ASSESSMENT OF THE EFFECTIVENESS OF ANTI-RABIES VACCINATION OF DOGS IN KIGALI CITY, RWANDA

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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LIST OF ABBREVIATIONS

AOR	- adjusted odds ratios
BAUEC	- Biosafety, Animal Use, and Ethics Committee, University of Nairobi
BHK-21	- baby hamster kidney cell line
BSA	- bovine serum albumin
$CD^{+}8$	- cluster of differentiation 8
DMSO	-dimethylsulfoxide
DNA	- deoxyribonucleic acid
EDTA-K2	- ethylene diamine tetra acetic acid – vitamin K2
ELISA	- enzyme-linked immunosorbent assay
EPG	- egg count per gram
ERA	- Evelyn Rokitnicki Abelseth strain, rabies virus
EU	- equivalent unit
fL	- femtolitre
H_2O_2	- hydrogen peroxide
IFNs	- interferons
IgG	- immunoglobulin G
IU	- international unit

LLEBN	- *Lleida Bat Lyssavirus*
NISR	- National Institute of Statistics of Rwanda
OD	- optical density
OIE	- World Organisation for Animal Health
PCR	- polymerase chain reaction
PCV	- packed cell volume
pg	- picogram
r	- Pearson correlation coefficient
RNA	- ribonucleic acid
RNEC	- Rwanda National Ethics Committee
RV-97	- rabies virus strain 97
RWF	- Rwandan Franc
s.q.f	- sufficient quantity for
SAD	- Street Alabama Dufferin strain, rabies virus
SAG2	- Street Alabama Gif strain, rabies virus
SEARG	- Southern and Eastern African Rabies Group
$TCID_{50}$	- median tissue culture infectious dose
Tris-EDTA	- Ethylenediamine Tetraacetic Acid; buffered solution

USSR	- The Union of Soviet Socialist Republics
w/v	- weight per volume
WHO	- World Health Organisation

ABSTRACT

In Rwanda, dogs receive annual rabies vaccination but it is not known whether vaccinated dogs respond well to rabies vaccines. A cross-sectional study was conducted to assess the effectiveness of anti-rabies vaccination of dogs in Kigali city, Rwanda to determine whether vaccinated dogs attain protective antibody levels. A face to face interview was conducted with dog owners regarding rabies disease and its control in Kigali city and blood samples for serum were taken to quantify levels of protective rabies sera titres in vaccinated and unvaccinated pet dogs. Factors influencing the response to rabies vaccination in dogs were also investigated through a questionnaire and analysing blood smears for parasites as well as faecal and whole blood samples. Based on dog distribution and vaccination history, 3 administrative sectors were chosen per district that is 9 study sectors were chosen across Kigali city; hence 137 dog owners were interviewed. Only 93 dogs were accessible for blood and faecal sampling, including vaccinated (80) and non-vaccinated (13). Although 95.5 % of the respondents were aware of rabies, only 43.7% knew of human and canine rabies. Nearly 74% knew that people can have rabies through dog-bites. Only 43% and 26% of respondents knew that clinical rabies is always deadly both in humans and dogs respectively while 20% reported they would wash dog-bites wounds wisely with water and soap before taking a dog-bite victim to a hospital. Of the study dogs (n=93), 39.8% and 100% tested positive for intestinal worms and negative for haemoparasites respectively. Of the vaccinated dogs (n=80), 35% did not have protective antibody levels. Age of dogs, deworming status, number of vaccinations against rabies influenced rabies antibody titres. Gaps in rabies knowledge and attitudes were revealed, i.e., awareness of the Rwandans about rabies needs to be strengthened. Vaccination programme needs to be monitored through regular serosurvey and take appropriate actions.

CHAPTER 1: INTRODUCTION

1.1 Background information

Rabies is a zoonosis that affects all warm-blooded animals including humans. Some countries have eradicated it, but it is still a challenge to many countries worldwide, including Rwanda. Every year, tens of thousands of people worldwide die of rabies, transmitted mainly by dog bites (World Health Organization (WHO), 2016). As stated by Okell *et al.*, (2013), rabies causes significant losses to livestock. In Rwanda, rabies is a very serious problem, and many challenges that hinder its effective control exist (Southern and Eastern African Rabies Group (SEARG), 2011). According to Rwanda Agriculture Board, 669 cases of human dog-bites were reported across Rwanda in 2016 (The New Times, 2017). Although Rwanda Agriculture Board Veterinary Laboratory can diagnose rabies, the surveillance is hampered by poor cooperation between medical and veterinary personnel. The involvement of veterinarians in cases of human dog-bites is limited and dogs that bite people and animals are rarely quarantined (Global Alliance for Rabies Control, 2017). Poor collaboration between veterinarians and medical personnel could also impact on awareness campaign of the public about rabies, and it is not known whether dog owners and the public at large have knowledge about rabies.

According to (World Organisation for Animal Health (OIE), 2011), controlling rabies disease in Rwanda, is accomplished by vaccinating pet dogs annually and eliminating stray dogs. In 2010, both dog and cat population in Rwanda was estimated to be 31, 448, and only 8,650 of them were vaccinated against rabies. The coverage rate of vaccination for both cats and dogs was at 27.5% (SEARG, 2011). According to Rwanda Agriculture Board, the number of dogs in Rwanda in 2016 was estimated to 18,117 including 11,375 that received rabies vaccination and 2870 culled, i.e., rabies vaccination coverage was 62.7% (The New Times, 2017).

Twelve percent (12%) (2,157 of 18,117) of the dogs were in Kigali city (Nyarugenge, Kicukiro and Gasabo districts records). Importation of rabies vaccines is controlled by Rwanda Agriculture Board, but their effectiveness in the field condition is not known. The weak surveillance of rabies in the country is a challenge to veterinary services in terms of tracking vaccination status of biting dogs. There is a target to eliminate hum an dog-mediated rabies by 2030 (WHO, 2016), but this goal may not be achieved if people are not aware of rabies burden, and if measures that are used to control rabies are not evaluated. Although rabies is mainly prevented through yearly dog vaccination in Rwanda it is not known whether vaccinated dogs respond well to vaccines.

Considering that it is not known whether dog owners and the public at large have knowledge about rabies and whether dogs receiving rabies vaccination respond well to vaccines; a study is therefore needed to address these gaps. Thus, the current study aimed to assess the effectiveness of antirabies vaccination of dogs and perceptions towards rabies disease in Rwanda.

1.2 Study objectives

General objective

To investigate the effectiveness of anti-rabies vaccination of dogs in Kigali city, Rwanda.

Specific objectives

1. To investigate knowledge, attitudes and practices of rabies disease and control in Kigali city, Rwanda,
2. To determine whether vaccinated dogs attained protective antibody levels against rabies disease in Kigali city, Rwanda,
3. To identify factors influencing the response to rabies vaccination in dogs in Kigali city, Rwanda.

1.3 Hypotheses of the study

1. Owning a dog for a long time did not impact on knowledge, attitudes or practices of rabies among dog owners in Kigali city, Rwanda.
2. Dogs receiving rabies vaccination in Rwanda attain protective antibody titres irrespective of type of vaccine utilised.

1.4 Study justification

In Rwanda rabies is controlled through annual vaccination and elimination of stray dogs. Although importation of rabies vaccines used in dogs and other domestic animals is regulated by Rwanda Agriculture Board, this cannot guarantee that rabies vaccination in dogs in Rwanda is successful.

Veterinarians have been vaccinating dogs but the vaccination outcome is based only on results given by manufacturers. It is not known whether vaccinated dogs in Rwanda develop protective antibody levels. This knowledge about vaccination effectiveness in dogs will assist authorities in developing effective control measures for rabies disease.

Success of vaccination is a multifaceted issue that may be related to the vaccine itself and its management as well as factors in vaccinated animals. The present study will carry out investigations regarding factors influencing vaccine response and determine whether vaccinated dogs produced protective levels of antibodies to rabies vaccines.

CHAPTER 2: LITERATURE REVIEW

2.1 Rabies overview

2.1.1 Aetiology

Being a viral zoonosis, rabies is invariably fatal disease which occurs in all warm blooded mammals and is usually transmitted to humans by dog bites (WHO, 2013b). Rabies disease is a lyssavirus infection (OIE, 2014b). Structurally, Lyssavirus are single-stranded, negative-sense RNA viruses exhibiting bullet shape (Rupprecht *et al.*,2017). Rabies virus genome is surrounded by a lipoprotein envelope (Abd-Elghaffar *et al.*, 2016).

Lyssavirus genus contains 14 species including *Rabies lyssavirus*. *Lyssavirus* are members of the Family *Rhabdoviridae* and order Mononegavirales (Afonso *et al.*, 2016). Rhabdoviruses usually comprise five main proteins: a large RNA-dependent RNA polymerase (L), a surface glycoprotein (G), a nucleoprotein (N), a protein component of the viral polymerase (P) and a matrix protein (M). The G protein forms the surface peplomers which interact with host cell receptors, facilitating endocytosis of the virion. In addition, the G protein induces virus-neutralizing antibodies and cell-mediated immunity (Quinn *et al.*, 2011).

Current *Lyssavirus species* include *West Caucasian bat lyssavirus, Shimoni bat lyssavirus, Rabies lyssavirus, Mokola lyssavirus, Lagos bat lyssavirus, Khujand lyssavirus, Irkut lyssavirus, Ikoma lyssavirus, European bat 1 lyssavirus, European bat 2 lyssavirus, Duvenhage lyssavirus, Bokeloh bat lyssavirus, Australian bat lyssavirus, Aravan lyssavirus* (Afonso *et al.*, 2016).

Lleida Bat Lyssavirus (LLEBV) is the new species waiting to be fully characterized whose RNA was detected from a bent winged bat (*Miniopterus* schreibersii) brain material in Spain (Ceballos *et al.*, 2013; Banyard *et al.*, 2014).

Phylogenetic investigations suggested that *Lyssaviruses* might have evolved in chiropterans some years back before spreading to carnivores (Badrane and Tordo, 2001). *Mokola lyssavirus* and *Ikoma lyssavirus* have not been known to directly cause infections of bats (Banyard *et al.*, 2014). Based on their antigenicity, Lyssaviruses have been suggested to form four separate phylogroups (Fooks *et al.*, 2014; Malerczyk *et al.*, 2014). Phylogroup 1 includes *Rabies lyssavirus, Khujand lyssavirus, Australian bat lyssavirus, Bokeloh bat lyssavirus, European bat lyssavirus* 1 and 2, *Aravan lyssavirus, Duvenhage lyssavirus, Irkut lyssavirus. Mokola virus, Lagos bat virus*, and *Shimon bat virus* belong to Phylogroup 2. *West Caucasian bat virus* belongs to Phylogroup 3 while phylogroup 4 encompasses *Ikoma lyssavirus* and *Lleida Bat Lyssavirus* (Malerczyk *et al.*, 2014).

Due to conserved antigenic sites on the surface glycoproteins of *Rabies lyssavirus, Duvenhage lyssavirus*, *European bat lyssaviruses* and *Australian bat lyssavirus*, rabies vaccination elicits cross-neutralisation and cross-protective immunity (OIE, 2013a). Rabies immunoglobulin and vaccine administered before and after exposure to *Irkut lyssavirus*, *Aravan lyssavirus*, and *Khujand lyssavirus* presented decreased protection (Hanlon *et al.*, 2005). Rabies vaccination against infection caused by *Mokola lyssavirus* and *Lagos bat lyssavirus* provokes little or no cross-protection (OIE, 2013a). Serologically, there is no cross-reaction between *West Caucasian bat lyssavirus* with any of *Rabies lyssavirus, Duvenhage lyssavirus, European bat lyssaviruses* and *Australian bat lyssavirus*, *Irkut lyssavirus*, *Aravan lyssavirus*, and *Khujand lyssavirus, Mokola lyssavirus* and *Lagos bat lyssavirus.* Most reported cases of animal and human rabies are caused by *Rabies lyssavirus*, but other lyssaviruses can cause clinical disease not typical of classical rabies (OIE, 2013a).

2.1.2 Epidemiology

2.1.2.1 Hosts

Rabies is a disease mainly affecting mammals, however all warm blooded animals are susceptible (WHO, 2013b). A report by Baby *et al.*, (2015), indicated that a bird species, i.e., *Gallus domesticus* suffered from natural rabies disease for the first time. Rat rabies has been reported from some countries in Asia but is very rare (WHO, 2013b). There are two epidemiological cycles of rabies, i.e. urban and sylvatic. Urban cycle is maintained by infected dogs while wildlife is responsible for sylvatic cycle. Dogs can spread rabies virus to wildlife and vice versa (WHO, 2012). Urban rabies is transmitted by pet animals (e.g., cats, cattle, dogs) while bats are considered significant maintenance hosts for sylvatic rabies; vampire bats are a significant source of rabies in North America (Chakraborty, 2013).

Susceptibility varies according to species, i.e., pet animals and people are moderately susceptible to the virus (Quinn *et al.*, 2011). Coyotes, foxes and wolves are highly susceptible; raccoons, skunks, insectivorous bats, and bobcats are considered intermediate. Opossums are quite resistant (Willey *et al.*, 2008). Bat lyssaviruses are of low risk to both humans and animals (Fooks *et al.*, 2014). Bats can transmit rabies to terrestrial animals (Burnett, 1989); red foxes were diagnosed with bat variants rabies (Daoust *et al.*, 1996) and skunks (Leslie *et al.*, 2006). Dogs and mongoose are the primary reservoirs of rabies virus in Africa; but additional wildlife species such as jackals, foxes can maintain the virus (Nel and Rupprecht, 2007). It was reported that Kudu antelope in Namibia, can horizontally get rabies through mucous membranes and maintain rabies virus (Scott *et al.*, 2012). Over 95% of human rabies cases are transmitted by dogs (WHO, 2016).

Rabies also causes considerable losses to livestock (Okell *et al.*, 2013) and is a risk to uncommon carnivores such as the Ethiopian wolf (*Canis simensis*) (Randall *et al.*, 2004) and the wild dogs of Africa (*Lycaon pictus*) (Kat *et al.*, 1996). *Duvenhage lyssavirus, Mokola lyssavirus, Lagos bat lyssavirus, Shimoni bat lyssavirus, Ikoma lyssavirus* are bat limited to Africa. *Lagos bat lyssavirus and Shimon bat lyssavirus* have never spread to humans from bats; Duvenhage virus from bats was involved in human fatalities (Banyard *et al.*, 2014). *Lagos bat lyssavirus* occurs in frugivorous bat species in the south of Africa. Rarely, it spreads to other mammals, for example, cats, dogs and mongooses (Markotter *et al.*, 2008).

2.1.2.2 Transmission

Transmission of rabies virus usually occurs through bites, but scratching and licking are also ways of spread. Before exhibiting clinical manifestations, saliva of rabid animals may contain the virus (Quinn *et al.*, 2011). According to (OIE, 2014b), spreading the virus through inhalation was reported in a cave overcrowded by bats. Animals and humans can rarely contract rabies disease via aerosols and through oral and nasal routes. Saliva of infected animals can spread human rabies through contaminating mucous membranes (e.g., conjunctiva, oral, genitalia), skin abrasions and open wounds (Hemachudha *et al.*, 2002).

Transplanting organs such as cornea and others can transmit human-to-human; corneas or organs should not be taken from a patient who died of rabies encephalitis or any unidentified neurologic disease (WHO, 2013b). As stated by Barnard *et al.* (1982), thousands of kudu antelopes that died of rabies in Namibia were believed to have contracted the disease through non-bite route. Transmission of rabies can also occur via manipulating and skinning of rabid animals (Kureishi *et al.*, 1992).

2.1.2.3 Rabies occurrence

Antarctica is the only continent where rabies is absent (OIE, 2014b), but over 95% of human deaths occur in Africa and Asia (Hampson *et al.*, 2015). Some countries have either been freed from rabies by controlling the disease in dogs and in wildlife or are historically regarded rabies-free (Knoop *et al.*, 2010). Fig.1 illustrates recent occurrence of dog-mediated rabies worldwide.

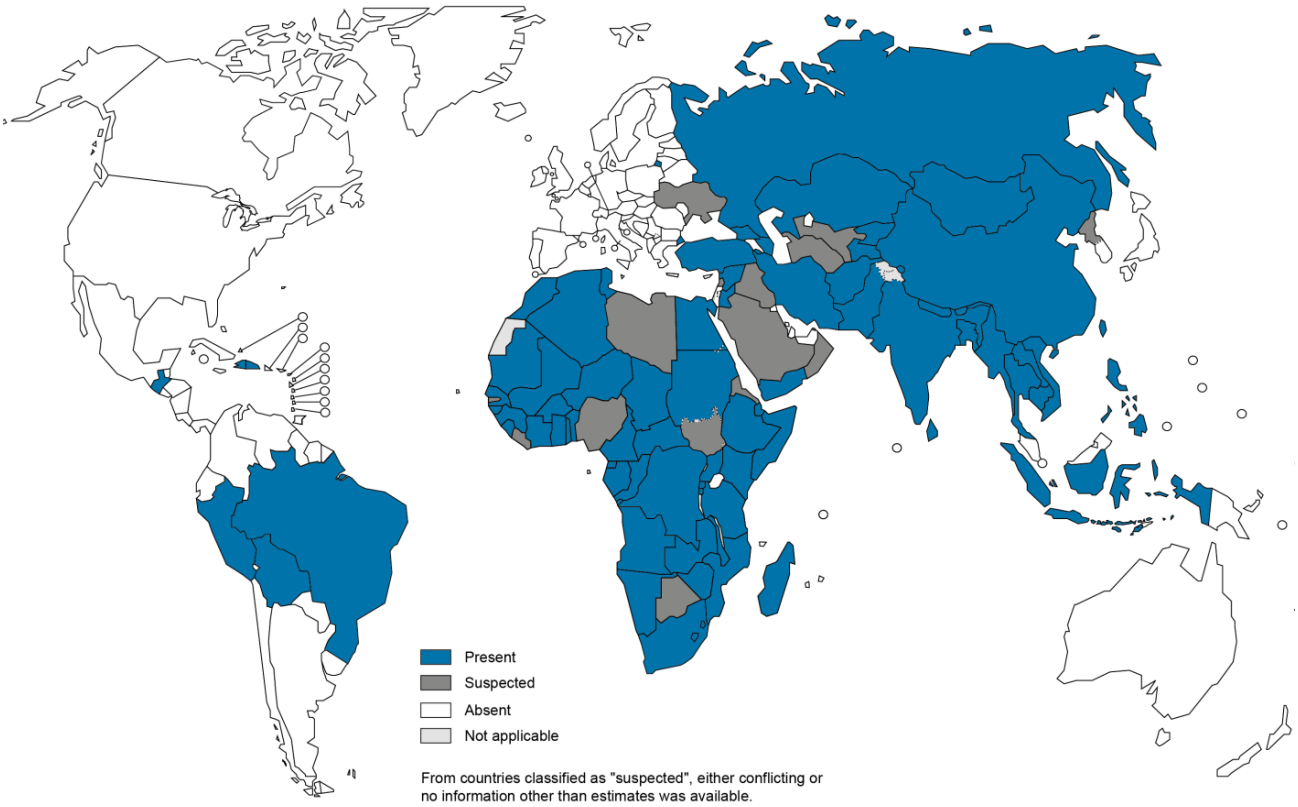

Figure 1 : Distribution of dog-mediated rabies in humans (Source: WHO, 2015)

2.1.3 Immune response

G protein mediates the binding of the virus particle to acetyl choline receptors in neural tissue and induces haemagglutination-inhibiting antibody. It is strongly antigenic, and antibody against G protein is protective. It reacts with virus neutralising antibody and also stimulates T lymphocytes expressing helper, suppressor, or cytotoxic activity (Chakraborty, 2013).

Rabies virus induces poor immunological response, immunity is mounted by T-helper cells but cytotoxic T cells do not participate in protection and may in fact be harmful to the host (Johnson *et al.,* 2010). During rabies infection, the Blood-Brain Barrier remains intact (Roy *et al.*, 2007). Rabies virus can use host innate immune respose to strategise its immunoevasion. The natural immunity promotes T cells infiltration and, concurrently favors elimanation of $CD8^+$ T cell (Chopy *et al.*, 2011). Rabies virus infection causes decrease in lymphocytes (Palomo *et al.,* 1995), and probably the production of cytokines in the central nervous system during infection, specifically tumor necrosis factor alpha, (TNF-α) is responsible for immunosuppression (Marquette et *al.,* 1996; Johnson *et al.,* 2010).

Rabies virus can defeat the antiviral activity of type I and II IFNs produced by the infected cells (Vidy *et al.,* 2005). Suppression of G protein expression in neurons helps in pathogenesis of rabies virus by depressing apoptosis (Morimoto *et al.*, 1999). High levels of G in fixed Rabies *lyssavirus* leads to apoptosis, enhanced permeability of the blood-brain barrier, and increased natural immunity in the central nervous system (Faber *et al.*, 2002). Virulent *Rabies lyssavirus* expresses low level of the G, and is able to evade the natural immunity of the host (Wang *et al.*, 2005).

Hindrance of neuronal apoptosis seems to be a subversive mechanism adopted by the virulent *Rabies lyssavirus* to resist removal and further persistent nonlethal infectious cycle in the host. This could be responsible for extended period of incubation observed in higher animals (Suja *et al.*, 2011). WHO has set global standards that any individual with antibody levels of less than 0.5 IU/ml is not adequately protected against rabies (WHO, 2012). The cutoff point for antibody response to rabies vaccination in dogs and cats is also 0.5 IU/ml (OIE, 2013a).

2.1.4 Diagnosis and sero-surveillance of rabies

2.1.4.1 Antemortem diagnosis

While the period of incubation for rabies varies and can be up to six months, the infective period for dogs, cats and ferrets is thought to commence ten days before manifesting initial clinical signs (OIE, 2013b). Although clinical findings are important, they may not help to clinically diagnose rabies disease beyond suspicion (OIE, 2013a).

Haematological and clinical chemistry changes are not very helpful (Hemachudha *et al.,* 2002) and the diagnostic protocol is risky and may expose the worker to infection. Furious (encephalitic) rabies accounts for 80% of cases of rabies in people, whereas paralytic rabies accounts for 20% of cases of rabies in humans (Mani and Madhusudana, 2013). The clinical course in domestic carnivores, may comprise prodromal, furious (excitative) and dumb (paralytic) phases. The furious form is observed more often in cats than in dogs. Foxes rarely exhibit this form of the disease. In dumb rabies, muscle weakness, difficulty in swallowing, profuse salivation and dropping of the jaw are the usual features (Quinn *et al.,* 2011).

Laboratory methods can be used to diagnose human rabies before death and specimens such as corneal smears, skin biopsy from nape of the neck or face, and saliva are usually taken. Immunofluorescence and molecular methods help demonstrate rabies virus antigen and DNA respectively while nucleic acid sequence based amplification on saliva and cerebrospinal fluid can be used to rapidly diagnose the infection as early as two days after symptom onset (Parija, 2012).

2.1.4.2 Postmortem rabies diagnosis

2.1.4.2.1 Direct Microscopy

Neurons infected with rabies lyssavirus show intracytoplasmic inclusion bodies referred to as Negri bodies and histologic tests can be used to demonstrate those viral particles aggregates (Meslin *et al.*,1996; OIE, 2013a). Unfixed tissue smears made from fresh specimens and sections of paraffin embedded brain tissues can be stained to demonstrate Negri bodies (Meslin *et al.*, 1996).

When unfixed tissue smears are stained by Sellers' stain, Negri bodies stain magenta to bright red with well-defined dark-blue to black basophilic inner bodies while neurons stain blue. Interstitial tissues stain pink whereas red blood cells stain copper red. Although certified biological stains are preferred, making a stock solution of Sellers' stain can be done by adding 10 grams of methylene blue and 5 grams of basic fuchsin in 1000 ml and 500 ml of absolute acetone-free methanol, respectively (Meslin *et al.*, 1996). Seller's staining and histopathologic techniques are still used to diagnose rabies, but in the absence of Negri bodies, they lead to false negatives (Singh *et al.*, 2017). Diagnosing rabies both in people and animals should consider immunochemical tests (OIE, 2013a).

2.1.4.2.2 Identification of viral antigen

2.1.4.2.2.1 Immunofluorescence

The direct immunofluorescence on acetone-fixed brain tissue smears is the diagnostic method of choice (Quinn *et al.*, 2011). If performed on fresh specimens, it is reliable and results are obtained within a few hours in over 97-99% cases (Barrat, 1992).

2.1.4.2.2.2 Rapid Rabies Enzyme Immunodiagnosis

In this technique, the solid phase is coated with polyclonal or monoclonal anti-N antibody and the rabies N protein comes from brain homogenate. The rabies N protein is captured by antibody coated over the solid phase. This test is considered sensitive and as specific as immunofluorescence; brain partially decomposed will not influence result of the test (Mani and Madhusudana, 2013).

2.1.4.2.3 Virus Isolation

The two methods that can be used are inoculation of mice and rapid tissue culture infection test (Meslin *et al.*, 1996).

2.1.4.2.3.1 Inoculation of mouse

About 10 mice aged 3-4 weeks and weighing 12-14 gr, or a 2-day-old newly born mice litter are used. Intra cerebral route is used to inoculate a 10-20 % (w/v) supernatant obtained from a brain homogenate mixed with an isotonic buffered solution and antibiotics. The mice are then monitored day-to-day for 28 days, i.e., after 5-7 days they begin exhibiting typical signs and symptoms of rabies based on incubation period. The brain of sick mouse is extracted for confirmatory test by immunofluorescence (OIE, 2013a).

2.1.4.2.3.2 Rapid Tissue Culture Infection Test

Isolation of virus in tissue culture is quick and can give results in 1-2 days. Neural origin cell lines are very appropriate for isolation of virus, and the murine neuroblastoma cell line Neuro-2a is the most frequently used cell line. Inoculation of the clinical specimen or homogenate of the brain can be done via shell vial cell culture or plates of 96 wells, incubation lasts for 24 hours, and staining is by direct immunofluorescence on acetone-fixed tissue (Madhusudana *et al.*, 2010).

Shell vial cell culture involves centrifuging a patient's specimen onto a cell monolayer contained in a vial to shorten the time to a positive culture result (Toronto medical laboratories / Mount Sinai hospital, 2003).

2.1.4.2.4 Antibodies demonstration

Rabies infection can be diagnosed indirectly through demonstration of antibody in the serum or in cerebrospinal fluid in non-vaccinated animals (Fooks *et al.*, 2009). Demonstration of antibodies is very valuable for evaluation of seroconversion after vaccination and epidemiological studies (Mani and Madhusudana, 2013). The rapid fluorescent focus inhibition test and the fluorescent antibody virus neutralization test are among the virus neutralization tests known globally (Quinn *et al.*,2011). Neutralization tests can be replaced by a technique that blocks required action of antibodies to the specific antigen (ELISA) (Moore and Hanlon, 2010). Commercially available indirect ELISAs, using rabies glycoprotein as antigen, are useful screening tests to determine if vaccinated cats and dogs have seroconverted (Quinn *et al.*,2011).

BioPro (O.K. SERVIS BioPro, s.r.o. Czech) is an indirect ELISA test kit that can be used to detect rabies virus antibodies to monitor oral vaccination campaigns for the fox (Wasniewski and Cliquet, 2012; Bedeković *et al.*, 2016). Platelia Rabies II (Bio-Rad, France) is another indirect ELISA kit that can detect glycoprotein antibodies in human serum and cerebrospinal fluid specimens. It is known to correlate with rapid fluorescent focus inhibition test (Feyssaguet *et al.*, 2007). The rapid neutralizing antibody detection test (RAPINA), is a rapid test that can detect 0.5 IU/ml antibodies against *Rabies lyssavirus* in human and animal sera or plasma (Nguyen *et al.*, 2015). Rabies ELISAs *per se* have a great potential for serological testing, but it seems the threshold of 0.5 IU/ml as an approximation of successful vaccination for animals needs to be reconsidered (Knoop *et al.*, 2010).

2.1.4.2.5 Detection of nucleic acid

Rabies virus RNA can be detected by reverse transcription PCR, PCR-ELISA *in situ* hybridisation, and by real-time PCR and diagnosing the virus with these methods is rapid and sensitive (Fooks *et al.*, 2009). Molecular diagnostic tests are not recommended at present for diagnosis of rabies after death (WHO, 2005), due to lack of standardisation and strict quality control since there is a high number of false positives or false negatives (OIE, 2013a).

2.1.5 Control of rabies

The dog is a major reservoir and accounts for most cases of rabies reported in people and animals (OIE, 2014a).

2.1.5.1 Sanitary prophylaxis

Surveillance for rabies in animals might be founded on danger that is focused on investigating and diagnosing unconfirmed cases (WHO, 2013b). Urban rabies can be effectively controlled by vaccination and restriction of dog and cat movement and by the elimination of stray animals.

Control of sylvatic rabies requires special measures, i.e., regional depopulation of reservoir species, which has rarely been successful, is ecologically unacceptable (Quinn *et al.*,2011).

Due to the existence of a number of lyssavirus species and the considerable position of chiropterans in worldwide ecology, it is impossible to eliminate bat rabies at the present time. Preventing people from getting rabies transmitted by bats should focus on creating awareness to the public (WHO, 2013b). Humane killing of a dog assumed to be rabid lessens the number of people at risk (WHO, 2013b), and in the case of unclear diagnosis, the dog can be quarantined and observed (OIE, 2016) for 10 days to rule out rabies (Tepsumethanon *et al.*, 2004). The salivary gland is an exit route and the animal will not survive the virus longer than 10 days once there is virus shedding in the saliva (Centers for Disease Control and Prevention , 2011).

2.1.5.2 Medical prophylaxis

Preventing animal and human rabies can uniquely be accomplished with preventive vaccination (Ondrejková *et al.*, 2015). Rabies vaccines contain a standardised preparation of immunogen amounts, i.e., they are either inactivated, live-attenuated or biotechnology-derived (OIE, 2013a). Rabies vaccine given orally has become a crucial means of controlling and eliminating the disease in wildlife species (WHO, 1990; WHO, 2013b). People with occupational risk (e.g., veterinarians, tanners) or recreational risk (dog handlers) of contracting rabies should be vaccinated against rabies before exposure (pre-exposure prophylaxis) (Chakraborty, 2013). After being bitten by a suspected dog or other rabid animal, preventive actions are taken up immediately (post-exposure prophylaxis) (Parija, 2012).

The measures taken include : (*a*) wounds are immediately cleaned with soap and water and then treated with quaternary ammonium compounds or aqueous solution of iodine or alcohol, (*b*) the animal is investigated to confirm weather is rabid or not, (*c*) administering hyperimmune serum, and (*d*) antirabies vaccine (Parija, 2012). Paralytic rabies of cattle transmitted by vampire bat can be controlled by vaccinating cattle (WHO, 2013b). Rabies vaccine virus strains used to challenge or manufacture vaccines are shown in Table 1.

Table 1: Rabies vaccine virus used for challenge or for vaccine manufacturing

Pasteur strain	Street Alabama Dufferin	Flury strain	Other strains
1882 France from a rabid cow infected by a dog	1935 USA from a dog	1939 USA from Miss Flury transmitted by a dog	
Passages in rabbits and mice then passages in cells at different levels: Pasteur virus (PV-12) Kissling (CVS-11) CVS challenge virus strain (CVS-27 Pitman-Moore (PM) RV-97	Primary cells of hamsters & pigs (10 passages) = ERA virus BHK 21 cell line passages: SAD Vnukovo (USSR Russia) SAD Vnukovo-32 SAD Bern (Switzerland) SAD-B19 SAG2 ERA 333	136 passages in 1 day-old-chicks 40/50 passages in embryonated eggs: low egg passage (LEP) 220/227 passages in embryonated eggs: high egg passage (HEP)	CTN: China from a dog (1956)

Source: (OIE, 2013a)

2.1.5.3 Control of rabies in Rwanda

In Rwanda, controlling rabies disease is accomplished with vaccinating owned dogs annually and depopulating stray dogs (OIE, 2011). Awareness of the public about rabies is done on the radio to announce campaigns for rabies vaccination. There is no pre-exposure vaccination for people at risk (veterinarians, medical and national park staff). In case of human dog-bites, post-exposure first aid is done at health centers before referring patients to hospitals. Wildlife species are not vaccinated (SEARG, 2011). A structured awareness campaign can improve knowledge of rabies in dogs (Castillo-Neyra *et al.*, 2017). Rwanda Agriculture Board Veterinary Laboratories can diagnose rabies, but dogs that bite people and animals are rarely quarantined (Global Alliance for Rabies Control, 2017). Five rabies vaccine brands for immunizing dogs and other domestic animals were on the market in Rwanda in 2016 (appendix 2).

2.1.6 Factors affecting success of rabies vaccination in dogs

Although the failure of rabies vaccination in animals might be higher, a study conducted on dogs received rabies vaccination in Nigeria rated it to 0.025% , i.e., 2.5 cases per 10,000 doses of vaccine (Oboegbulem *et al.*,1987; Tepsumethanon *et al.*, 2016).

A number of factors can impact on achievement of canine rabies vaccination such as the brand of vaccine applied, number of rabies vaccinations, dog's breed and size, as well as age at vaccination. Larger dog breeds require a booster vaccination schedule (Berndtsson *et al.*, 2011). Other factors that influence titre of antibody include status of nutrition, sex and the excellence of vaccine preservation (Kennedy *et al.*, 2007; Jibat *et al.*, 2015).

A case-control study done on efficacy of rabies vaccines in dogs over 5 years found that 10.7% did not have protective antibody levels (< 0.5 IU/ml) and vaccine brands, dog ages and interval between vaccination and sampling influenced antibody levels (Nokireki *et al.*, 2017).A study done in by Tresamol *et al.*(2016) in India, 90% of both dogs received rabies vaccines through intramuscular and subcutaneous injections produced protective antibody levels.

2.1.6.1 Vaccine related factors

The preservation of the immunological properties of rabies vaccines necessitates storing them according to the producers' guidelines. Above all, cold chain should be maintained; sunshine and temperature changes should be prevented. Vials containing killed vaccines should be used within 2-3 days of opening, and taking vaccine from multidose containers should be done aseptically (WHO, 2013b). Immunity of the dog population is accomplished when vaccination coverage rate is not less than 70%. During vaccination campaigns cats should also be vaccinated. Vaccinating teams should consider vaccinating puppies, including those newly born, to ensure satisfactory coverage of the herd (WHO, 2013b). Vaccination campaign turn-out can be increased through public commitment and mobilization. If a number of dogs are difficult to restrain, dog owners can be assisted by skilled dog handlers to grasp and contain dogs compassionately for vaccination(WHO, 2013b). If dogs cannot be handled or grasped, administration of vaccine orally may be used to increase coverage (WHO, 2007).

2.1.6.2 Dog-related factors

Various factors can impact on production of antibodies including amounts of antigens, way of application, and especially animal status (Moore and Hanlon, 2010). Health status of dogs should be improved through vaccinating them against other diseases, deworming, spaying or neutering them (WHO,2013b). Neutralizing antibodies against rabies virus usually peak 4-6 weeks after primary antigenic stimulation. Many weeks after vaccination, there is a rapid decline in antibody titers and which below can go the level of detection (WHO, 2013b).

Different nematodes can parasitize pets, but ascarids, ancylostomatids and *Trichuris vulpis* are the most common (Traversa, 2011;Traversa, 2012). Although helminths can suppress immune system, deworming reverses their immunoregulatory effects (Hewitson *et al.,* 2009). Before vaccinating puppies and dogs against rabies, they must regularly be dewormed (WHO, 2013a). Dogs suffering from subclinical ancylostomosis were found to have changes in inflammation markers and iron metabolism (Schmidt *et al.,* 2016). Larvae of *Ancylostoma caninum* and *Toxocara canis* can regulate canine immune system through modifying antigen-specific and polyclonal T cell responses and maturing dendritic cell (Junginger *et al.,* 2017).

Health and disease of companion animals can be assessed through performing a complete blood count (Becker *et al.,* 2008). According to Sharp (1996), human haematology analyzers can be used to determine various blood parameters (PCV, total white cells, total platelet) in dogs blood samples.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

The study was carried out in Kigali city in Rwanda and involved nine administrative sectors across the 3 districts of the city namely Gasabo, Kicukiro and Nyarugenge. The study was carried between September 2016 and March 2017.

The land area of Rwanda is 26,338 square kilometers; administratively, Rwanda is divided into provinces, districts, sectors, cells, and villages. Rwanda is subdivided into 5 provinces including Kigali city as well as into 30 districts (National Institute of Statistics of Rwanda **(**NISR), 2014). Kigali city is located at latitude 1º 57'S and on longitude 30º 04' E (Kigali Institute of Science Technology and Management, 2001). Kigali city is subdivided into three districts namely Gasabo, Kicukiro and Nyarugenge. Gasabo district is divided into 15 sectors, Kicukiro into 10 sectors, Nyarugenge into 10 sectors (NISR, 2014).

The dogs vaccination records were used to select three sectors per district based on dog distribution and vaccination history. In Nyarugenge district Mageragere, Kigali and Nyamirambo sectors were chosen. In Gasabo district, Gisozi, Kacyiru and Kimironko sectors were picked; in Kicukiro district Niboye, Gatenga and Kicukiro sectors were chosen (Fig.2).

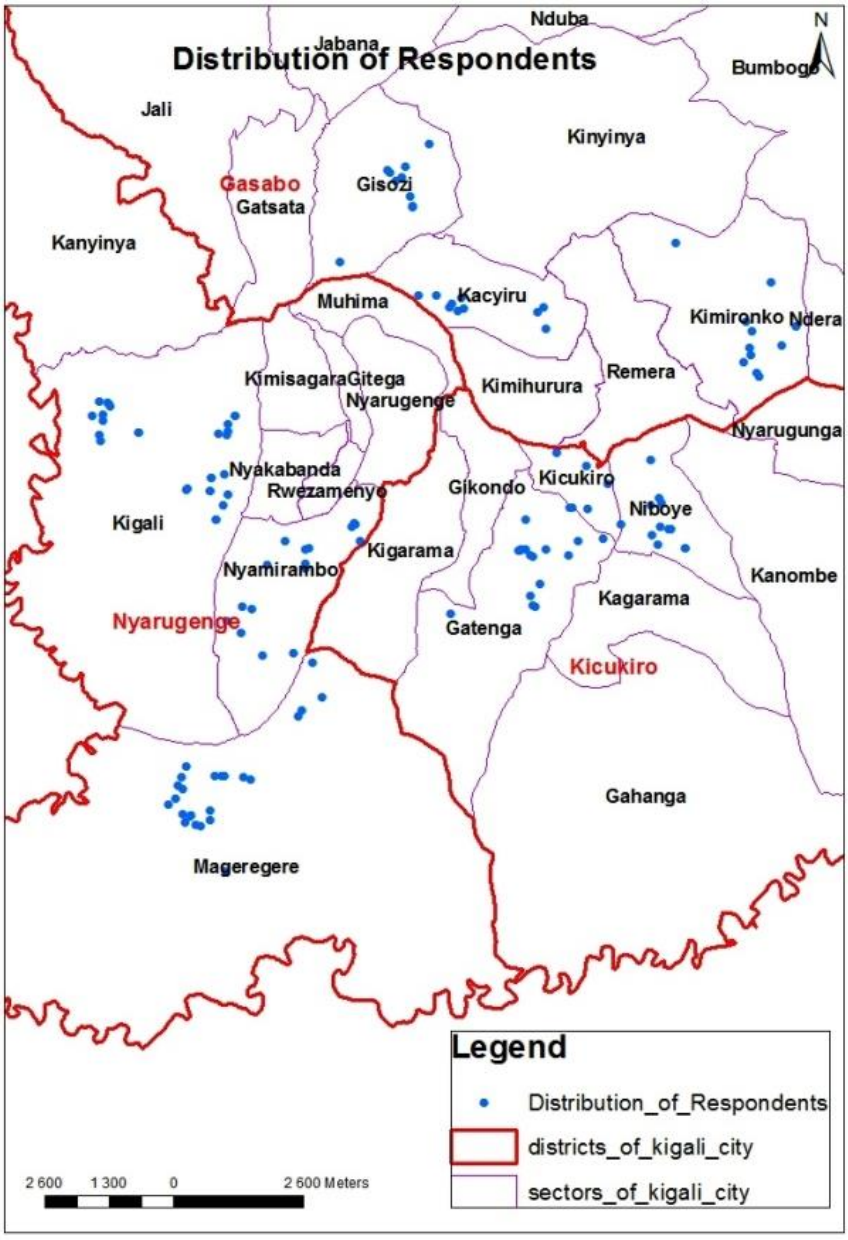

Figure 2 : Map of the study area and location of respondents (Drawn from GPS data by authors using ArcGis10.2 software)

3.2 Study design

This was a cross-sectional study involving interviewing dog owners of vaccinated or unvaccinated dogs on rabies disease and its control as well as collecting plain tube and EDTA.K2 blood and faecal samples and making blood smears.

At district level, cluster sampling was used to select study sectors while at lower level entities in cells and villages, snowballing sampling was used to reach dog owners households for interview and collection of samples from dogs, i.e., one dog per household of interviewed respondent.

3.3 Computing sample size

A standard formula by Chomel *et al.*,(1987) was used to compute the study respondents and dogs. The number of dogs needed for this study corresponded to the respondents included in the study.

$N= t^2 \times pq/d^2$; where

N= sample size,

t = 1.96 (95 % confidence level),

d= .05 (5% precision),

p = Ratio of target population with feature being measured, q = 1-p

According to Chomel *et al.*, (1987), when samples are collected between 1 and 9 months after vaccinating agaist rabies, at least 90% of the population has a protective level of rabies antibodies. When samples are taken 12 months after a vaccination campaign, 85% of the populations have a protective level. A *p* value of 0.85 was adopted in the formula sample size calculation for the study.

Determining the number of dogs needed for this study was done as follow:

$N= (1.96)^2 \times (0.85)\ (1-0.85) / (0.05)^2 = 196$ dogs

3.4 Ethical approval

Permission to conduct this study was given by the Biosafety, Animal Use, and Ethics Committee of the Faculty of Veterinary Medicine at the University of Nairobi in Kenya (Ref: FVM BAUEC/2017/126) and by the Rwanda National Ethics Committee (Review Approval Notice: No.15/RNEC/2017).

3.5 Processing data

After interviewing dog owners and performing laboratory work, all the data was entered in Statistical Package for Social Sciences (SPSS) Statistics version 20 for statistical analysis. During analysis of the interview findings, descriptive statistics (frequency), chi-square tests of associations regarding knowledge, attitudes and practices (KAP) of rabies with dog owner's specific information, namely sex, level of education, and dog ownership length. Using the 3 components of interests, i.e., level of rabies knowledge, attitudes, practices; an index was constructed for each of the component, basing on the dog owners' responses to applicable questions. Principal components factor analysis was used to construct indexes (Niragire and Nshimyiryo, 2017), and for each of the KAP status indexes, a simple binary logistic regression model was fitted to data for each of respondent's sex, level of education and dog ownership length. Regarding interpretation, dog owners' rabies knowledge was classified as sufficient or insufficient, attitude was categorised as positive or negative while practices of rabies among dog owners were considered appropriate or inappropriate. Identification and quantification of net associations of each of the components with the dog owners' information were done by conducting binary logistic analyses for each indicator (Stoltzfus, 2011).

To determine the direction and extent of the effects of dog owner's specific information, i.e., sex, level of education, dog ownership length and residential district on status of the 3 indices, a binary logistic regression analysis was performed for each of rabies knowledge, attitude and practice.The results of faecal and blood smears examination as well as of complete blood count were processed using descriptive statistics (frequency, percentage, arithmetic mean and standard deviation). Descriptive (percentage and geometric mean) and inferential (chi-square tests and regression analysis) statistics were used to analyse and interpret sera analysis data. The confidence interval of 95% was considered and the level of significance was set to 0.05% for anlysing both interview and sera analysis data.

3.6 Collecting data and samples

Permission was obtained from the district administration for data collection. The go-ahead allowed the investigator to access vaccination records at the district offices and to get assistance from study sectors authorities. From the district the investigator visited sectors and the sector administration connected him to the executive secretary of cells, village chiefs and finally to dog owners whom the village chief thought took their dogs for vaccination. Communicating to dog owners by phone or in person allowed the investigator to introduce him-self to them and confirm whether the dog owner had taken the dog for vaccination and if he would wish to take part in the study.

3.6.1 Administration of the questionnaire to dog owners

The number of dog owners and study dogs targeted for interview and collection of samples was 196, but due to difficulties in meeting respondents and time constraints, only 137 respondents were interviewed with a survey questionnaire (appendix 1).

The investigator had trouble meeting some dog owners in sectors located in or near city center as they were very busy and this justify why few dog owners were interviewed in center city sectors compared to those in suburb. During each interview, information on respondent's knowledge, attitudes and practices towards rabies disease and its control in Rwanda was gathered. Each dog owner a signed certificate of consent before being interviewed (Fig.3).

Figure 3 : A dog owner (right) seeking clarification before signing certificate of consent

During each interview, dog owners were asked about the rabies disease and its control. For a dog to be considered vaccinated the owner presented a certificate of vaccination to the investigator. Owners of unvaccinated dogs were also interviewed to allow sampling of their dogs.

3.6.2 Identifying factors affecting response to anti-rabies vaccination in dogs in Rwanda

Ninety three (93) whole blood and faecal samples were collected and blood smears were made to determine the health status of the study dogs and check whether they had infections that could influence sero-conversion level after vaccination against rabies.

Dogs' data regarding age, sex, deworming history, time since rabies vaccination, number of vaccinations, vaccine type used was also collected recorded during interview with dog owners.

3.6.2.1 Collection of blood and faecal samples

During sampling, a dog owner or his worker put a leash on the neck of dog and applied a dog muzzle upon instructions by the investigator. The investigator and a dog owner or his employee placed a dog in lateral recumbency and three handlers excluding the researcher helped to maintain a dog in the position. The first person held the hind limbs with one of his hand and placed the other hand over the hip joint. The second person used one of his hands to hold the head and one of the fore limbs of a dog and placed the left hand over the scapula region. The third handler immobilized the fore limb in contact with the ground as illustrated in Fig. 4.

Figure 4 : Restraint of dogs during collection of samples

Prior to collecting blood from cephalic vein, the main investigator shaved site of venipuncture with a scissor and disinfected it with cotton swab soaked with 70% alcohol. Before venipuncturing, the investigator applied gloves on the lower part of the fore limb to distend the vein.

Using a multi-sample needle holder and respective needles, clotted blood samples were collected in plain vacuum collection tubes while whole blood samples were collected in EDTA-K2 vacuum tubes. Blood smears were made on frosted slides using drops of blood escaping from cephalic vein after collection of blood sample.

Fig. 5A **Fig. 5B**

Figure 5 : Smear making after taking blood sample

Faecal samples were directly collected from the rectum and placed into faecal jars using a gloved finger soon after blood samples and smears were made (Fig. 6).

Figure 6 : Collecting faecal samples with gloved fingers

3.6.2.2 Preserving the samples

After sampling, the used materials (needles, disposable gloves, cotton wool) were kept in non-breakable container and brought back to the laboratory for incineration. Faecal and blood samples were kept in a cooler box without ice packs (Fig. 7). EDTA-K2 samples were immediately taken to Good Sheperd Polyclinic, a private human clinic located in Kigali in Rwanda for complete blood count while plain tube blood and faecal samples were ferried to the National Veterinary Laboratory of Rwanda. Blood smears were air-dried and then wrapped in dry piece of paper until fixed.

Figure 7 : Blood and fecal samples in a cooler box without ice packs in the field

3.6.2.3 Analysis of whole blood samples

A human haematology analyzer was used to determine various blood parameters. At the clinic, blood samples were processed with an automated haematology analyser (URIT-3000 Plus, India, shown below, Fig.8A & 8B) calibrated for humans. As shown in Fig. (8A), the technician would hold an EDTA-K2 blood sample tube, open it and then put the tube sample so as to have the sample probe of URIT-3000Plus inside the sample tube for aspiration. The machine would have previously been programmed for the full count of blood parameters. The URIT-3000Plus haematology analyser printed the results immediately.

The print outs (Fig. 8B) were read and the data manually collated for the different blood parameters.

Fig. 8A **Fig. 8B**

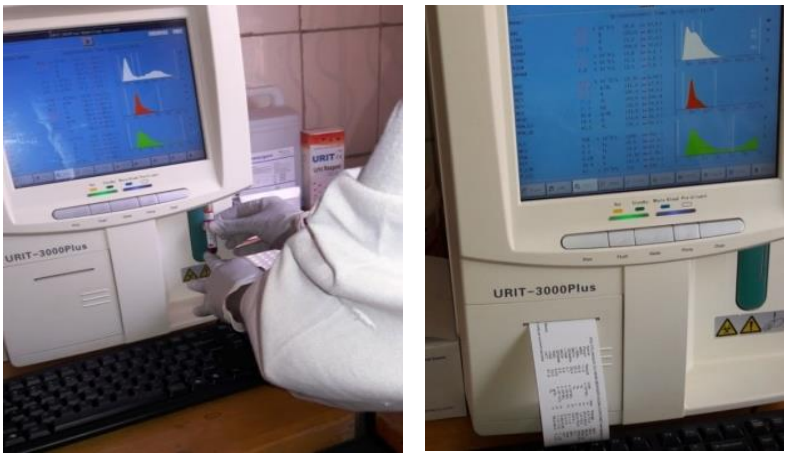

Figure 8 : Automated haematology analyser used for analysing EDTA-K2 blood samples

3.6.2.4 Examination of faecal samples

Samples were collected across the nine study sectors from nighty three dogs of which, 55% were restricted while 45% were free. The floating fluid was prepared after the method by García *et al.* (2014), where by 360 grams of sodium chloride was added in one litre of tap water. Faecal examination was done by McMaster technique (Hansen and Perry, 1994); preparation of faecal suspension involved putting 2 grams of faeces in 28 millilitre of float fluid. Identification of worms 'eggs was done on microscope at 10× magnification and nematodes were differentiated on the basis of their eggs' shape and shell thickness as well as presence of morulae (Soulsby, 1982). Diagnosis of *Taenia* species can be achieved through finding worm segments and fecal flotation (Bowman and Nelson, 2014). In this study, live cestode and proglottid were detected with the naked eye (Fig. 9A and 9B). Ribbon-shaped flatworm consisting of scolex, neck and strobila were identified as cestodes (Baron, 1996; Roberts and Janovy, 2009) while proglottids exhibited a cucumber seed like shape (Ballweber, 2001).

Fig. 9A **Fig. 9B**

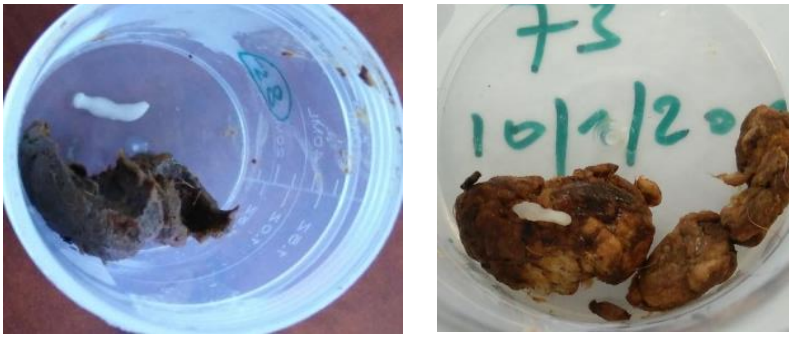

Figure 9 : Detection of cestodes live worm and proglottid with the naked eye

3.6.2.5 Examination of blood smears

The smears were fixed with pure methanol in a coplin jar for five minutes and air-dried before staining. Giemsa technique was used to stain blood smears; Giemsa solution was prepared by adding one part of working solution in nine part of buffer solution (Brar *et al.*, 2002). The smears were stained for 35 minutes and after being air-dried, were examined on the microscope at 100 x magnification with oil immersion.

3.6.3 Determining the level of antibodies in vaccinated and unvaccinated dogs

Due to aggressiveness, death of dogs as well as dog owners misreporting vaccination status, it was not possible to take samples from all the 137 dogs, rather only 93 apparently healthy dogs were sampled, namely: 80 vaccinated and 13 unvaccinated. Dogs were sampled sometime after vaccination as indicated in Table 2.

Table 2 : Interval between rabies vaccination and blood sampling

Sampling time	Months post-vaccination
A	1-5
B	6-9
C	10-12

Table 2 shows that based on interval between vaccinations and sampling time; study dogs were categorized into three groups.

3.6.3.1 Serum preparation

After taking blood, the tubes were inclined until a clot was formed and then put in a cooler box without ice packs. Upon arrival in the laboratory, the samples were centrifuged and the serum obtained preserved at -80^{o}C until used.

3.6.3.2 ELISA assay

ELISA test was carried out according to the protocol of the manufacturers (Platelia™ Rabies II; Bio-Rad, France) (appendix 3) with a total of 93 serum samples. Prior to carrying out the ELISA, test serum samples were kept at room temperature to thaw. After defrosting, samples were homogenised with IKA® VORTEX (Genius 3). As prescribed by Bio-Rad, the investigator prepared the quantification standards (S6-S1). Reagent S6 was prepared first, that is, 2 µl of the 4 EU/ml calibrated positive control was diluted into 198 µl of the sample diluent (1/100). Reagent S6 was then diluted 1:2 where 100 µl from S6 was mixed with an equal volume of the sample diluent to get S5). Through the same dilution pattern (1/2) S4 was prepared and then S3, S2, and S1. Two (2) µl of the 0.5 EU/ml calibrated positive control was added to 198 µl of the sample diluent. Preparing the negative control involved putting 2 µl of the negative control sample into 198 µl of the sample diluent while sera samples were also prepared by putting 2 µl of the control serum sample into 198 µl of the sample diluent (1:100 dilutions).

The 100 µl of diluted negative control, 0.5 EU/ml calibrated positive control and the quantification standards were distributed to the microplate sensitised with the rabies virus glycoprotein. The microplate was covered firmly with an adhesive film and incubated for one hour at 37°C.

The wash solution (1 volume to 10 volume of distilled water) and the dilution of the concentrated conjugate (1 volume to 9 volumes of prepared 1X wash solution) were prepared before end of incubation. After incubation, the adhesive film was removed and the microplate was washed thrice and then 100 µl of the diluted conjugate was added to each well before covering the microplate with an adhesive film and held to 37°C for one hour. During this incubation the investigator prepared enzymatic development solution that is diluting the chromogen (R9) to 1/11 in peroxidase substrate buffer (R8).

After incubation, the adhesive film was eliminated and then the microplate was washed five times and then 100 µl of the enzymatic development solution was distributed to each well. The microplate was incubated for 30 minutes at room temperature and then 100 µl of the stop solution was added to each well to discontinue the reaction. The spectrophotometer was used to read the reactions in the microplate at 450 nm and then optical densities were copied and pasted into the Bio-Rad conversion tool to get final antibody titres. Equivalent units per ml (EU/ml), i.e., unit equivalent to the international units defined by sero-neutralization were used to express serum titres. The cutoff point was 0.5EU/ml and four sero-conversion levels were recognised, namely high (>4EU/ml), sufficient (0.5-4EU/ml), insufficient (0.125-0.5EU/ml) and undetectable (<0.125EU/ml).

CHAPTER 4: RESULTS

The findings include data on interview, complete blood count, faecal and blood smears examination and serum analysis.

4.1 Interview results

The results focused on knowledge, attitudes, practices (KAP) of rabies disease and its control.

4.1.1 Respondents education background

Four percent (4%) of respondents did not get formal education, while 31% and 28% finished the primary and secondary schooling, respectively and while 37% finished or were doing the university education.

4.1.2 Sourcing information on rabies disease

Table 3 indicates that the respondents mainly sourced rabies information neighbours (39%), the media (29.9%), community meetings (13.1%) and parents (7.2%). The rest of them acquired the information from education at school (5.2%), veterinarians (4.8%) and workplace (0.8%) such as medical pharmacy and police through serving victims of dog-bites or their relatives.

Table 3 : Respondents' sources of information on rabies

Sourcing information for respondents	Positive responses (N=137)	Percent
Neighbours / friends	98	39.0
The media	75	29.9
Community meetings	33	13.1
Parents during childhood	18	7.2
Education at school	13	5.2
Veterinarians	12	4.8
Workplace	2	0.8
Total		100

4.1.3 Rabies susceptibility

Table 4 shows that 22.4% and 21.3% of the respondents knew of canine and human rabies while 12.3% and 11.1% were aware that cats and jackals can develop rabies. Approximately 7.4% and 7% knew of rabies in cows and goats, respectively while 6.6% and 6.2 knew that sheep and pigs can respectively suffer from rabies. Nearly 5.2% knew that rabbits can have rabies while 0.5% did not have knowledge of hosts that can get rabies.

Table 4 : Perceptions on susceptibility to rabies virus infection

Category of animals perceived to be susceptible	Positive responses (N=137)	Percent
Dogs	133	22.4
People	126	21.3
Cats	73	12.3
Jackal	66	11.1
Cows	44	7.4
Goat	41	7
Sheep	39	6.6
Pigs	37	6.2
Rabbits	31	5.2
Do not know	3	0.5
Total		100

4.1.4 Methods of rabies virus transmission to humans

Fig 10 shows that show that of the respondents, 74% were aware that dog-bite is a way through which people can contract rabies. Licking wounds (16%) and scratching skin (8%) were also known by respondents as ways through which human dog-transmitted rabies can spread. One percent (1%) indicated they did not know about ways through which dogs can transmit rabies to people.

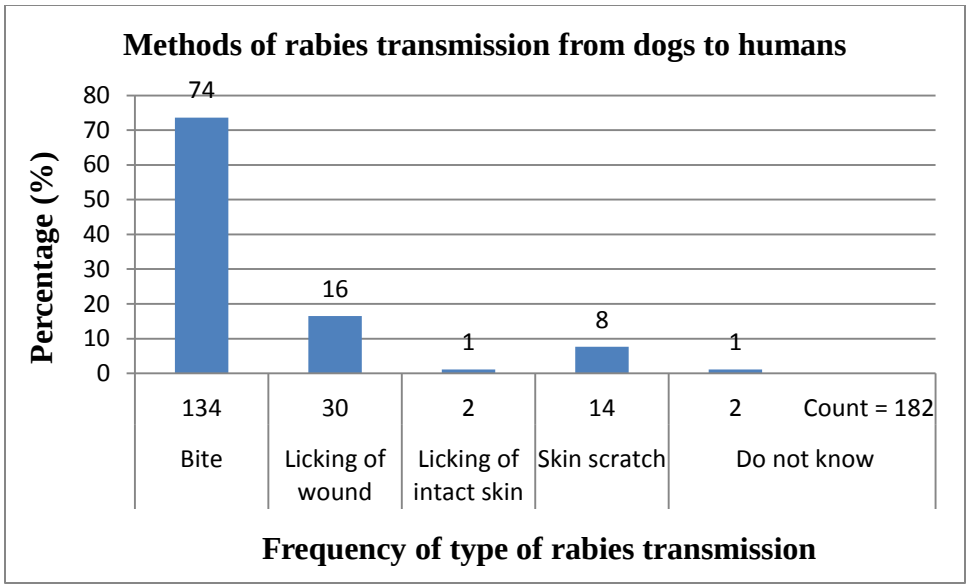

Figure 10 : Methods of rabies transmission from dogs to humans

4.1.5 Transmitting rabies virus from dogs to other animals

Table 5 shows that, of the respondents, 85% were aware of the real possible routes through which transmission of rabies between dogs and other animals can occur (bite, licking of wound, skin scratch). Ten percent (10%) were misinformed about the routes as they transmission of rabies between dogs and other animals can occur through food, licking intact skin, inhalation and coitus. Five percent (5%) reported that they did not know about possible ways of transmitting rabies between dogs and other animals.

Table 5 : Transmitting rabies between dogs and other animals

Rabies transmission methods mentioned	Positive responses (N=137)	Percent
Bite	120	65
Licking of wound	29	16
Skin scratch	8	4
Food	12	7
Licking of intact skin	1	1
Coitus	2	1
Inhalation (aerosolized saliva)	2	1
Do not know	10	5
Total		100

4.1.6 Clinical manifestations of canine rabies

Table 6 shows that 99% of respondents knew at least one rabies clinical manifestation and that aggressiveness (27%), hyper-salivation (23%) and wandering over long distances (20%) were famous clinical manifestations. Approximately 10% indicated they knew that a rabid dog manifested pica and dropping of the jaw for each clinical sign while alteration of sound and dysphagia were reported by 7% and 2%, respectively.

Table 6 : Clinical manifestations of canine rabies

Known clinical manifestations of rabies	Positive responses (N=137)	Percent
A dog is aggressive	112	27
A dog drools profusely	85	20
Pica	43	10
Dysphagia	7	2
A dog roams over long distance	97	23
Alteration of sound	31	7
A dog presents with dropped jaw	41	10
Do not know	2	1
Total		100

4.1.7 Ways in which dog-mediated rabies can be controlled in people

Table 7 reveals that, of the respondents, 82% indicated that vaccinating dogs regularly would be the best method of fighting against human dog-transmitted rabies whereas 6% and 5% thought that killing stray dogs and educating the public would be the best method. Three percent (3%) thought that restricting dogs completely and vaccinating people at risk regularly could be the best methods for each option whereas 1% thought that prophylaxis undertaken after exposure would be the best option.

Table 7 : Ways in which dog-mediated rabies can be controlled in people

Methods of controlling dog rabies	Frequency (N=137)	Percent
Vaccinating dogs regularly	112	82
Restricting dogs completely	4	3
Educating the public	7	5
Killing stray dogs	8	6
Vaccinating people at risk regularly	4	3
Prophylaxis undertaken after exposure	2	1
Total	137	100

4.1.8 Controlling rabies in dogs

Table 8 indicates regular vaccination was the preferred method of preventing dogs from contracting rabies (81%), followed by killing stray dogs (11%), restricting dog movements completely (6%) and castrating dogs (2%).

Table 8 : Preferred method for rabies control in dogs

Rabies control methods	Frequency (N=137)	Percent
Killing of stray dogs	15	11
Restriction of dog movements	8	6
Regular vaccination	111	81
Castration	3	2
Total	137	100.0

4.1.9 Clinical rabies in dogs

Fig.11 shows that, of the respondents, 54% believed in treating rabies successfully while 26% indicated they knew that rabies is always a deadly disease. Approximately 20% were unaware of whether rabies is a curable or a fatal disease.

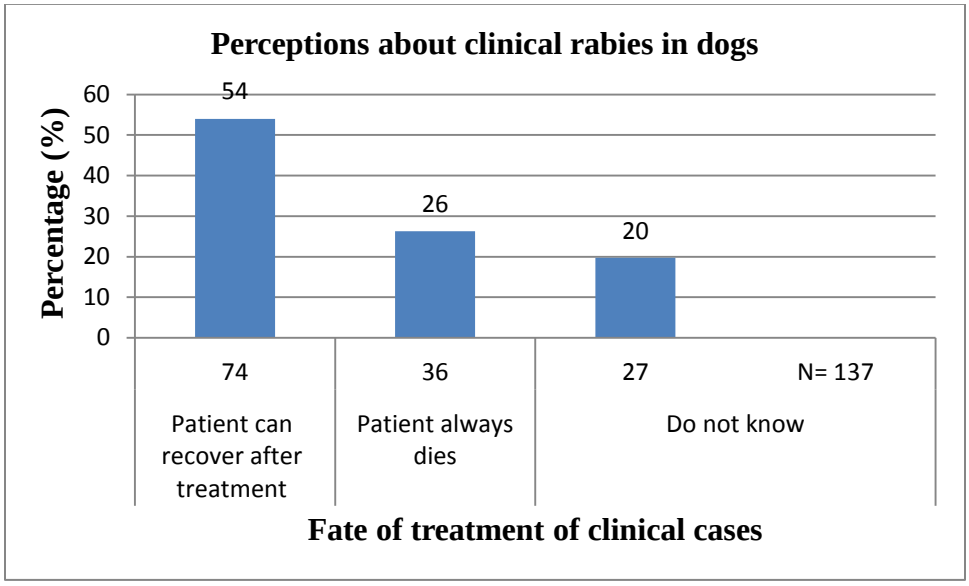

Figure 11 : Perceptions about clinical rabies in dogs

4.1.10 Treating rabies in humans

Fig. 12 shows that 42% of the respondents thought rabies in people can be treated successfully while 43% believed rabies is always fatal and 15% were unaware of whether rabies is treatable or not.

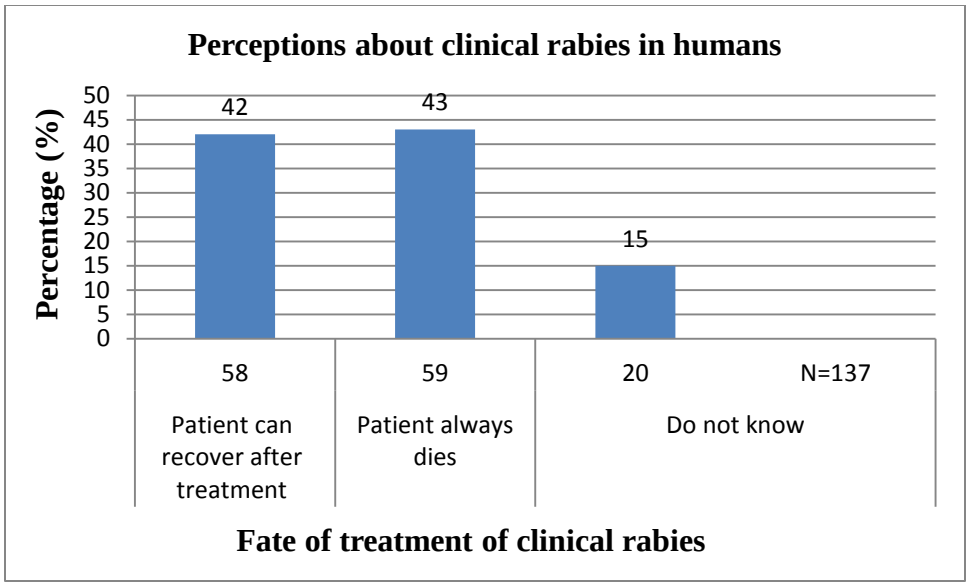

Figure 12 : Perceptions about clinical rabies in humans

4.1.11 Dealing with cases of dog-bites

Table 9 shows that in order to confirm whether a suspected dog is rabid or not, 69% of respondents would confine it for 10 days, irrespective its vaccination status. Killing or releasing the dog had equal but less weighting at 15%.

Table 9 : How deal with a dog in case of human dog bite

Action to take in case of human dog bite	**Frequency (N=137)**	**Percent**
The dog is vaccinated and the owner is known : immediate release	21	15.5
Vaccination status of the dog and the owner are unkown : immediate killing	21	15.5
Irrespective of vaccination status of the dog : confining it for 10 days to confirm it is rabid or not	95	69
Total	**137**	**100**

4.1.12 Options taken before taking a victim of dog-bite to a health care facility

Table 10 shows that, of the respondents, 68.6% would not do anything before taking the victim of dog-bite to a health care facility while 20.4% would use water alone or with soap if it is available to wash the wound. The percentage of those who cover the victim's wound with dressings and bandages was 8% while those who would apply the salt to the wound accounted for or clean it with 70% alcohol or with povidone-iodine accounted for 1.5% for each.

Table 10 : Options taken before taking a victim of dog-bite to a health care facility

Action taken before taking a dog-bite victim to a health care facility	**Frequency (N=137)**	**Percent**
Washing the wound wisely with water alone or with soap if avaible	28	20.4
Covering the wound with dressings and bandages	11	8.0
Applying the salt to the wound	2	1.5
Taking the patient to a health care facility without doing anything	94	68.6
Cleaning the wound with 70 % alcohol or povidone-iodine	2	1.5
Total	**137**	**100**

4.1.13 Rabies vaccination in dogs

Availability, accessibility and preservation of rabies vaccines as well as cost of vaccination, etc are discussed here.

4.1.13.1 Vaccine used

Table 11 illustrates five brands of rabies vaccines that were available on the Rwandan market. The vaccines were coded as follows: Rabies Veterinary Vaccine Inactivated B.P. (VET.) (A), Vaxipet R (B), Vaxipet DHPPi+LR (C), Rabisin (D), and Nobivac Rabies (E) (appendix 2).

The table shows the two most used vaccine brands were A and B representing 57% and 14 %, respectively while D and E accounted for 12.2% for each respectively. Vaccine brand C was found to be the least used brand and was the only polyvalent vaccine as the other four brand vaccines are monovalent.

Table 11 : Vaccine brands used to protect dogs against rabies

Vaccine brand	Frequency (N=107)	Percent
A	61	57
B	15	14
C	5	4.6
D	13	12.2
E	13	12.2
Total	**107**	**100**

4.1.13.2 Receiving vaccination for dogs

Table 12 shows that 57.9% of the dog owners had their dogs vaccinated by veterinarians at home whereas those who took their dogs to sites during vaccination campaign accounted for 41.2%. Approximately 0.9% had their dogs vaccinated at a veterinary clinic.

Table 12 : Process for dog vaccination

Dog owners' options for dog vaccination	Frequency (N=107)	Percent
Taking a dog to a site during vaccination campaign	44	41.2
A veterinarian comes at home	62	57.9
Taking a dog to a veterinary clinic	1	0.9
Total	**107**	**100**

4.1.13.3 Preserving rabies vaccines at time of purchase and administration

Table 13 shows that 86% of respondents indicated that at time of vaccinating their dogs, veterinarians kept vaccines in a cooler box while 12.1% their veterinarians preserved vaccines on ice in plastic bag. The rest of respondents (1.9%) purchased and brought home the vaccines and administration was done by veterinarians.

Table 13 : Preservation of rabies vaccines at time of purchase and administration

Means of preservation	**Frequency (N=107)**	**Percent**
On ice in a cooler box	92	86.0
On ice in a plastic bag	13	12.1
Purchase and taking home by a dog owner & administration by a veterinarian	2	1.9
Total	**107**	**100.0**

4.1.13.4 Cost of vaccination for dogs

Table 14 shows that dogs of owners who were charged nothing and those who paid 1-1,500 RWF (41%) had their dogs vaccinated during vaccination campaign at sites by public veterinarians. Owners whose dogs were vaccinated by private veterinarians at owners' homes accounted for 43% and paid 5,001-10,000 RWF or 10,001-20,000 RWF or 20,001-30,000 RWF. Dogs of owners who paid 1,501-5,000 RWF (16%) had their dogs vaccinated by both public and private veterinarians either at owners' home or at a veterinary clinic.

Table 14 : Rabies vaccination costs

Vaccination fees (RWF)	Frequency (N=107)	Percent
Free of charge	20	19
1- 1, 500	24	22
1,501-5,000	17	16
5,001 -10,000	19	18
10,001-20,000	25	23
20,001-30,000	2	2
Total	**107**	**100**

4.1.13.5 Judgment on vaccination fees

Table 15 shows that 56% and 22% of the respondents indicated that the cost of vaccination was affordable and minimal, respectively whereas 22% complained about the cost, saying it was considering it expensive.

Table 15 : Status of vaccination fees

Judgment on vaccination fees	Frequency (N=107)	Percentage
Fee was minimal	24	22
Fee was affordable	60	56
Fee was too much money	23	22
Total	**107**	**100**

4.1.13.6 Age of dogs at first vaccination

Table 16 shows that most (79%) of the respondents did not think puppies aged less than three months could be vaccinated and therefore did not take them to vaccination. However, 21% of the dog owners took their puppies for vaccination within 3 months of birth.

Table 16 : Age of dogs at first vaccination

Age of the dog at first vaccination	**Frequency (N=107)**	**Percent**
Less than or up to three months old	22	21
Older than three months	85	79
Total	**107**	**100**

4.1.13.7 Reasons for not taking dogs to be vaccinated for owners of unvaccinated dogs

Table 17 shows that those who owned unvaccinated dogs indicated that the leading factors that hindered them from vaccinating their dogs were lacking information (40%); neglecting (37%) and lacking adequate knowledge of rabies (12%). The other factors for not vaccinating dogs were the cost of vaccination and vaccination sites localized far from household representing 9% and 2%, respectively.

Table 17 : Reasons for having unvaccinated dogs for dog owners

Reason for having unvaccinated dogs	Positive answers (N=30)	Percent
Lack of information	17	40
Lack of knowledge on rabies	5	12
Difficulty in catching dogs	0	0
Vaccination fees too high	4	9
Sites of vaccination set far during vaccination campaign	1	2
Negligence	16	37
Total		100.0

4.1.14 Findings of multiple regression analyses

Table 18 reveals that, of the predictor variables, i.e., dog owner's sex, level of education, residential district or length of dog ownership, none was statistically associated with the dog owner's rabies knowledge. Also, none of respondents' attitudes or practices was statistically significant associated with any of the predictor variables. On the other hand, different relationships between dog owner's status of KAP towards rabies and the chosen dog owner's classes existed. First, dog owners' who finished or were still continuing university education were less likely to have sufficient knowledge of rabies compared to those who were less educated. Sufficient knowledge odds were more than twice among dog owners who did not receive formal education, and 24% higher among dog owners who finished secondary education compared to those who finished university education. Sufficient knowledge odds were 40% lower among male dog owners compared to female ones.

The respondents who had owned dogs for 5-10 years were less likely to be sufficiently knowledgeable about rabies than those who had owned dogs for more than 10 years (AOA=0.96). Though, the respondents who had kept dogs for less than five years were more likely to get sufficient rabies knowledge (AOR=1.23). Sufficient knowledge odds among dog owners who lived in Kicukiro and Nyarugenge were respectively 41% and 58% lower than that of those residing in Gasabo. Second, male dog owners' were more likely to adopt positive attitudes towards rabies (AOR=1.47). Positive attitude's odds were more than 40% lower among dog owners who finished any educational level except for university education. In the same way, compared to other dog owners, those who had kept dogs for more than 10 years were more likely to adopt positive attitudes towards rabies. Especially, rabies positive attitudes' odds among the respondents who had owned dogs for 5-10 years were 65% lower compared to those who had kept dogs for more than 10 years. Third, practices of rabies among the dog owners, were more likely to be appropriate for male respondents (AOR=1.40), residing in Gasabo and who had at least finished primary education (AOR=1.41), or who had at least owned dogs for 5 years (AOR=1.46).

Table 18: Adjusted odds ratios with 95% confidence intervals

Characteristics	Category	Knowledge	Attitude	Practice
Administrative District	Gasabo	*Reference*	*Reference*	*Reference*
	Kicukiro	0.59(0.23,1.54)	0.65(0.18,2.33)	0.55(0.21, 1.44)
	Nyarugenge	0.42(0.15,1.18)	1.74(0.46,6.51)	0.98(0.32, 2.98)
Respondent's sex	*Female*	*Reference*	*Reference*	*Reference*
	Male	0.60(0.27,1.34)	1.47(0.49,4.42)	1.40(0.62, 3.13)
Level of education level	*Tertiary*	*Reference*	*Reference*	*Reference*
	No education	2.12(0.27,16.45)	0.41(0.03,5.40)	0.71 (0.09, 5.66)
	Primary	0.97 (0.37,2.54)	0.50(0.14,1.85)	1.42 (0.52,3.88)
	Secondary	1.24 (0.51,3.05)	0.59(0.18,1.97)	1.51(0.59,3.86)
Dog ownership length	*> 10 years*	*Reference*	*Reference*	*Reference*
	< 5 years	1.23 (0.54, 2.79)	0.39(.14, 1.11)	0.97(0.42,2.27)
	5-10 years	0.96 (0.37, 2.50)	0.35(.10, 1.26)	1.46(0.52,4.13)
Constant		2.33	0.38	1.53

4.2 Whole blood parameters

The data for all the blood parameters was obtained from three references and used to compute hematologic reference range for dogs.

4.2.1 Computed haematologic reference intervals for dog blood

For each blood parameter, the lower value was calculated by taking the arithmetic mean minus the standard deviation (SD) and computed the upper value through adding the standard deviation to the arithmetic mean (Table 19).

The table was used to evaluate the observed blood parameters for the samples of the dogs in the study.

Table 19 : Computed hematologic reference intervals for dog blood

No	Blood parameters	Mean ± SD	Study computed intervals	Intervals from reference (*)
1	WBC (10^9 cells/L)	13.077 ± 6.26	6.8-19.3	6-17[2*]
2	LYM%	45.644 ± 25.75	19.9-71.4	15-30[1*]
3	MID%	18.885 ± 6.25	12.6-25.1	
4	GRAN%	35.471 ± 25.41	10.1-60.9	
5	LYM(10^9 cells/L)	5.585 ± 3.78	1.8-9.4	
6	MID (10^9 cells/L)	2.397 ± 1.22	1.2-3.6	
7	GRAN (10^9 cells/L)	5.096 ± 5.36	0.3-10.5	
8	RBC (10^{12} cells/L)	6.778 ± 1.48	5.3-8.3	5-8[1*]
9	HBG (g/dL)	16.461± 3.77	12.7-20.2	12-18[2*]
10	HCT (%)	51.748 ± 12.08	39.7-63.8	41 - 58[3*]
11	MCV (fL)	76.284 ± 4.92	71.4-81.2	60-77[2*]
12	MCH (pg)	24.215 ± 1.63	22.6-25.8	19-24[1*]
13	MCHC (g/dL)	31.918 ± 2.79	29.1-34.7	30-36[1*]
14	RDW- CV (%)	16.142 ± 1.71	14.4-17.8	
15	RDW-SD (fL)	54.705 ± 6.92	47.8-61.6	
16	PLT (10^9 cells/L)	202.344 ± 132.86	69.5-335.2	186 - 545[3*]
17	MPV (fL)	10.789 ± 2.62	8.2-13.4	8.4 - 14.1[3*]
18	PDW (fL)	13.063 ± 3.07	10-16.1	
19	PCT (%)	0.195 ± 0.11	0.1-0.3	
20	P-LCR (%)	30.771 ± 15.75	15-46.5	
21	P-LCC (10^9 cells/L)	53.032 ± 27.42	25.6-80.4	

***Reference sources: the numbers/asterisks refer to the following sources**

1) Brar *et al.,* 2002; 2) Schalm *et al.*,1975; 3) Cornell University, 2014

Legend: GRAN: Granulocyte, HCT: Haematocrit, HGB: Haemoglobin, LYM: Lymphocyte, MCH: Mean Corpuscular Hemoglobin Concentration, MCV: Mean Corpuscular Volume, MID: Monocyte, MPV: Mean Platelet Volume, PCT: Plateletcrit, PDW: Platelet Distribution Width, P-LCC: Platelets Larger than 12 fL and smaller than 30 fL, P-LCR: Platelet Large Cell Ratio, PLT: Platelet, RBC: Red Blood Cell. RDW-CV: Red Blood Cell Distribution Width - Coefficient of Variation, RDW-SD: Red Blood Cell Distribution Width - Standard Deviation, WBC: White Blood Cell.

4.2.2 Effect of helminthoses on leucocytes in study dogs

Table 20 indicates that 22.2% and 11.1% of dogs that presented with leucopenia were diagnosed with *Ancylostoma spp* and cestodes respectively. Fifty percent (50%) and 16.6% of those that had leucocytosis were infected with Ancylostoma spp and cestodes respectively. Approximately 50% of the dogs that had lymphocytosis were parasitised with cestodes while 18.2% of those having lymphopenia were infected with *Ancylostoma spp* and cestodes for each.

Table 20 : Effect of helminthoses on leucocytes in study dogs

Effect	Frequency (n=93)	Ancylostomosis	Cestodes
Leucopenia	9	2(22.2%)	1(11.1%)
Leucocytosis	6	50%	16.6%
Lymphopenia	11	18.2%	18.2%
Lymphocytosis	12	-	50%

4.2.3 Helminthoses and blood parameters in the study dogs

Table 21 reveals that 50% of dogs that presented with low hematocrit value were parasitized with *Ancylostoma spp* while 7% of them were diagnosed with cestodes. Seven percent (7%) of the dogs that had higher level of haematocrit were infected with *Ancylostoma spp*. Approximately 90% of dogs that presented with low MCV were infected with *Ancylostoma spp* while 10 % a mixed infection involving *Ancylostoma spp* and *Toxocara canis.* Eighty percent (80%) and 20% of the dogs with high levels of MCV were parasitised with *Ancylostoma spp* and cestodes respectively. Coefficients of Pearson correlation indicated a significant relationship between haematocrit and helminthiasis at the 0.05 level, r= 0.263, *p*= 0.011. Mean Corpuscular Volume (MCV) correlated significantly with helminthiasis at the 0.05 level, r= 0. 207, *p*=0. 046.

Table 21 : Helminthoses and blood parameters in the study dogs

Blood parameter	**Number of the dogs considered**	**Worm infection**		
		Ancylostoma spp	Cestodes	*Ancylostoma spp - T. canis*
Low HCT	14	50%	7%	-
High HCT	15	7%		
Low MCV	10	90%		10%
High MCV	5	80%	20%	-

4.3 Faecal examination findings

The feed regimes recorded for the dogs showed 44% were given raw meat while 56% were not offered raw meat rather the owners boiled the meat before distribution. Deworming was done for 46% against 54% that were not dewormed.

4.3.1 Prevalence of intestinal helminth in dogs

Table 22 shows that 60.2% of the cases were negative against 39.8% that were positive for worm burden in dogs. *Ancylostoma spp* was the predominant parasite representing 32.3%, followed by tapeworms that occupied 6.5% and a mixed infection *Ancylostoma spp - Toxocara canis* accounting for 1 %. The overall prevalence of parasites was 39.8%.

Table 22 : Identified gastrointestinal worms in dogs

Group of identified species	**Frequency (N=93)**	**Percent**
Ancylostoma spp	30	32.3
Cestodes	6	6.5
Ancylostoma spp and Toxocara canis	1	1
Negative	56	60.2
Total	**93**	**100**

4.3.2 Egg count distribution in dogs

Figure 13 shows that the dogs that had an EPG range of 50-500 and 501-1000 accounted for 58% and16% respectively while those having an EPG range varying from 1001-2500 and 2501-3750 represented 13% for each.

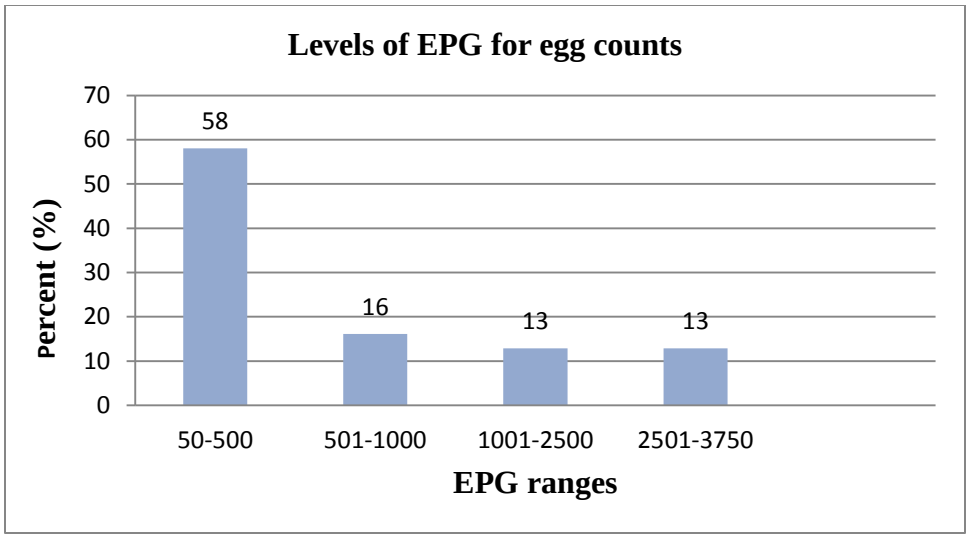

Figure 13 : Egg count distribution in dogs

4.4 Findings from the examination of blood smears

All the nighty three (100%) blood smears tested negative for haemoparasites. Of the study dogs, 61.3% were sprayed regularly or irregularly with ectoparasiticides while 38.7% that were not.

4.5 Results of serum samples analysis

Antibody titres for the 93 sera samples were quantified and converted to equivalent unity to assess the vaccine responsiveness. Factors that could influence response of dogs to rabies vaccination were investigated, namely status and frequency of vaccination, vaccine brand, time between vaccination and sampling, deworming status, and blood parameters.

4.5.1 Vaccination status of dogs

Figure 14 shows that of the sampled dogs, 86% were vaccinated against rabies while 14% were not.

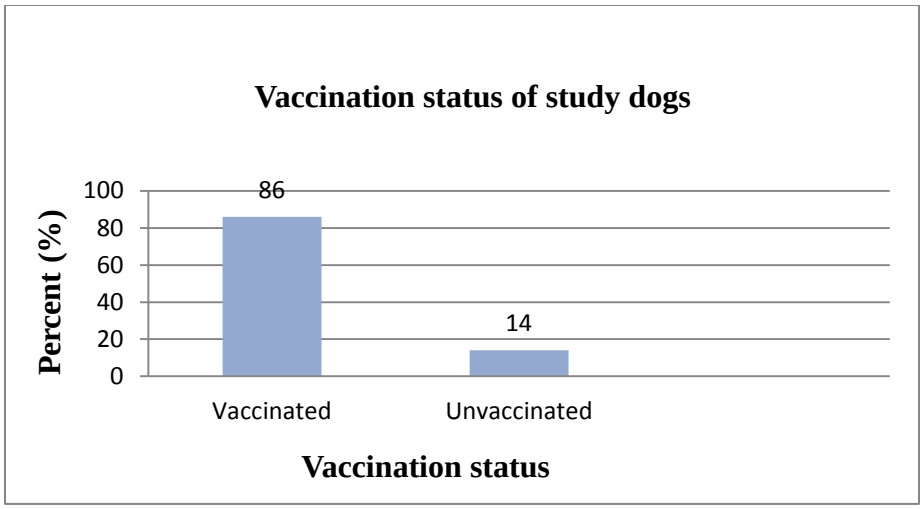

Figure 14 : Vaccination status of dogs

4.5.2 Frequency of vaccination in dogs

Table 23 shows that dogs had different vaccination frequencies with 59% of them being vaccinated once or twice while 11% and 12 % had three or four vaccinations and 18% had at least five vaccinations.

Table 23 : Frequency of vaccination in dogs

Number of vaccination	Frequency (N=80)	Percent
Once	25	31
Twice	22	28
Thrice	9	11
Quadruple	10	12
At least five times	14	18
Total	**80**	**100**

4.5.3 Interval between vaccination and sampling among the dogs

Figure 15 shows that, of the vaccinated dogs, 59% and 26% were sampled 10-12 and 1-5 months after vaccination, respectively, while 15% were sampled 6-9 months following vaccination.

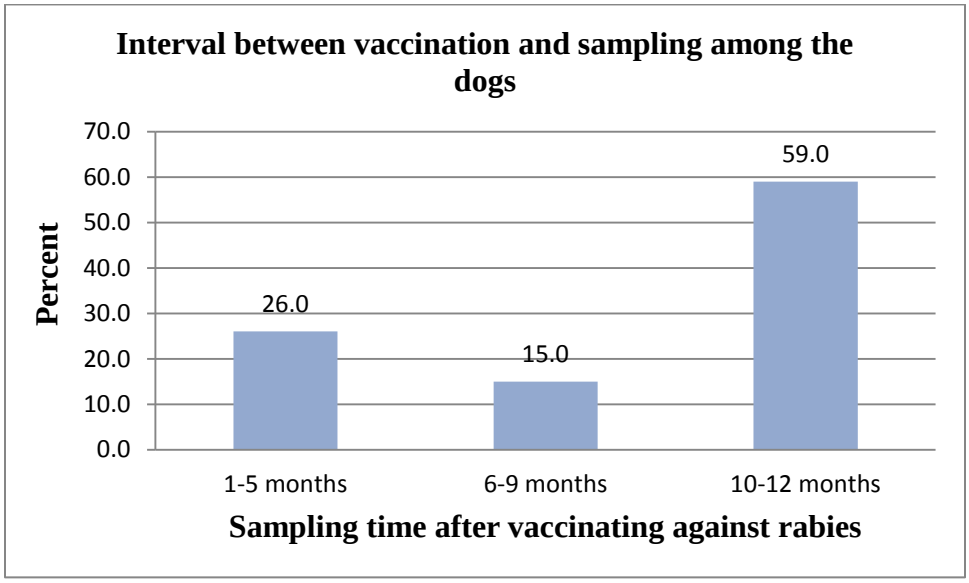

Figure 15 : Interval between vaccination and sampling among the dogs

4.5.4 Sero-conversion in vaccinated dogs

Based on the fact that 0.5 IU/ml of rabies antibodies was confirmed to be the cutoff point for a satisfactory response to rabies vaccination in dogs and cats (OIE, 2013a), 65% had adequate level of protective antibodies while 34% had insufficient level of protection. Approximately 1% did not have detectable sero-conversion (Table 24).

Table 24 : Sero-conversion in dogs received rabies vaccination

Antibody titres	Clarification	Frequency (N=80)	Percent
> 4 EU/ml	High level of sero-conversion	18	22.5
0.5 - 4 EU/ml	Sufficient level of sero-conversion	34	42.5
0.125 - 0.5 EU/ml	Insufficient level of sero-conversion	27	34.0
< 0.125 EU/ml	Undetectable level of sero-conversion	1	1.0
Total		**80**	**100**

4.5.5 Sero-conversion in unvaccinated dogs

Table 25 indicates that 6 dogs had undetectable level of sero-conversion (< 0.125 EU/ml); 7 dogs of which dog coded 2 (0.192 EU/ml or 0.189 OD), 16 (0.17 EU/ml or 0.166 OD), 29 (0.238 EU/ml or 0.236 OD), 42 (0.161 EU/ml or 0.157 OD), 63 (0.133 EU/ml or 0.13 OD), 79 (0.179 EU/ml or 0.192 OD), and 90 (0.185 EU/ml or 0.181 OD) had detectable antibody titres though considered insufficient in terms of sero-conversion (less than the cutoff point : 0.5 EU/ml).

Table 25 : Sero-conversion in unvaccinated dogs

Antibody titres	Frequency (N=13)	Percent
0.125-0.5 EU/ml	7	53.8
< 0.125 EU/ml	6	46.2
Total	**13**	**100**

4.5.6 Sero-conversion in dogs sampled 10-12 months following rabies vaccination

Figure 16 indicates that 60% obtained adequate level of protective antibodies while 40% did not have sufficient level of protective rabies antibody.

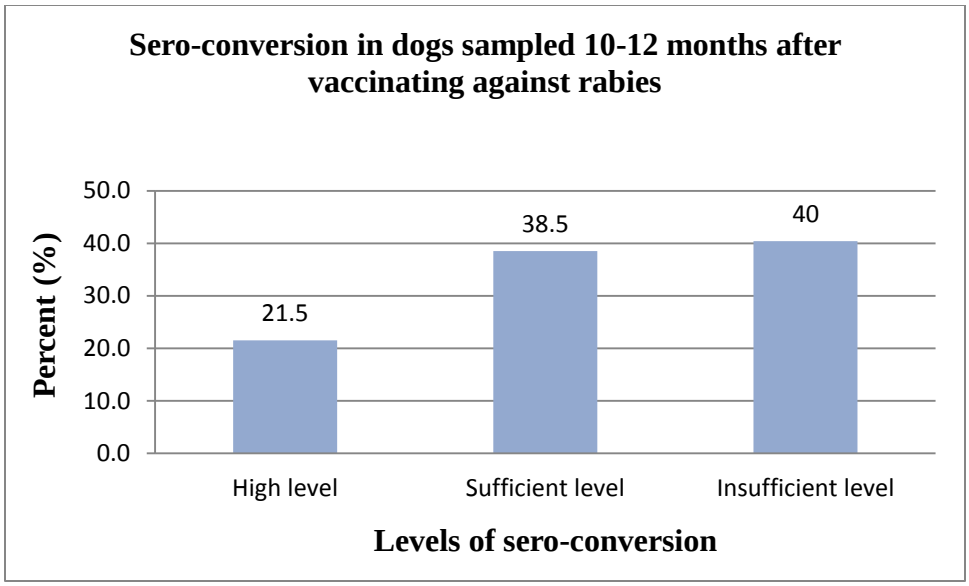

Figure 16 : Sero-conversion in dogs sampled 10-12 months after vaccinating against rabies

4.5.7 Sero-conversion in dogs sampled 6-9 months after receiving rabies vaccination

Figure 17 shows that 58% of the dogs sampled 6-9 months after receiving rabies vaccination had adequate level of protective antibodies against 42% that had insufficient level of protection.

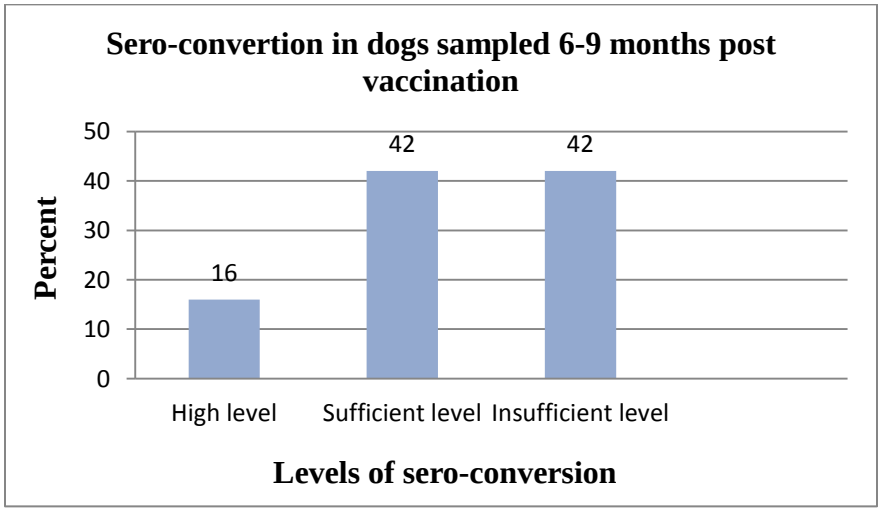

Figure 17 : Sero-conversion in dogs sampled 6-9 months after receiving vaccination

4.5.8 Sero-conversion by period of sampling post-vaccination

Table 26 shows that 81% of the dogs sampled 1-5 months after receiving vaccination had protective antibody levels while 19% did not have. Of the dogs sampled 6-9 months following rabies vaccination, 58% were protected while 42% were not. Of the dogs sampled 10-12 months post-vaccination, 60% were protected while 40% were not. Chi-square test showed that the difference between post-vaccination sampling time and sero-conversion levels, $X^2(9) = 12$, $p=0.213$ was not statistically significant.

Table 26 : Summary table for sero-conversion by period of sampling post-vaccination

Post-vaccination months	**High**	**Sufficient**	**Insufficient**
1-5	29	52	19
6-9	16	42	42
10-12	21.5	38.5	40

4.5.9 Sero-conversion by sampling time and vaccination response

Table 27 shows that 81% of the dogs sampled 1-5 months after receiving rabies vaccination had protective levels of sero-conversion (>0.5EU/ml) while 19% did not have. Of those sampled 6-9 months following rabies vaccination, 58% had protective sero-conversion levels while 42% did not have. Of the dogs sampled 10-12 months post-vaccination, 60% had protective antibody levels while 40 did not have. Chi-square test showed that the difference between sero-conversion levels, $X^2(9) = 12$, $p=0.213$ was not statistically significant. Chi-square test showed that the difference between post-vaccination sampling time and implication of sero-conversion, $X^2(9) = 12$, $p=0.213$ was not statistically significant.

Table 27 : Sero-conversion by sampling time and vaccination response

Post-vaccination sampling time	Percent of sero-conversion in vaccinated dogs	
	+ve	-ve
1-5	81	19
6-9	58	42
10-12	60	40

4.5.10 Sero-conversion in dogs sampled 1-5 months post vaccination

Fig 18 indicates that 81% of the dogs sampled 1-5 months following vaccination had protective antibodies while 14% and 5% had inadequate and undetectable levels of sero-conversion respectively.

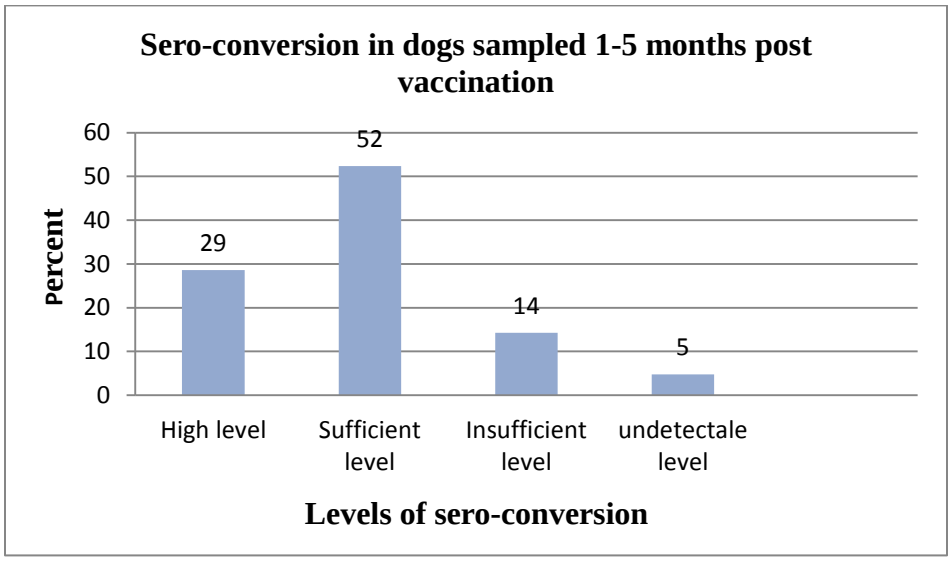

Figure 18 : Sero-conversion in dogs sampled 1-5 months post vaccination

4.5.11 Time of sampling post vaccination and insufficient sero-conversion in vaccinated dogs

Table 28 shows the high number of vaccinated dogs that did not have adequate rabies protective antibodies (< 0.5 EU/ml) was recorded in dogs sampled 6-9 months (42%) after vaccination and in those sampled 10-12 months (40%) after vaccination.

The least number was recorded in those sampled 1-5 months (19%) after vaccination. The difference between protected and non-protected dogs in terms of time of sampling pot vaccination, $X^2(9) = 9$, $p = 0.213$ was not statistically significant.

Table 28 : Time of sampling after vaccination and protection against rabies

Sampling time	**Protected**	**Non-protected**	**Total**
1-5 months	17(81%)	4(19%)	21
6-9 months	7(58%)	5(42%)	12
10-12 months	28(60%)	19(40%)	47

4.5.12 Vaccination frequency and insufficient sero-conversion in vaccinated dogs

Table 29 indicates from one to four times of anti-rabies vaccination, the number of vaccinated dogs that did not have adequate rabies protective antibodies (< 0.5 EU/ml) decreased by frequency of anti-rabies vaccinations. The highest number of vaccinated dogs (52%) that were not protected against rabies had received vaccination once, while 33% and 29% of them were vaccinated three times and at least five times respectively. Nearly 27% and 20% had obtained rabies vaccination twice and four times respectively. The difference between protected and non-protected dogs in terms of time in terms of frequency of vaccinations, $X^2(25) = 30$, $p = 0.224$ was not statistically significant.

Table 29 : Vaccination frequency and insufficient sero-conversion in vaccinated dogs

Vaccination frequency	Protected	Non-protected	Total
Once	12(48%)	13(52%)	25
Twice	16(73%)	6(27%)	22
Thrice	6(67%)	3(33%)	9
Four times	8(80%)	2(20)	10
At least five times	10(71%)	4(29%)	14
	52	**28**	**80**

4.5.13 Mean titres versus deworming status

Table 30 indicates deworming status influenced the antibody response. The geometric mean titre in dewormed dogs (1.094) was higher than the geometric mean titre in dogs that were not dewormed (0.633). The mean titre computed from dewormed dogs was higher than the overall mean (0.815) while that computed from dogs that were not dewormed was lower than the overall mean titre.

Table 30 : Mean titres versus deworming status

Status of deworming	Dewormed	Non-dewormed	Overall GM
Geometric mean titres (GM)	1.094	0.633	0.815
Titre range	0.125-4.1	0.125-4.1	

4.5.14 Frequency of deworming and production of antibodies

Table 31 shows that the frequency of deworming did not show effect on production of antibodies. This data shows a fact of increased response with shorter frequency, twice=1.738; once a year= 1.529.

Table 31 : Frequency of deworming and production of antibodies

	Every month*	**4 times a year**	**3 times a year**	**Twice a year**	**Once a year**	**Irregularly**	**Overall GM**
Geometric mean titres (GM)	4.100	0.767	0.796	1.738	1.529	0.901	1.094
Titre range	4.1	0.139-4.1	0.349-1.815	0.246-4.1	0.248-4.1	0.125-4.1	

***** Only one dog was dewormed monthly

4.5.15 Mean titres versus identified worms

Table 32 shows that the species of worms did not influence antibody production; the geometric mean titre of dogs parasitized with Tapeworms was higher (1.211) than that computed from dogs that tested negative for worms (0.920) however the latter one was higher than the mean titre of dogs infected with *Ancylostoma spp* (0.570) and overall mean titre (0.815). However *Ancylostoma spp* influenced blood parameters; *Ancylostoma spp is* haematophage, and was found to cause anaemia.

Table 32 : Mean titres versus identified worms

	Negative	***Ancylostoma spp***	**Tapeworms**	***Ancylostoma spp - Toxocara canis***	**Overall GM**
Geometric mean titres (GM)	0.920	0.570	1.211	4.100*	0.815
Titre range	0.125-4.1	0.125-4.1	0.251-4.1	-	

* Infection with *Ancylostoma spp - Toxocara canis* was detected in one dog

4.5.16 Egg count distribution in dogs and mean titres

Table 33 shows the EPG range was to some extent related to the production of antibodies where the geometric mean was expected to decrease with high EPG, but it was not fully matching. The lowest geometric mean titre was computed from the third higher EPG range (0.318).

Table 33 : Egg count distribution and mean titres

EPG	50-500	501-1000	1001-2500	2501-3750	Overall GM
Geometric mean titres (GM)	0.659	0.629	0.318	0.768	0.607
Titre range	0.125-4.1	0.179-3.676	0.125-1.089	0.125-4.1	

4.5.17 Mean titres versus vaccination status

Table 34 shows the mean titre of vaccinated dogs (1.071) was higher than the mean titre of unvaccinated dogs (0.151).

Table 34 : Mean titres versus vaccination status

Status of vaccination	Vaccinated	Unvaccinated
Geometric mean titres	1.071	0.151
Titre range	0.125-4.1	0.125-0.238

4.5.18 Percent of dogs vaccinated per vaccines given and sampling time

Table 35 shows that 75% and 81.8% of dogs vaccinated with vaccines A and D, respectively, were sampled 10-12 months post-vaccination. All dogs vaccinated with both vaccines E and C were sampled 1-5 months following vaccination, while 22% of dogs vaccinated with vaccine B were sampled 10-12 months after vaccination.

Table 35 : Percent of dogs vaccinated per vaccines given and sampling time

Vaccine brand	Total number of dogs per vaccine	Dogs sampled 10-12 months	Dogs sampled 6-9 months	Dogs sampled 1-5 months
A	48	36(75%)	7(14.6%)	5(10.4)
B	9	2(22.2%)	3 (33.3%)	4(36.6%)
D	11	9 (81.8%)	2(18.2%)	0
C	3	-	-	3(100%)
E	9	-	-	9(100%)
Total	**N = 80**			

4.5.19 Percentage protected for each sampling intervals for each vaccine

Table 36 shows that regardless of time of sampling, 88.9% and 77.8% of dogs vaccinated with vaccines E and B were protected while 66.6% and 63.7% of those immunized with vaccines C and D were protected. Vaccine A had the lowest number of protected dogs accounting for 58.3%. Considering dogs vaccinated with vaccine A, out of 10 dogs vaccinated once and were sampled 10-12 months after vaccination, 40% were protected. Out of 4 and 10 dogs vaccinated twice and were sampled 6-9 and 10-12 months after vaccination, 75% and 60% were respectively protected. Of 6 dogs vaccinated three times and were sampled 10-12 months following vaccination, 50% were protected while 3 in 4 (75%) of those in their fourth vaccination that were sampled 10-12 months after receiving rabies vaccination were protected. Four of six (67%) of those vaccinated at least five times and were sampled 10-12 months were protected.

Table 36 : Percentage protected for each sampling intervals for each vaccine

Vaccines	**A (n=48)**		**B (n=9)**		**C(n=3)**		**D(n=11)**		**E(n=9)**	
Vaccination number	Protection		Protection		Protection		Protection		Protection	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Once										
1-5	0	2	1*	0	0	1*	0	0	5	0
6-9	0	0	0	0	0	0	0	2	0	0
10-12	4	6	1*	1*	0	0	1*	1*	0	0
Twice										
1-5	1*	0	0	0	0	0	0	0	1*	0
6-9	3	1*	1*	1*	0	0	0	0	0	0
10-12	6	4	0	0	0	0	4	0	0	0
3 times										
1-5	0	0	0	0	1*	0	0	0	1*	0
6-9	1*	0	0	0	0	0	0	0	0	0
10-12	3	3	0	0	0	0	0	0	0	0
4 times										
1-5	1*	0	1*	0	1*	0	0	0	0	0
6-9	1*	0	0	0	0	0	0	0	0	0
10-12	3	1*	0	0	0	0	1*	1*	0	0
$\geq$ 5 times										
1-5	1*	0	2	0	0	0	0	0	1*	1
6-9	0	1*	1*	0	0	0	0	0	0	0
10-12	4	2	0	0	0	0	1*	0	0	0
Total	**28 (58.3%)**	**20**	**7 (77.8%)**	**2**	**2 (66.6%)**	**1**	**7 (63.7%)**	**4**	**8 (88.9%)**	**1**

*: Sampling intervals with one dog were not considered for inspecting trends

4.5.20 Percent of protection of vaccinated dogs at time of sampling for various vaccines

Table 37 shows that 6.3% of dogs vaccinated with vaccine A and sampled 1-5 months after vaccination were protected against 10.4% and 42% sampled 6-9 and 10-12 months respectively and were protected. Sixty-four percent (64%) of dogs vaccinated with vaccine D and sampled 10-12 months after vaccination were protected. Nearly 44.5% and 22.2% of dogs vaccinated with vaccine B and sampled 1-5 and 6-9 months respectively, were protected while only 11.1% of those sampled 10-12 months following vaccination were protected. Sixty-seven (67%) and 89% of dogs vaccinated with vaccines C and E and sampled 1-5 months for each vaccine were protected. Chi-square test showed that there was difference between protected and non-protected vaccinated dogs in terms of sampling time, $X^2(9)=10$, $p= 0.350$), but it was not statistically significant.

Table 37 : Protection of vaccinated dogs versus sampling time for various vaccines

Vaccine	**1-5 months**	**6-9 months**	**10-12 months**	**Total number of vaccinated dogs**
	Protected	Protected	Protected	-
A	3(6.3%)	5(10.4%)	20(42%)	48
B	4(44.5%)	2(22.2%)	1(11.1%)	9
D	0	0	7(64%)	11
C	2(67%)	0	0	3
E	8(89%)	0	0	9

4.5.21 Percent of protection for type of vaccines and frequency of vaccination

Table 38 shows that (28/52) 53.8% of protected dogs were vaccinated with vaccine A and that the number of dogs protected by the vaccine brand A increased by number of vaccinations, except at the third and at least fifth vaccinations. The percentage of dogs protected by vaccine A was higher in dogs vaccinated twice (20.9%), four (10.4%) and at least five (10.4%) times compared to 8.3% recorded in dogs vaccinated once and twice respectively. The number of dogs vaccinated with vaccine B and were protected was lower (22.2%) in dogs vaccinated once compared to the number of dogs that were protected on at least five times of vaccinations (33.3%).

Table 38 : Percent of protection for type of vaccines and frequency of vaccination

Frequency of vaccinations	**Vaccine A (n=48)**	**Vaccine B (n=9)**	**Vaccine D n=11)**	**Vaccine C (n=3)**	**Vaccine E (n=9)**
	Protected	Protected	Protected	Protected	Protected
Once	4(8.3%)	2(22.2%)	1(9%)*	0	5(55.6%)
Twice	10(20.9%)	1(11.1%)*	4(36.7%)	0	1(11.1%)*
Three times	4(8.3%)	0	0	1(33.3%)*	1(11.1%)*
Four times	5(10.4%)	1(11.1%)*	1(9%)*	1(33.3%)*	0
At least five times	5(10.4%)	3(33.4%)	1(9%)*	0	1(11.1%)*
Percentage	**28(58.3%)**	**7(77.8%)**	**7(63.7%)**	**2(66.6%)**	**8(77.8%)**

*****: Cases represented by one dog were not part of trend analysis

4.5.22 Mean titres compared to vaccine brands

Table 39 shows that the vaccine brands induced different antibody levels as indicated by the varying geometric mean titres thus impacting the immunity in the vaccinated dogs differently.

Vaccines A and D were the types that yielded the lowest mean titres, namely: 0.897 and 0.814 respectively and these titres were below the overall mean (1.071). Approximately 44.4% and 36.4% of dogs vaccinated with both vaccines A and D, respectively, had levels of rabies protective antibodies below 0.5 EU/ml. Higher mean titre (2.115) was scored by vaccine E and then by vaccines B (1.850) and C (1.261).

Table 39 : Mean titres compared to vaccine brands

Vaccine brand	**A**	**B**	**C**	**D**	**E**	**Overall GM**
Geometric mean titres (GM)	0.897	1.850	1.261	0.814	2.115	1.071
Titre range	0.125 -4.1	0.391 -4.1	0.27 4-4.1	0.14-4.1	0.46-4.1	

4.5.23 Mean titres and intervals between vaccination and sampling time

Figure 19 shows interval between vaccination and sampling impacted on antibody response and antibody titres decreased by post vaccination time. Dogs sampled 1-5 months after receiving vaccination had the highest mean titre (1.559), followed by dogs sampled 6-9 months post vaccination (0.949) and finally by those sampled 10-12 months (0.934).

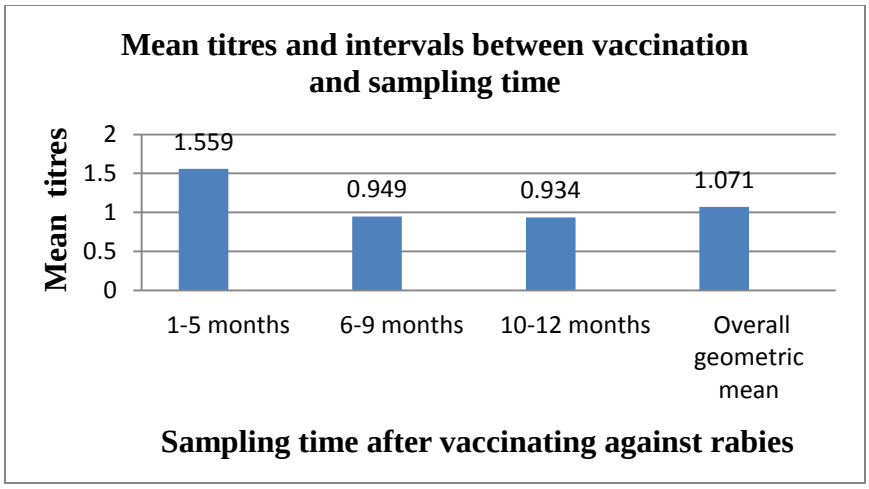

Figure 19 : **Mean titres and intervals between vaccination and sampling time**

4.5.24 Mean titres and the number of vaccinations applied

Figure 20 shows that antibody production increased by number of vaccinations given; from one to four times of vaccination mean titres went up gradually, i.e., 0.608, 1.320, 1,395 and 1.787. On at least five times mean titre decreased (1.243) compared to the second, third and fourth times of vaccination.

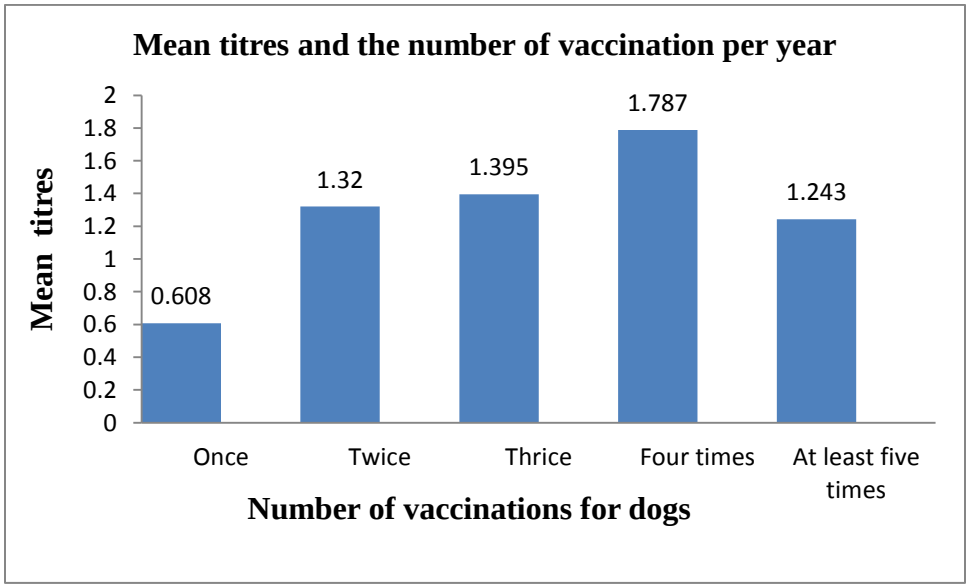

Figure 20 : Mean titres and the number of vaccinations applied

4.5.25 Age of dogs and antibody titres in vaccinated dogs

Figure 21 shows that mean titres went up by years; mean titre of dogs younger than one year (0.638) was lower than that of dogs aged between 1-2.5 years old (0.82) while that was also lower than that of dogs aged between 2.5-4 years old (1.515). Mean titre of dogs aged at least five years (1.227) was lower than that of dogs aged 2.5-4 years old; however was higher than the means titres of dogs younger than one year old (0.638) or aged between 1-2.5 years old (0.82). Out of 80 vaccinated dogs, 10% were younger than 1 year old, 32% were 1-2.5 years old while 29% were 2.5-4 years old and at least five years old for each.

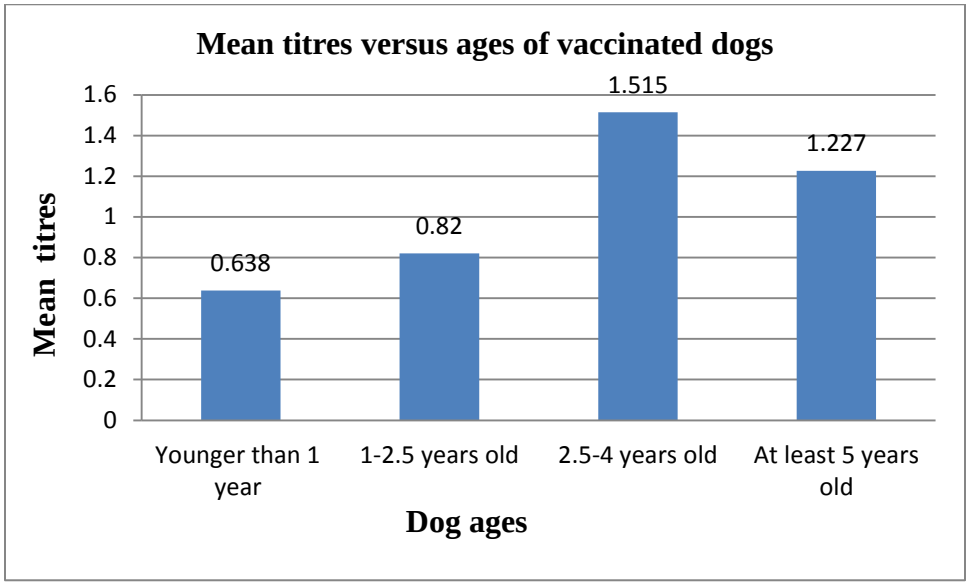

Figure 21 : Impact of ages of dogs on antibody response

CHAPTER 5: DISCUSSION

5.1 KAP survey on rabies disease and control in Rwanda

5.1.1 Knowledge about rabies disease and control

The 43.7% of the respondents who were familiar with human and canine rabies in this study, was lower than 70% of respondents reported by Sambo *et al.* (2014) in Tanzania who knew that humans and dogs can contract rabies. The respondents who were aware of feline rabies in this study (12.3%) was lower than 23.1% of respondents who knew that cats can have rabies that was reported by Guadu *et al.*(2014) in Ethiopia. A study on dog rabies vaccination conducted by Mucheru *et al*. (2014) in Kenya found that 29% of participants knew of animal rabies; this was comparable to 32.4% of respondents who knew that domestic animals can have rabies as reported in our study. Our study shows that 11.1% of respondents knew that jackals can develop rabies and this figure could be compared to the findings by Kabeta *et al.* (2015) in Ethiopia who found that 47.7% of respondents knew that wildlife species can suffer from rabies.

We found that 46.2% of respondents sourced rabies information from neighbours and parents whereas 43% acquired the information through the media and community meetings. The rest of respondents acquired rabies information from education at school (5.2%), veterinarians (4.8%), and workplace (0.8%). In a KAP study by Tschopp *et al.* (2016) involving urban and pastoralist interviewees in Eastern Ethiopia, 92.1% of urban respondents sourced the information from families while 5.8% and 1.8% sourced it from school and the media respectively.

Another KAP study about rabies conducted by Guadu *et al.*(2014) found that 86.6% of interviewees acquired rabies information from neighbours, friends and relatives while 10.7% and 2.4% sourced the information from the mass media and mixed sources, respectively.

Our study found that 74% of respondents were aware that human dog-transmitted rabies is spread through bites while 16% and 8% knew that it can be transmitted through licking wounds and skin scratching respectively. Only 1% of the respondents did not know how human dog-transmitted rabies can be spread. In a study by Jemberu *et al.* (2013) in Ethiopia, 98% of respondents were aware that transmitting rabies occur through bite while 84% thought that spreading rabies can occur through contact of saliva of rabid individual with damaged or intact skin. The same study reported that 32% thought that transmitting rabies can occur through inhalation. A KAP study about rabies conducted by Fenelon *et al.*(2017) in Haiti, showed that 73% and 20% of respondents knew that rabies can be spread through biting and coming in contact with saliva respectively.

This study showed that 85% of respondents knew of the correct ways through which animal rabies (between dogs and other animals) can be transmitted, i.e., bites (65%), licking wounds (16%) and scratching skin (4%). Of the rest of respondents, 10% believed in wrong routes through which animal rabies can be transmitted: food (7%), licking intact skin (1%), coitus (1%), and inhalation (1%) while 5% did not know about possible ways of transmitting animal rabies. In a study by Jemberu *et al.*(2013), 84% and 32% of respondents thought that rabies can be spread through exposing saliva of rabid individual to damaged or intact skin and inhalation respectively. We found that of the respondents, 99% knew at least one clinical manifestation of rabies; the most known signs were aggressiveness (27%), wandering over long distances (23%) hypersalivation (20%). In a study by Guadu *et al.*(2014) in Ethiopia, 76.8% of respondents considered biting and changing behaviors, paralysis, salivation and hydrophobia the clinical findings of rabies.

Our findings showed that 82% of respondents believed that vaccinating dogs regularly can prevent people from getting rabies while 18% thought that other prophylactic measures (killing stray dogs, educating the public, restricting dogs, vaccinating people at risk, prophylaxis undertaken before exposure) would be enough to prevent and control human rabies. A study conducted by Muriuki *et al.*(2016) in Kenya revealed that 66% of respondents knew that vaccinating dogs help to prevent and control rabies.

We found that 81% of respondents knew that vaccinating dogs regularly can prevent them from developing rabies while 19% believed in other prophylactic measures (killing stray dogs: 11%, confining dogs: 6% and castrating dogs : 2%). A study by Kabeta *et al.* (2015) in Ethiopia, found that 53.9% and 41.7% of respondents thought that confining dogs and vaccinating them could prevent them from developing rabies.

The findings of this study showed that 26% and 43% of respondents knew that clinical rabies is always a deadly disease both in dogs and humans respectively and the percentages were lower than 63% of respondents who reported that clinical rabies is invariably fatal as it was reported by Sambo *et al.*(2014).

Our study revealed that dog owners who did not get formal education or those who completed secondary school were more likely to be knowledgeable about rabies than those who finished university education. This was inconsistent with previous studies by (Sambo, 2012; Costa and Fernandes, 2016) who reported that rabies knowledge among respondents steadily went up from lower to higher education. Considering that all of our respondents kept dogs and that they mainly sourced rabies information from neighbours, we think that the respondents could gain rabies knowledge through interaction among themselves rather than going to school.

This study indicated that sufficient knowledge odds were 40% lower among male dog owners compared to female ones. A previous study by Costa and Fernandes (2016) concluded that sex did not impact on rabies knowledge among the respondents. Our study also found that owning a dog for a long time was associated with the respondents' rabies knowledge; this was in agreement with the study by Mucheru *et al.* (2014) in Kenya, who found that through keeping a dog for a long time, the respondents could acknowledge the benefits of regular vaccination of dogs.

5.1.2 Attitudes and Practices about rabies disease and control

Regardless of dog's vaccination status against rabies, 31% of the respondents did not think that dogs that bite people and animals should be retained for sometime (10 days) for confirmation as to being rabid. A KAP study on behaviors of animal bite victims by Kabeta *et al.* (2015) in Ethiopia, reported that 47.7% of respondents indicated that dogs that bit people and animals were killed after biting. This study showed that 20.4% of the respondents were aware of cleaning dog-bites wounds with water alone or with soap if it was available before taking a dog-bite victim to a health care facility. The percentage of 20.4% was lower than 43.07% of the victims who washed dog-bites wounds with soap and water before seeking medical care reported by Dhiman *et al.* (2016); however it was higher than 5% of the respondents reported by Sambo *et al.* (2014) who knew that it is important to wash dog-bites wounds before attending a health care facility. This study showed that 79% of the respondents were not aware that puppies can receive rabies vaccination within 3 months of birth and that only 21% vaccinated their dogs when they were younger than three months old. A KAP study on rabies by Ameh *et al.*(2014) in Nigeria reported that 86% of respondents were not aware of age at which dogs can receive rabies vaccination for the first time.

Our study showed that the dog owners' sex and level of education were more likely to influence their attitudes towards rabies and that all the three variables (sex, level of education and length of dog ownership) were more likely to influence the dog owners' practices towards rabies.

5.2 Quantifying rabies antibody titres in vaccinated and unvaccinated dogs

The current study reveals that 35% of dogs that received rabies vaccination did not have adequate level of protective antibodies while 53.8% of unvaccinated dogs had detectable rabies antibody titres ranging from 0.133 EU/ml to 0.238 EU/ml. The cutoff point was 0.5EU/ml (OIE, 2013a), hence they were not protected. Failing of rabies vaccination in animals might be higher, but a study conducted on dogs received rabies vaccination in Nigeria rated it to 0.025% (Oboegbulem *et al.,*1987; Tepsumethanon *et al.,* 2016).

This study showed that 65% of the vaccinated dogs were protected while 34% were not and 1% did not sero-convert and were at risk of developing rabies. Thirty-five percent (35%) that did not have adequate level of protective antibodies were higher than 4.62% and 30% detected in vaccinated pet dogs and were inadequately protected against rabies that were reported by Ondrejková *et al.* (2015) and by Fernandes *et al.* (2017).

Probably interval between vaccination and sampling time could justify the higher percentage of vaccinated dogs that had inadequate level of protective antibodies recorded in the current study. We took dog blood samples from one up to twelve month post-vaccination, while Ondrejková *et al.* (2015) collected dog blood samples on the 30th day following rabies vaccination. This study shows that 53.8% (n=13) of non-vaccinated pet dogs had measurable antibodies though were considered insufficient sero-conversion; the percentage was higher than 7.4% (n=576) and 13.04% of pet dogs that did not receive rabies vaccination and had measurable rabies antibody titres as reported by Cleaveland *et al.* (1999) and Ondrejková *et al.* (2015).

Prager *et al.* (2012) reported that occurrence of rabies virus antibodies in healthy animals, but it was not ascertained whether such animals produced the antibodies after having an abortive infection or surviving from the disease. According to El-Sayed (2018), non-encephalic rabies strains may cause abortive animal and human rabies which does not leave any health abnormalities.

5.3 Factors affecting response to anti-rabies vaccination in dogs in Rwanda

In a study by Berndtsson *et al.* (2011) time between vaccination and sampling, age at vaccination, size of dog breed, number of antirabies vaccinations and administered vaccine brand were found to be factors influencing success of rabies vaccination. According to Kennedy *et al.* (2007) size, age and breed of an animal as well as sampling time and vaccine brands were important to antibody response in dogs received rabies vaccination.

In the current study, 100% of the dogs tested negative for haemoparasites; while the prevalence of gastrointestinal worms was 39.8%; it was higher than 5.9% reported by Pullola *et al.* (2006) in Finland, but was lower than 78.1% and 75.26% found by (Getahun and Addis, 2012; Abere *et al.*, 2013) in Ethiopia.

Ancylostoma spp (32.3%) was the predominant species, followed by tapeworms (6.5%) and a mixed infection by *Ancylostoma spp* and *Toxocara canis* (1%). Occurrence of *Ancylostoma spp* (32.2%) reported in the current study was comparable to 31.9% found by Onyeabor (2014) in Nigeria; but this prevalence was lower than 71.4% reported by Ngui *et al.*(2014) in Malaysia. Possibly, husbandry practices, namely deworming status of dogs, controlling dog movements could have influenced the prevalence reported in our study. The occurrence of tapeworms (6.4%) found in this study lower than 33% (*Taenia spp*) reported by Minnaar *et al.*(2002) in South Africa; however it was higher than 0.4% found by Swai *et al.*(2010) in Tanzania.

Changes in prevalences of tapeworms could be related to methods of sampling and identification. We investigated live dogs and detected tapeworms and proglottids with the naked eye while Minnaar *et al.*(2002) sampled euthanized dogs and utilised adhesive tape swabs on perianal site to recover tapeworms eggs and segments.

We detected *Ancylostoma caninum* and *Toxocara canis* at the rate of 1% and this can be compared to 7.5% of *Toxocara spp* found by Alvåsen *et al.* (2016) in Malawi. We found that haematocrit (r= 0.263, *p*= 0.011) and Mean Corpuscular Volume (r= 0. 207, *p*=0. 046) correlated positively with helminthiasis although it was low. Mean corpuscular volume is key index for erythrocyte estimation and is used to describe anemia in dogs (Cowell, 2004).

This study showed that EPG range was not associated with antibody response in dogs infected with gastrointestinal helminths; the lowest geometric mean titre (0.318) was yielded by the third higher EPG (1000-2500). Our study shows that deworming status matched with antibody response; dewormed dogs yielded a geometric mean titre of 1.094 and it was higher than 0.633 recorded in non-dewormed dogs.

The results of the current study indicated that the status of vaccination influenced production of antibodies and the average number of rabies antibodies recorded per vaccinated dogs (11.776059735) was eight times than that recorded in unvaccinated dogs (1.41579378). Dog ages was associated with antibody response; the titres were as follow : dogs younger than one year (0.638), dogs aged between 1-2.5 years old (0.82), dogs aged between 2.5-4 years old (1.515), dogs aged at least five years (1.227). This study indicates that time of sampling impacted on antibody titres, i.e., the titres were 1.559, 0.949 and 0.934 in dogs sampled 1-5 months, dogs sampled 6-9 months and in dogs 10-12 months after vaccination respectively.

We found that regardless of the vaccine brands used, 98% of the vaccinated dogs produced antibodies against rabies; however not all achieved protective levels. The geometric mean per vaccine brand were as follows - 2.115 (Nobivac Rabies), 1.850 (Vaxipet R), 1.261 (Vaxipet DHPPi+LR), 0.897 (Rabies Veterinary Vaccine Inactivated B.P. VET.) vaccine A), and 0.814 (Rabisin). The geometric mean titres showed that number of vaccinations influenced antibody response, i.e., the titres per number of vaccination were as follows - 0.608 (dogs vaccinated once), 1.320 (dogs vaccinated twice), 1.395(dogs vaccinated thrice), 1.787 (dogs vaccinated four times), 1.234 (dogs vaccinated at least five times). Correlation between number of vaccinations against rabies for dogs, r=0.255, p=0.013 was statistically significant.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

1. This study exposed gaps in rabies knowledge regarding susceptibility, transmission, control and treatment,
2. None of respondents' sex, level of education, and length of dog ownership was statistically associated with their rabies knowledge, attitudes or practices,
3. Dogs produced rabies antibodies irrespective of vaccine brand used,
4. Sixty-five percent (65%) of all the vaccinated dogs had protective antibody levels, whereas 35% of them did not have protective antibody levels indicating poor vaccination response and high vulnerability to rabies infection.

6.2 Recommendations

Based on the findings of the present study we would recommend the followings:

1. Strengthening awareness of rabies disease among the people of Rwanda through the mass media and public meetings where veterinarians should participate in disseminating rabies information
2. Studies be carried out to determine why some dogs were poor responders to rabies vaccination and find ways to remedy the situation
3. Dogs should be dewormed before receiving rabies vaccination and regularly thereafter.

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APPENDICES

Appendix 1 : Survey questionnaire

SURVEY QUESTIONNAIRE FOR DOG OWNERS ON ASSESSMENT OF KNOWLEDGE, ATTITUDES, PRACTICES ON RABIES DISEASE AND CONTROL IN RWANDA

Interview date:…...../……../2016

Phone cell: …….…….……

Dog owner ID: Code:……...

I) DOG OWNER PARTICULARS

1. District…… Sector……..... …….

Cell……...……Village…….……..

2. GPS reading: (1) land mark number :……(2) Longitude……

(3) Latitude…… (4) Elevations……

3. Sex: (1) Male ☐ (2) Female ☐

4. Education level: (1) No formal education ☐ (2) Primary ☐ (3) Secondary ☐ (4) Tertiary ☐

II) DOG DATA

5. How many dogs do you keep?

6. If you own more dogs; chose one dog to be sampled randomly

7. Sex: (1) Male ☐ (2) Female ☐

8. Age: (1) <6 months ☐ (2) 6-11.99 months ☐ (3) 1-2.49 years ☐ (4) 2.5-4.99 years ☐ (5) ≥ 5 years ☐

9. Breed: (1) Local ☐ (2) Cross ☐ (3) Pure ☐ ,specify……

10. Body condition score: (1) emaciated ☐ (2) thin ☐ (3) moderate ☐ (4) stout ☐ (5) obese ☐

III) DOG KEEPING

11. How long have you been keeping dog (s)? ***Tick one*** (1) < 5 years ☐ (2) 5-10 years ☐ (3) >10 years ☐

12. Why do you keep dog (s)? ***Tick one*** (1) Security ☐ (2) pet ☐ (3) herding (4) accidentally ☐

13. How do you control dog (s) movement? ***Tick appropriately***

(1) The dog lives freely in a fenced place ☐ (2) The dog lives in a kennel within fenced place and is free during night ☐ (3) There is no fence and kennel, and the dog lives at liberty ☐ (4) There is no fence and kennel, but the dog is kept on a leash during daytime ☐

14. What kind of diet do you give dog (s)? ***Tick appropriately***

(1) Commercial dry food ☐ (2) Commercial canned wet food ☐ (3) Raw diet (raw meat + mixed with bones) ☐ (4) Home cooked food ☐ (5) Remnant from human food at home ☐ (6) Relics from restaurant ☐ (7) Other ☐, specify……

IV) HEALTH MANAGEMENT

15. Do you deworm your dog (s)? (1) Yes ☐ (2) No ☐

16. If yes, how often? ***Tick one*** **(**1) every 3 months ☐ (2) semiannual ☐ (3) annual ☐ (4) irregular ☐ (5) every month ☐ (6) Every 4 months ☐ (7) every 2 months ☐

17. When was the last date of deworming? ……...../……../20……

18. Do you apply ectoparasiticide (ticks, lice, fleas, and keds) on your dog (s)?

(1) Yes ☐ (2) No ☐

19. If yes, how often do you apply it (them)? ***Tick one***

(1) Thrice a week ☐ (2) twice a week ☐ (3) once a week ☐ (4) once a month ☐ (5) once ☐ two months ☐ (6) every three months ☐ (7) Irregularly ☐ (8) thrice a month ☐ (9) twice a month ☐

V) KNOWLEDGE ABOUT RABIES

Q1. Have you heard about rabies disease? (1) Yes ☐ (2) No ☐

Q2. If yes, how did you get information about rabies disease? ***Tick as appropriate***

(1) On the radio ☐ (2) Reading of hard or online newspapers, books, etc ☐ (3) Public meeting ☐ (4) neighbours ☐ (5) Parents ☐ (6) via veterinarians ☐ (7) schooling ☐

(8) Other, ☐ specify

Q3. Do you know susceptible hosts to rabies? (1) Yes ☐ (2) No ☐

Q4. Which of the following hosts can suffer from rabies? ***Tick appropriately***

(1) Dogs ☐ (2) cat ☐ (3) cows ☐ (4) sheep ☐ (5) goat ☐ (6) pigs ☐ (7) rabbits ☐ (8) People ☐ (9) Jackal ☐ (10) Other, specify….

Q5. How rabies can be transmitted between dogs and other animals? ***Tick as appropriate***

(1) Bite ☐ (2) licking of wound ☐ (3) skin scratch ☐ (4) food ☐ (5) licking of intact skin ☐ (6) other, ☐ specify,

Q6. How rabies can be transmitted from dogs to humans? ***Tick as appropriate***

(1) Bite ☐ (2) licking of wound ☐ (3) licking of intact skin ☐ (4) skin scratch ☐ (5) do not know ☐

Q7. Do you know clinical signs of rabies in dogs? (1) Yes ☐ (2) No ☐

Q8. Which of the following clinical signs are seen in dogs with rabies? ***Tick as appropriate***

(1) Aggressiveness (biting without any provocation) ☐ (2) Profuse salivation ☐ (3) Pica ☐ (e.g., sticks, nails, faeces, etc) ☐ (4) Difficulty in swallowing ☐ (5) Roaming over long distances (running for no apparent reason) ☐ (6) Change in sound (e.g., hoarse barking or inability to make sound) ☐ (7) Dropping of the jaw ☐ (8) Other, ☐ specify 9) do not know ☐

Q9. What is the prognosis for rabies in dogs showing clinical signs? ***Tick one***

(1) They can be treated successfully ☐ (2) they always die ☐ (3) do not know ☐

Q10. What is the prognosis for dog mediated rabies in people showing clinical signs? ***Tick one***

(1) They can be treated successfully ☐ (2) they always die ☐ (3) do not know ☐

Q11. What is the most effective method for rabies control in dogs? ***Tick one***

(1) Killing of stray dogs ☐ (2) restriction of dog movements ☐ (3) regular vaccination ☐ (4) castration ☐

VI. ATTITUDES TOWARDS RABIES

Q12. If a dog bites a man, what would you wish to happen to the biting dog if is caught? ***Tick one***

(1) Immediate release if the owner is known and the dog is vaccinated ☐

(2) kill the dog directly if the owner and vaccination status are unkown ☐

(3) Keep the dog for 10 days to see if is rabid; regardless of its vaccination status ☐

Q13. If your colleague is bitten by a dog, what can you do before you take him to a health care facility? ***Tick one***

(1) Careful wash of the wound with water alone or with soap if available ☐ (2) Covering the ☐ wound with dressings and bandages (3) Apply the salt to the wound ☐ (4) Take the patient to a health care without doing anything ☐ (5) Apply 70 % alcohol to the wound ☐ (6) apply other type antiseptics to the wound ☐, specify…..

Q14. In your thinking, how best dog-mediated rabies can be controlled in humans? ***Tick one*** (1) Regular vaccination of dogs ☐ (2) Complete restriction of dogs ☐ (3) Education of the public ☐ (4) Killing stray dogs ☐ (5) regular vaccination of people at risk (e.g., veterinarians) ☐ (6) Post-exposure prophylaxis ☐

(VII) PRACTICES ABOUT RABIES

Q15. Do you bring your dog (s) to vaccination against rabies? (1) Yes ☐ (2) No ☐

Q16. How old was your dog at first vaccination?

(1) Little than or three month old ☐ (2) older than three months ☐ (3) do not know ☐

Q17. How often do you bring your dog (s) to vaccination? ***Tick one***

(1) Twice a year ☐ (2) once a year ☐ (3) irregular ☐ (4) unvaccinated dog ☐

Q18. How often have your dogs been vaccinated? (Number of vaccinations) ***Tick one***

(1) Once ☐ (2) twice ☐ (3) thrice ☐ (4) four times ☐ (5) five times ☐ (6) over 5 times ☐

Q19. How do you proceed to have your dog (s) vaccinated? ***Tick one***

(1) Take a dog to a site during vaccination campain ☐ (2) veterinarian comes at home ☐ (3) both approaches ☐ (4) Take a dog to a veterinary clinic ☐

Q20. Who vaccinates your dog (s)? ***Tick one***

(1) Private veterinarians ☐ (2) public veterinarians ☐

Q21. What vaccine brand was used last time? ***Tick one***

(1) Rabies Veterinary Vaccine Inactivated B.P. (VET.) ☐ (2) VAXIPET R ☐ (3) CANVAC®R ☐ (4) VAXIPET DHPPi+LR ☐ (5) RABISIN ☐ (6) NOBIVAC RABIES ☐

Q22. How a veterinarian who vaccinated your dog last time did carry the vaccine? ***Tick one***

(1) In a cooler box ☐ (2) on ice without cooler box (e.g., in a plastic bag) ☐

(3) The vaccine was purchased and carried on ice by "the dog owner" and then administered by a veterinarian ☐

Q23. How much did you pay to have your dog vaccinated last time? ***Tick one***

(1) 1- 1, 500 FRW ☐ (2) 1,501-3,000 FRW ☐ (3) 3,001 – 5,000 FRW ☐ (4) 5,001 - 10,000 FRW ☐ (5) 10,001 – 20,000 FRW ☐ (6) 20,001-30,000 FRW ☐ (7) free of charge ☐

Q24. What do you think of the vaccination fees? ***Tick one***

(1) Too little money ☐ (2) too much money ☐ (3) affordable ☐

Q25. Why have you not taken your dog (s) to vaccination? "Owners of unvaccinated dogs"- Tick *as appropriate*

(1) Lack of information ☐ (2) lack of knowledge on rabies ☐ (3) difficulty in catching dogs ☐

(4) too much vaccination fees ☐ (5) Sites of vaccination set far during vaccination campaign

(6) Negligence ☐

Appendix 2 : Brands of rabies vaccines used in animals that were on Rwandan market

i) Rabies Veterinary Vaccine Inactivated B.P. (Vet.) (Indian immunological limited, India). Each dose (1 ml) comprises killed Rabies viral antigen with a strength ≥ 1 IU per dose and aluminium hydroxide gel as an adjuvant. Thiomersal 0.01% w/v added as preservative. The vaccine contains cultured rabies virus produced in BHK 21 cell line, originated from Baby Hamster Kidney cells and inactivated with aziridine compound. It is manufactured by Indian Immunologicals Limited for Kenya Veterinary Vaccines Production Institute.

ii) Vaxipet R (inactivated vaccine against rabies) (Laprovet, France): it contains:

Virus rabiei inactivated, SAD Vnukovo-32 strain…… min. 2 IU

Excipient ……..……s.q.f 1 ml

iii) Vaxipet DHPPi+LR are polyvalent vaccine (Laprovet, France)

DHPPi component is freeze-dried containing:

Virus febris contagiosae canis …….min. $10^{3.0}$ TCID50, max. $10^{4.5}$ $TCID_{50}$

Virus laryngotracheitidis contagiosae canis……. min. $10^{3.5}$ TCID50, max. $10^{4.5}$ $TCID_{50}$

Parvovirus enteritidis canis……min. $10^{4.5}$ TCID50, max. $10^{5.5}$ $TCID_{50}$

Virus parainfluensis canis …… min. $10^{3.0}$ TCID50, max. $10^{4.2}$ $TCID_{50}$

Excipient …….s.q.f. 1 dose (1 ml)

LR component, in suspension:

Virus rabiei inactivated, SAD Vnukovo-32 strain……..min. 2 I.U.

Leptospira icterohaemorrhagiae inactivated……min. titre 32 defined MAT*

Leptospira canicola inactivated……... min. titre 32 defined MAT*

Leptospira grippotyphosa inactivated ……. min. titre 32 defined MAT*

Excipient……..s.q.f. 1 dose of 1 ml

*geometric mean of titres of specific antibodies defined by microagglutination test

iv) Nobivac® Rabies (Intervet India Pvt. Ltd):

Each dose contains Rabies strain Pasteur RIV inducing more than 2 I.U. in the mouse potency test. The virus is grown on the BHK-21 clone CT cell line inactivated with ß -propiolactone and adsorbed on aluminium phosphate.

v) Rabisin (Merial, France): a one ml dose contains:

Active substance……..… Inactivated rabies virus, strain G52 ≥ 1 IU*

Excipient: …….Aluminum (as hydroxide) 1.7 mg.

* Minimum titles in accordance with the requirements of the European Pharmacopoeia.

Appendix 3 : Composition of the Platelia™ Rabies II ELISA kit

Labeling	Type of reagent
R1	Microplate: 12 strips of 8 wells sensitized with the rabies virus glycoprotein
R2	Wash solution: 10 fold concentrated Tris NaCl buffer Preservative: ProClin ^{TM} 300 (0.01%)
R3	Negative control: Non-reactive control TRIS-EDTA Preservative: ProClin™ 300 (0.1%)
R4a	0, 5 EU/ml Positive control: 0.5 EU/ml calibrated positive control. Glycine buffer Containing BSA and dog serum with anti- rabies IgG; yellow coloured. Preservative: ProClin™ 300 (0.1%)
R4b	4 EU/ ml Positive control: 4 EU/ml calibrated positive controls. Glycine buffer containing BSA and dog serum with anti- rabies IgG, blue coloured. Preservative: ProClin™ 300 (0.1%)
R6	Sample diluent: Ready-to use TRIS - EDTA buffer for sample dilution; red coloured. Preservative: ProClin™ 300 (0.1%).
R7	Conjugate: Solution containing Protein A-Peroxidase and purified bovine protein; Concentrated 10 times and green coloured. Preservative: ProClin™ 300 (0.1%).
R8	Peroxidase substrate buffer: Solution of citric acid and sodium acetate containing 0.015% H_2O_2 and 4% dimethylsulfoxide (DMSO)
R9	Chromogen: Tetramethylbenzidine (TMB) solution 0.25%
R10	Stop solution: 1 N sulphuric acid solution

Appendix 4 : Quantification of antibody titres with spectrophotometer at 450 nm