dog exposure estimate much higher than previous national estimates consisting of 12 exposure cases per 100,000 population (Deressa *et al*., 2010) and of 1±5 exposure rates per 100,000 population as reported by Yibrah and Damtie (Yibrah and Damtie, 2015). Underreporting in Ethiopia primarily occurs due to the deep-rooted traditional practice of treating rabies by healers, which as such interferes with assessing the real magnitude of the problem.

In the present study, the cost estimation per sufficient post exposure treatment (PEP) it accounted around 9.2 ±22.8 for children and 11.8±27 for adults in dollar. This study is comparable with the study conducted by Beyene *et al* 23.4, 31.5 and 40.1 USD in in Bishoftu, Lemuna-bilbilo and Yabelo respectively (Beyene *et al*., 2018). The highest contribution direct health care cost for adults were the tetanus immunization whereas the indirect health care cost for the year 2014-2017 was the transportation cost while 2018-2019 was the productivity loss. The availability of the post exposure vaccine in each sub cities helpful which minimize the cost of transportation as well as the productivity loss in searching treatment.

Application of the WHO recommended cell culture PEP cell culture PEP by a 5-dose vaccine regimen instead of the currently applied 17 doses NTV, would result in considerably higher health care costs (average of 172.4 USD per five doses) versus maximum (2 USD per 17 doses NTV-PEP). The non-healthcare costs will, however, is reduced due to the reduction in number of doses and therefore the required number of visits to the health centers (5 visits versus 17 visits). In addition to its cost we can minimize the fatality rate and disability rate due to application of NTV. This increase in costs is primary related to the difference in price of the imported cell culture-based PEP and the locally produced NTV. Comparable costs were also reported in the study conducted by Beyene *et al* in 2018 (Beyene *et al*., 2018) and in Tanzania (Shim *et al*., 2009).

# 6 CONCLUSION AND RECOMMENDATIONS

In conclusion, although overall level of knowledge of the majority of the respondents was good and attitude and practice towards rabies is moderate, which gained from the informal sources, there is still KAP gaps regarding mode of transmutation, clinical sign, deadly nature of the disease, not aware that cure for rabies is not available after symptoms appear and lack of awareness on the first aid measures to be taken after a case of rabies suspected animal bite. Further this study showed that the presence of high free roaming and unvaccinated dog populations cause a relevant health and economic burden in Addis Ababa. The incidence of rabies increasing as compared from the previous decade. High incidence rate was recorded in the year of 2019 and in Kolfe Keraniyo sub city over the period of six years. The post exposure vaccine uses in Ethiopia called Nervous Tissue Vaccine which was banned by WHO due to its known for its fatality rate and disability rate due to the occurrence of severe and sometimes fatal allergic encephalomyelitic reactions still produced in EPHI. Our estimations on the burden and status of rabies in Addis Ababa could provide important information for evidence-based decision-making process with insights into the potential benefits of implementing cost-effective and coordinated intervention activities.

Therefore, based on the above conclusion the following recommendations are forwarded:

- ❖ Mass vaccination of dogs, the prevention of free movement of dogs and the management of dog populations should be carried out in order to achieve zero rabies in humans and animals.
- ❖ Quality cell culture anti-rabies vaccine (WHO recommended vaccine) must be available and accessible to the affected community as a whole.
- ❖ Awareness-creation and social mobilization programs and events related to rabies problems should be undertaken using different media and other tools for the Community

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# 8 ANNEXES

## Annex 1: Forms and Questioner used

You are invented to participate to research study being conducted by Elsa Zerabiruk from Addis Ababa University. The purpose of the study is to assess the status of rabies in animals and humans in Addis Ababa, Ethiopia. Your participation on this study is voluntary.

If you are willing please sign here ----------------------------

**Part one socio-demographic characteristic**

1. **Age group**

   A. 1-15
   B. 16-30
   C. 31-45
   D. >46

2. **Gender**

   **A. Female**
   **B. Male**

3. **Educational Status**

   A. Illiterate
   B. Elementary
   C. Secondary
   D. University

4. **Dog Ownership Dog**

   A. Own Dog
   B. Do not own dog

5. **Type of employment**

   A. Private Employment
   B. Governmental
   C. Self-employment
   D. No employment

**Part two knowledge attitudes and practice of respondents in relation to cause, host range, clinical sign and transmission of rabies**

1. **Heard about rabies**

   A. Yes
   B. No

2. **If yes for Q#1, what was the source of the information**?

   A. Media
   B. 2. Friends
   C. Health department
   D. Health care workers
   E. Training
   F. Community conference

G. Any other meeting

**3. Cause of rabies**

A. Virus
B. Starvation
C. Sprit
D. Any other

**4. Species affected by rabies**

A. Animals
B. human
C. human and animals
D. I don’t now

**5. Transmit from animal to human, human to human**

A. Yes
B. No

**6. Means of transmission in animal and human**

A. Bite only
B. Contact with saliva only
C. Bite and saliva contact with open wound
D. Raw meat and milk
E. Do not know

**7. Sign and symptom in animals**

A. stop eating and drinking
B. Biting and change in behaviour
C. Salivation
D. Paralysis
E. don't know

**8. Is rabies fatal?**

**A. Yes**
**B. No**

**9. Action for rabid animal**

A. Tie
**B. Killing**
**C. I don’t know**

**10. Preferred action taken for bitten human**

A. post exposure vaccination
B. Tradition treatment
C. Spiritual healer

**11. First aid for bitten human**

A. wash with water and soap
B. Tying with cloth
C. herbal extract
D. herbal extract
E. Nothing

**12. Anti-rabies vaccine in human after exposure**

A. immediate
B. Later
C. any time
D. don’t know

**13. Easily treatable after onset of clinical signs**

A. Yes
B. No
C. don’t know

**14. How rabies transmissions can be prevented /controlled in animals?**

A. Vaccination
B. Application of herbal remedies
C. Killing rabid animals
D. Others, please specify-

**15. Knows that suspected pet head to be submitted to a veterinarian**

A. Yes
B. No
C. Do not know

**16. Annoyed by the presence of stray dogs**

A. Yes
B. No

**17. Ownership of exposing animal**?

A. Own (family) domestic animal
B. Neighbour’s domestic animal
C. Freely roaming domestic animals
D. Didn’t recognize the domestic animal (stray)
E. Other wild life

**18. Type of exposure**?

A. Bite (with close or without close)
B. lick or discharges contact with broken skin or mucous membrane
C. scratch or abrasions without bleeding
D. Contact with human rabies patient

**19. Exposing/suspected animal status**

A. Under clinical observation (quarantine)
B. Free from rabies after 10 days of observation
C. Positive for rabies
D. runaway/i let it go
E. Stray dog
F. Killed by owner/community

**For dog owners**

**1) For what Purpose do you keep your dog/s?**

A. Guarding
B. Pets,
C. Breeding
D. Don't know
**E.** Others

**2) How do you manage your dog/s in your household?**

A. In a cage
B. Dog is free to roam in side
C. Dog is free to outside compound
D. Tied outside the cage
E. Cohabit with owner
F. Free roam sometimes in compound

**3) How do you care your dog/s health?**

A. Vaccination
B. Visit to vet clinic
C. Home treatment traditionally
D. Don't know

**Annex 2: Relevant data to calculate PET cost**

**Transportation cost in Ethiopian birr.**

| **Sub city** | **2014** | | **2015** | | **2016** | | **2017** | | **2018** | | **2019** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | max | min | max | min | max | Min | max | Min | max | Min | Max |
| Addis Ketema | 3 | 6 | 3 | 6 | 3 | 6 | 3 | 5 | 3 | 5 | 3 | 5 |
| Nifas silk Lafto | 5 | 9 | 5 | 9 | 5 | 9 | 10 | 12 | 10 | 12 | 10 | 12 |
| Gullele | 3 | 6 | 3 | 6 | 3 | 6 | 6 | 10 | 6 | 10 | 6 | 10 |
| Yeka | 8 | 10 | 8 | 10 | 8 | 10 | 12 | 15 | 12 | 15 | 12 | 15 |
| Bole | 6 | 9 | 6 | 9 | 6 | 9 | 9 | 12 | 9 | 12 | 9 | 12 |
| Lideta | 3 | 4 | 3 | 4 | 3 | 4 | 5 | 6 | 5 | 6 | 5 | 6 |
| Kirkos | 3 | 4 | 3 | 4 | 3 | 4 | 5 | 6 | 5 | 6 | 5 | 6 |
| Arada | 3 | 6 | 3 | 6 | 3 | 6 | 6 | 10 | 6 | 10 | 6 | 10 |
| Kolfe keraniyo | 5 | 9 | 5 | 9 | 5 | 9 | 8 | 12 | 8 | 12 | 8 | 12 |
| Akaki Kality | 12 | 15 | 12 | 15 | 12 | 15 | 18 | 20 | 18 | 20 | 18 | 20 |

Note: Within sub city the transportation cost minimum 3 birr and maximum 7 birr.

**Health care cost and Non-health care cost for each year**

| | 2014 | | 2015 | | 2016 | | 2017 | | 2018 | | 2019 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Health care cost** | Min | Max | min | Max | Min | Max | Min | max | min | Max | min | Max |
| | | | | | | | | | | | | |
| **PET cost** | Free | Free | Free | Free | Free | Free | Free | Free | 20 | 60 | 20 | 60 |
| **Antibioti Cs** | 15 | 25 | 15 | 25 | 20 | 30 | 20 | 30 | 28 | 35 | 28 | 35 |
| **Tetanus** | 30 | 50 | 40 | 70 | 40 | 70 | 40 | 100 | 48 | 158 | 48 | 158 |
| **Non-health care cost** | | | | | | | | | | | | |
| **Producti vity cost** | 3.36 | 6.8 | 3.78 | 7.65 | 4.2 | 8.5 | 4.6 | 9.35 | 4.62 | 9.35 | 5.02 | 10.2 |

**GDP per capital and Average exchange rate dollar to birr for each year**

| | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 |
|---|---|---|---|---|---|---|
| **GDP per capital** | 567 | 641 USD | 717 USD | 769 USD | 772 USD | 858 USD |
| **GDP per capital per day** | 1.6 USD | 1.8 USD | 2 USD | 2.2 USD | 2.2 USD | 2.4USD |
| **Average exchange rate dollar to birr** | 19.67USD | 20.96 USD | 21.8USD | 23.96USD | 27.66 USD | 29.2 USD |