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Using a One Health Approach for the Assessment of Rabies Control in Rural Victoria Falls, Zimbabwe

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Abstract

In rural Victoria Falls, Zimbabwe, rabies vaccinations in dogs are conducted annually but it is unknown whether dogs in this area have adequate protection to prevent an outbreak. A One Health approach was used to evaluate the rabies immune status of rural dogs and collect information about rabies knowledge, attitudes, and practices among dog owners. In 2020, blood was collected from dogs and a survey was implemented. A BioPro Rabies ELISA Ab kit was used to test sera, with a percentage of blocking ≥70% classified as having protective rabies antibodies (PrAbs). At the time of sampling, 32.1% (160/498) of dogs had PrAbs. The proportion of dogs with PrAbs decreased with time since vaccination, with <1 year (87.5%), 1-2 years (45.1%) and >2 years (31.2%). Only 3.8% of respondents would wash the wound with soap and water; the recommended first step in post-bite care. Vaccinations should be increased in this area.

Keywords

One Health, rabies, dogs, BioPro Rabies ELISA Ab Kit, rural areas, Victoria Falls, Zimbabwe

Summary for Lay Audience

Rabies is a fatal viral disease, distributed globally, and transmitted to people primarily through dog bites. Rabies is widespread in Zimbabwe, with human fatalities reported annually in the country. To supplement the Government's effort on rabies control, Victoria Falls Wildlife Trust and Veterinarians for Animal Welfare Zimbabwe have joined forces to carry out dog rabies vaccinations in rural communities adjacent to the major tourist attraction of The Victoria Falls. This thesis uses a One Health approach, to address the challenges rabies poses to dogs, people, and wildlife in the specific ecosystem of Victoria Falls, Zimbabwe. The research project aimed to determine the rabies immune status among dogs and collect information about knowledge, attitudes, and practices (KAP) to better understand behaviours and interactions between dogs, people, and wildlife in rural Victoria Falls (VF). In 2020, as part of this masters' project, blood samples were obtained from dogs and a survey was implemented among dog owners in rural VF. Rabies antibodies were evaluated for 498 dogs, with a percentage of blocking ≥70% classified as having protective rabies antibodies (PrAbs). In total, 500 surveys were completed by 342 dog owners. At time of sampling in 2020, 32.1% (160/498) of dogs had PrAbs. Among previously vaccinated dogs, 44.8% (133/297) had PrAbs. The proportion of dogs with PrAbs decreased with time since vaccination, with <1 year (87.5%), 1-2 years (45.1%) and >2 years (31.2%). Prior rabies vaccination, body condition and herding cattle were factors associated with having PrAbs. KAP responses from dog owners indicated that most respondents knew that rabies is fatal to people (91.8%) and knew where to get post-exposure treatment for rabies (90.6%). Although most (87.4%) dog owners would seek medical attention if bitten by a dog, only 3.8% would wash the wound with soap and water, the recommended first-step for post-bite treatment. Evaluating the rabies antibody levels in the dog population is important to guide the process to re-vaccinate these animals. Rabies vaccinations efforts in dogs should be increased in this area to protect and prevent rabies in dogs and thus prevent human infection and deaths.

Co-Authorship Statement

For Chapter 2 of this thesis, research collaborators involved in the study contributed to the field and laboratory work components of the project. Following is the name of the collaborator, their duty and role in this research project. Jessica Dawson, the in-field project manager, lead, and coordinate, oversaw all activities conducted in the field in Zimbabwe, including the implementation of the survey, including collection of consent from dog's owners and activities related to dog's vaccinations and blood samples. Dr. Chris Foggin and Dr. Isaac Moyo, Field Veterinarians, supervised all field activities related to dog vaccinations. Included but not limited to taking blood samples for serology testing, determining vaccination status, carry out physical and clinical assessments. Dr. Moyo surveyed study participants (dog owners), assisted in data collection and entry. Dr. Claude Sabeta, virologist, oversaw the laboratory analysis of the dog sera samples. Ryan LaPenna conducted all epidemiological analyses of these data and wrote up results in a format suitable for peer-review publication.

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Dr. Moyo helped in the collection and entry of the paper surveys, and cleaning of the data, prepping it for analysis. Ms. Dawson and Dr. Foggin helped in the design of this study and provided great resources about the local area and the ongoing rabies control efforts. Dr. Foggin and Dr. Moyo provided great insights into the "veterinary world" of rural Victoria Falls, Zimbabwe through the exchange of emails, pictures, and documents. I am thankful for Dr. Sabeta and his lab's diligent work on analyzing the dog sera samples and presentation of the serology data. I am thankful for the comments and critique provided by our collaborators.

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1 Background

1.1 Rabies and One Health

Rabies is an acute progressive encephalitis caused by neurotropic RNA viruses from the genus *Lyssavirus*. Rabies is spread to humans primarily through bites or scratches of infected animals, usually via saliva (Fooks et al., 2014). Rabies is one of the oldest recognized zoonoses (diseases that can be transmitted between other animals and humans). Rabies has been known to humankind for at least 4000 years. The Sumerian Laws of Esnunna, Northern Babylonia dated 1930 BCE, recounts the earliest written record of rabies death in dogs and humans, where it is described, that owners must pay a fine if their dog contracts rabies and bites another person and causes their death (Tarantola, 2017; Yuhong, 2001).

Rabies has the highest case fatality rate of any infectious disease (Fooks et al., 2014). Once an individual shows clinical signs or symptoms, the disease is almost always fatal (> 99% fatality) (Jackson, 2018b). However, rabies is a 100% preventable disease (Fooks et al., 2014). In humans, rabies can be prevented through immunization (vaccines), rabies elimination in animal reservoirs (such as dogs), and increased awareness of rabies enabling prevention (Wandeler et al., 2013; World Health Organization, 2018). People can receive the rabies vaccine prior to exposure (pre-exposure prophylaxis, PrEP) or after exposure (post-exposure prophylaxis, PEP) (World Health Organization, 2018). Globally, PrEP is uncommon, given only to individuals in high-risk occupations who frequently come into close contact with potential rabies reservoirs or its vectors (Wandeler et al., 2013). PEP, the recommended treatment for a bite victim following rabies exposure, prevents virus entry into the nervous system and subsequent death (Wandeler et al., 2013). PEP consists of 1) extensive wound washing for at least 15 minutes with soap and water, 2) prompt and timely administration of a series of human rabies vaccines, and 3) if needed, the infiltration of rabies immunoglobulin into bite wounds (World Health Organization, 2018). Vaccinations of dogs is the most cost-effective way to achieve reduction in the number of human deaths (Cleaveland et al., 2006; Lembo et al., 2010). Numerous studies have confirmed that vaccinating dogs against rabies reduces the number of human deaths from dog-mediated rabies and, thus, the need for PEP for bite victims (Cleaveland et al., 2003; Kaare et al., 2009; Zinsstag et al., 2017) . Education on dog behaviour and bite prevention can reduce the incidence of rabies in people and the financial burden associated with post-bite treatment (World Health Organization, 2018). Community engagement is important to disseminate information concerning rabies prevention and control measures, including how to prevent dog bites and what to do after a bite (World Health Organization, 2018).

Despite the existence of an effective and safe vaccine, rabies continues to cause unnecessary suffering and deaths in animals and humans. Rabies is seriously underreported and remains one of the most neglected of diseases (World Health Organization, 2018). According to the World Health Organization (WHO), rabies causes 59,000 human deaths every year, with 40% of victims being children <15 years old (Hampson et al., 2015). Rabies is found on every continent except for Antarctica, with 95% human rabies deaths occurring in Asia and Africa (World Health Organization, 2018). Approximately, 80% of all human cases occur in rural areas, where there is limited or no access to the human rabies vaccine (Singh et al., 2017; World Health Organization, 2018).

Globally, dogs are the most common source for human exposure and account for up to 99% of all human rabies transmissions (World Health Organization, 2018). The implementation of mass dog vaccination (MDV) has successfully eliminated the disease in dogs in many countries, thereby leading to a reduction in human deaths (Cleaveland et al., 2006; Lembo et al., 2010). It has been shown that at least 70% of the dog population needs to be immunized to prevent outbreaks of rabies (Coleman & Dye, 1996). MDVs, together with community awareness of rabies and accessibility to PEP, are the cornerstones of the internationally led “Zero by 30” rabies elimination strategy, that uses a One Health approach and brings together multiple stakeholders to achieve the target goal of zero human deaths from dog-mediated rabies by 2030 (World Health Organization et al., 2019). According to the United States Centre for Disease Control and Prevention (CDC), “One Health is a collaborative, multisectoral, and transdisciplinary approach—working at the local, regional, national, and global levels—with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment” (*One Health | CDC*, 2022). In 2021, a newly formed operational definition of One Health was developed by the One Health High-Level Expert Panel (OHHLEP), that states: “One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent. The approach mobilizes multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems, while addressing the collective need for clean water, energy and air, safe and nutritious food, taking action on climate change, and contributing to sustainable development" (*Joint Tripartite and UNEP Statement on Definition of "One Health,"* 2021).

The rationale for using a One Health approach for rabies stems from the fact that this zoonosis can be transmitted across the animal-human interface, and rabies prevention is influenced by socio-cultural and economic factors. Thus, it is essential that human and animal health sectors, government, non-governmental organizations, and communities work in close collaboration to achieve rabies control and elimination. It is based on this rationale that the use of the One Health approach for rabies is at the core of this thesis work.

1.2 Rabies virus

Lyssaviruses are the causative agent of rabies. The genus *Lyssavirus* (Family *Rhabdoviridae*) currently consist of 17 viral species and one putative species. All lyssaviruses share a similar morphology and genome structure. Lyssaviruses have an unsegmented negative strand single-stranded RNA genome, with a length of approximately 12,000 nucleotides that is encapsulated in a bullet-shaped envelope. The genome encodes five structural proteins (3′-N-P-M-G-L-5′). The virion envelope is comprised of the membrane matrix protein (M) and glycoprotein (G) molecules. The G protein forms spikes that cover the surface and is the primary antigenic determinant. The viral RNA is tightly associated the nucleoprotein (N) and, together with the phosphoprotein (P) and the polymerase (L), comprises the ribonucleoprotein (RNP) complex which can start transcription and replication (Rupprecht et al., 2017; Scott & Nel, 2021).

The lyssavirus of greatest public health and animal health concern is the rabies virus. All mammals are susceptible to rabies virus infection, but only a few species (particularly carnivores and bats) are recognized as reservoirs for the disease and play an important role in the persistence of rabies. Two epidemiological cycles have been described for the maintenance of rabies infection. The urban cycle is maintained by domestic dogs, who act as the reservoir. The urban cycle predominates in areas of Africa, Asia, and Central and South America. The sylvatic (wildlife) cycle involves a variety of terrestrial mammals who can maintain rabies virus transmissions. The sylvatic cycle is the predominant cycle in the Northern Hemisphere. There are areas where both the urban and sylvatic cycles are present (Rupprecht et al., 2017; Scott & Nel, 2021).

1.3 The course of a rabies infection

1.3.1 Transmission

Rabies virus is transmitted primarily via bites from a rabid animal, which sheds infectious virus from the saliva of the infected animal into the person being bitten. Non-bite transmission can also occur if infected saliva or other potentially infectious material (i.e. nervous system tissue) enters open wounds, cuts, or mucosal membranes such as eyes, nose, or the mouth. However, these non-bite or scratch mode of transmission are uncommon (*Rabies - Nervous System*, 2020; Wandeler et al., 2013).

1.3.2 Pathobiology

Virus inoculation occurs following a bite (entry site) from a rabid animal, where a break in the skin allows the virus-laden saliva to be delivered into the muscle tissue. To access the peripheral nervous system, the virus enters via a motor neuron through the neuromuscular junction (NMJ), or via a sensory nerve through nerve spindles. The nicotinic acetylcholine receptor (nAChR), the neuronal cell adhesion molecule (NCAM) and the p75 neurotrophin receptor (p75NTR) are considered as rabies virus receptors as these receptors have been shown to bind to the rabies virus glycoprotein and/or be involved in viral entry (Lentz et al., 1983; Thoulouze et al., 1998; Tuffereau et al., 1998). To enter the nervous system, rabies virus can directly bind to neuronal receptors (NCAM and p75NTR) present on motor neurons. Alternatively, prior to entry and infection of motor neurons at the NMJ, virus can bind to nAChR present on muscle cells. Viral entry of muscle cells would lead to local replication, infection and budding into the presynaptic cleft. In turn, this could concentrate extracellular virus for presentation to NCAM and p75NTR on the motor neuron. However, it remains unclear whether one or more of these receptor-mediated pathways occur (Davis et al., 2015; Lafon, 2005).The transport of the rabies virus from the peripheral to the central nervous system relies on microtubule dependent retrograde fast axonal transport (Gillet et al., 1986). The time from exposure to the first appearance of clinical signs of rabies is termed the incubation period. Most incubation periods of dogs in experimental conditions are between 3 and 6 weeks (Wandeler et al., 2013). The incubation period in humans is usually 2-3 months, but it can be highly variable and can range from a week to years (World Health Organization, 2018). The variability of the incubation period is due to the many factors, such as the site of exposure, rabies virus variant, or host immune status. The virus replicates and spreads from neuron to neuron and continues this progression to the central nervous system and brain. In the central nervous system, retrograde axonal transport continues but is now facilitated by metabotropic glutamate receptor subtype 2, an abundant cellular entry receptor in the central nervous system (Wang et al., 2018). Viral replication in the central nervous system (CNS) allows centrifugal spread to peripheral organs, including the salivary glands, skin, cornea, and other organs (Murphy et al., 1973). Viral spread along nerves (via anterograde transport) to salivary glands leads to viral excretion in the saliva for transmission to a new host. The WHO recommends an observational period of 10 days for clinically healthy dogs that have bitten a person, to rule out rabies infection (Rupprecht et al., 2017; Scott & Nel, 2021; Wandeler et al., 2013).

1.4 Rabies serology

1.4.1 Serology as a tool to measure the immune response to rabies

Both the cellular and humoral arms of the immune system are involved in the rabies immune response. The humoral immune response is commonly assessed by serology. Serology is the study of blood serum and other bodily fluids, in people or other animals, to investigate the presence of antibodies (S. Moore & Gordon, 2020). Antibodies, also known as immunoglobulins, are proteins secreted by plasma cells that can become activated in response to antigens on microorganisms, such as viruses, to protect the host. In addition to the primary role of antibodies, which is to bind to antigens, antibodies can also elicit secondary or effector functions (Sundberg & Mariuzza, 2002). Effector functions of antibodies include neutralization, opsonization, complement activation, and antibody dependent cellular cytotoxicity (ADCC) (Forthal, 2014). Neutralization of pathogens occurs when antibodies bind to and inactivate surface proteins used for attachment or entry into cells, thereby preventing the pathogens from infecting cells. These antibodies are called neutralizing antibodies. Opsonization is the process whereby a pathogen tagged by antibodies induces phagocytosis of the organism through Fc receptor binding on immune cells, such as macrophages (Bournazos et al., 2020). The complement system (classical pathway) can become activated by antigen-antibody complexes. Downstream of this pathway are other immune effector actions, such as initiating the formation of the membrane attack complex which destroys the pathogen by forming pores in the microbial cell membrane (Dunkelberger & Song, 2010). ADCC is a cellular immune process whereby an Fc receptor bearing effector cells lyses and destroys antibody-coated target cells. Not all antibodies have effector functions, and sometimes antibody-antigen binding may not lead to a biological effector because they are not effective in eliciting an effector function. Whether antibodies can elicit an effector function/action depends upon the individual characteristics of a specific antibody structure such as the class, subclass, or variable region of the antibody (S. Moore & Gordon, 2020). Exposure to an antigen will lead to the activation of multiple immune cell clones and the production of a polyclonal antibody response. As the course of the immune response develops, affinity maturation occurs, resulting in antibodies with increased affinity being produced (Mishra & Mariuzza, 2018; S. Moore & Gordon, 2020; Rajewsky, 1996).

RABV-specific antibodies are produced by the immune system in response to infection or vaccination. It has been shown that protection against rabies is largely dependent on the presence of RABV-neutralizing antibodies (RVNA) against the viral glycoprotein (Aubert, 1992; Hooper et al., 1998, 2009; Katz et al., 2017). In humans, the main isotype of immunoglobulin induced by the rabies vaccine is IgG, with the activities of IgG antibodies dependent on both the IgG subclass (four subclasses found in humans: IgG1-4) and Fc glycosylation. (Arnold et al., 2007; Katz et al., 2017). A study reported a difference in glycosylation profiles on IgG1 antibodies between neutralizing and non-neutralizing IgG1 antibodies in human sera from vaccinated individuals, suggesting that glycosylation plays an important role in antibody neutralization activity (Koike et al., 2021).

Virus neutralization assays are the “gold standard” method for the detection and analysis of RVNA, which includes the fluorescent antibody virus neutralization test (FAVN) and the rapid fluorescent foci inhibition test (RFFIT). A variety of research areas, including studies on rabies pathogenesis, immunity, vaccine development, and routine immunity monitoring of rabies vaccinated individuals, rely on these gold standard RVNA assay tests. Although these tests will remain the gold standard, there is a need for additional, less costly methods that can be used in resource poor areas, especially lower resourced areas where rabies is endemic, if the global initiative to eliminate human deaths caused by dog-mediated rabies by 2030 is to be achieved (S. Moore & Gordon, 2020).

1.4.2 Rabies ELISA serology

Enzyme-linked immunosorbent assays (ELISA) and virus-neutralization assays (VNA) represent the most common methods used for the detection of rabies-specific antibodies. Both the rapid fluorescent foci inhibition test (RFFIT) and fluorescent antibody virus neutralization test (FAVN) rely on the same principle, in which antibody concentration is measured by the ability of sera to neutralize a rabies challenge virus, visualized by the reduction in fluorescence (Cliquet et al., 1998). In comparison, ELISA-based tests rely on the interaction between antigen and antibody, regardless of the ability of the antibodies to neutralize virus. Thus, ELISA-based tests measure the binding of rabies-specific antibodies. In recognition that virus neutralization assays are expensive, use live rabies virus, requires extensive training of personal, and are difficult to access to lower resourced areas (Rodriguez et al., 2021), ELISA-based tests have gained popularity as an alternative test to measure rabies antibodies. The WHO has recognized ELISA-based tests as acceptable for antibody detection and measurement when RFFIT and FAVN testing is not feasible (World Health Organization, 2018). Different ELISA-based test kits are now commercially available, but with varying degree of sensitivity and specificity (Ciconello et al., 2022; Gold et al., 2020; Welch et al., 2009).

1.4.3 BioPro Rabies ELISA Ab Kit

The BioPro Rabies ELISA Ab kit (O.K. SERVIS BioPro, Prague, Czech Republic) is a commercially available blocking ELISA kit used for the detection of rabies antibodies in serum of domestic and wild carnivores (*BioPro Rabies ELISA Ab Kit*, 2013). Estimation of seroprevalence in vaccinated populations by BioPro ELISA is a suitable method for the evaluation of oral rabies immunisation campaigns in wildlife and is used in many countries as part of their rabies elimination programs (*BioPro Rabies ELISA Ab Kit*, 2013). Dog sera are tested to determine if the animal is sufficiently immunized to be protected against rabies infection. The World Organization for Animal Health (WOAH, formally known as the Office International des Épizooties or OIE) recognized the use of rapid ELISA-based tests, like BioPro ELISA, for rapid screening if correlating with the "gold standard tests", such as the FAVN and RFFIT ("Terrestrial Manual

Online Access," 2018). Many studies have reported good sensitivity, specificity, and correlation with gold standard tests for BioPro ELISA in both for the evaluation of rabies antibodies in domestic dogs and cats (Wasniewski & Cliquet, 2012) and in wildlife, such as foxes and raccoon dogs (Wasniewski et al., 2013, 2014). This is especially important for the analysis of wildlife samples, that are often autolyzed, bacterially contaminated, or diluted. Wasniewski and Cliquet (2012) evaluated the BioPro ELISA for detection of rabies antibodies in the sera of domestic carnivores (dogs and cats) and reported a specificity of 100%. A 86.2% concordance was found when comparing the BioPro ELISA to the FAVN test (Wasniewski & Cliquet, 2012).

The procedure for BioPro ELISA is similar to other blocking ELISA-based tests, whereby the rabies antibodies from the test sera competes with an enzyme-labeled rabies antibody (antiglycoprotein) for binding with an inactivated rabies antigen present on the well surface of commercially-prepared microplate (*BioPro Rabies ELISA Ab Kit*, 2013; S. Moore & Gordon, 2020). The antigen coated on the wells of the BioPro ELISA microplate is a crude glycoprotein G. The amount of enzyme-labeled antibody is detected by adding an enzyme substrate and allowing the plate to incubate for colour development. The amount of rabies antibodies in the test serum is inversely related to the intensity of colour development. The rabies antibody in test serum can be quantitated by the use of a standard curve and an optical density (OD) reader (*BioPro Rabies ELISA Ab Kit*, 2013).

The BioPro ELISA kit uses a glycoprotein as coated-antigen and a streptavidin-biotin detection system. First, serum samples are added to glycoprotein-coated wells, allowing for antibody-antigen binding (Rodriguez et al., 2021). Second, biotinylated anti-rabies antibodies are added and will interact with antigen not blocked by the test sample. Third, a streptavidin peroxidase conjugate is added and will interact with the biotinylated anti-rabies antibodies. Fourth, tetramethylbenzidine, a peroxidase substrate, is used as a visualizing reagent. The peroxidase reacts with the substrate leading to colour development. The higher the intensity of colour development, the lesser the antibody amount in the test sample. Lastly, a stop solution in added and optical density is measured at a wavelength of 450nm using an ELISA microplate reader. ELISA results are calculated using the OD measurement provided by the microplate reader and presented as percentage of blocking (PB). The percentage of blocking (PB) must be calculated for each sample, either manually, using a software on a plate reader, or using an evaluation sheet (*BioPro Rabies ELISA Ab Kit*, 2013; S. Moore & Gordon, 2020).

Sera of domestic pets, such as dogs, are tested to evaluate their immune response and determine if the animal is adequately protected against rabies infection. A rabies neutralization antibody titre ≥0.5 IU/ml is defined by the WHO and WOAH as a reliable indicator of an adequate immune response, post-vaccination, to guarantee protection ("Terrestrial Manual Online Access," 2018; World Health Organization, 2018). It was demonstrated that samples with a PB ≥ 70% correlates with a titre level of ≥0.5IU/ml based on a virus neutralization test (Wasniewski & Cliquet, 2012). The test interpretations of the BioPro Rabies ELISA Ab Kit are presented in Table 1.1 (*BioPro Rabies ELISA Ab Kit*, 2013; Wasniewski & Cliquet, 2012).

Table 1.1: Interpretation of BioPro Rabies ELISA Ab Kit for domestic animals

Percentage of blocking (PB) result	Interpretation
PB < 40%	Negative for rabies antibodies
40% ≤ PB > 70%	Either the sample should be re-tested using a virus neutralization test or considered directly as a negative sample, requiring a booster vaccination
PB ≥ 70%	The antibody titre should be considered as positive, indicating satisfactory protection against rabies

1.5 History of rabies and its control in Zimbabwe

The early history (Pre-20th century) of rabies in Zimbabwe is not well documented (Shone, 1962). According to Edmonds[1] (1922), the indigenous inhabitants could recall that the disease was prevalent prior to European settlement in the country (Edmonds, 1922; Shone, 1962). Zimbabwe was apparently free of rabies in 1900 (Shone, 1962). The first definitive evidence of rabies was of an outbreak in dogs, which began in 1902 after the disease was introduced from Zambia via the heavy traffic along a major trade route connecting these two countries at the time (Shone, 1962; Swanepoel et al., 1993). The outbreak was eventually controlled with the implementation of muzzling orders (later abandoned), large-scale culling of strays and unowned dogs, dog registration, dog confinement, and, most importantly, the application of a Dog Tax Ordinance (Shone, 1962). This dog tax introduced in 1912, resulted in an enormous decrease in the number of dogs (Shone, 1962). In 1913, there was only one outbreak of rabies, and the disease was declared eliminated in 1914. Other than an incident of imported dog rabies in Victoria Falls in 1938, Zimbabwe remained free of rabies from 1914 until 1950 (Foggin, 1988; Shone, 1962).

In 1950, rabies was reintroduced into Zimbabwe by infected dogs who were accompanied by people moving freely across the south-western and southern borders from Botswana and the Transvaal (present day South Africa) (Swanepoel et al., 1993). Rabies quickly engulfed the country, disseminating among the more densely populated communal farming areas and, by 1954, the disease had reached the northern areas of Zimbabwe. In 1952, the first serious outbreaks in wild animals, especially jackals, were confirmed (Shone, 1962). Since 1913, the growth of the human (and dog) population made tie-up orders and culling of strays and ownerless dogs increasingly difficult to enforce. As a result, these measures played a secondary role in controlling the outbreak (Shone, 1962; Swanepoel et al., 1993). Rather, the main method used to gain control of this outbreak was mass vaccination of dogs. In 1951, mass immunization campaigns were started using the Flury low-egg passage vaccine, which had been recently approved (Shone, 1962).

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[1] Edmonds (1922) was the assistant chief veterinary surgeon of Rhodesia

By the early 1960s, rabies control was largely achieved in most of the country, apart from eastern and western border areas with Mozambique and Botswana. A breakdown of control measures occurred between 1965-1980 due to unrest and civil war, resulting in a resurgence of rabies cases as it became challenging to vaccinate dogs in communal farming areas. A record 861 positive cases, including humans (n=25), domestic (primary dogs, n=260 and cattle, n=101) and wild animals (primarily jackals, n=428), were reported in 1981, one year after the cessation of war and independence in 1980 (Foggin, 1988; Swanepoel et al., 1993). Following the end of war, the control of dog rabies was complicated by continued unrest in the southwest of the country, the influx of refugees (and "refugee dogs") from the civil war in Mozambique, and rabies outbreaks in jackals (Foggin, 1988). Starting in the 1980s, the vaccination coverage in dogs progressively improved, but declined after 1990, probably due to other major disease programs being prioritized and so fewer resources being allocated to rabies control programs (there were multiple other major disease outbreaks, including foot-and-mouth disease in 1989 and Newcastle disease in 1994) (Bingham et al., 1995). Presently (2022), it is understood that the vaccination coverage of dogs in Zimbabwe is insufficient, as there are rabies cases in dogs, and related human rabies fatalities, reported every year.

1.6 Rabies in dogs in Zimbabwe

1.6.1 Epidemiology of rabies in dogs in Zimbabwe

After the re-introduction of rabies into Zimbabwe in the early 1950s, the disease became established in both the domestic dog (*Canis familaris)* population and in wild carnivores (principally side-stripped jackals (*Lupulella adustus*) and black-backed jackals (*Lupulella mesomelas*)) (Swanepoel et al., 1993). While both jackals and dogs can serve as hosts for rabies virus, the majority of rabies cases have originated from dogs, which are the most important vector for human rabies infection in Zimbabwe (Bingham et al., 1999a, 1999b). Epidemiologic data collected from 1950-1996 reported that dogs (45.7%) were responsible for the most animal rabies cases in Zimbabwe, followed by jackals (25.2%) (Bingham et al., 1999a, 1999b). Importantly, Foggin (1988) reported that 90% of human rabies fatalities between 1950-1988 were from exposure to rabid dogs (Foggin, 1988). Additionally, between 1992-2003, dog bites were responsible for 90.5% (38/42) of human rabies cases (Pfukenyi et al., 2007). Several closely related factors have been associated with the incidence of dog rabies in Zimbabwe, including the percentage vaccination coverage, land use, human/dog population density, jackal population, and proximity to international borders (Madzima, 1995).

Two previously conducted surveys of Zimbabwe's dog population were used to estimate vaccination coverage in dogs. In 1986, Brooks estimated that 40% of the dog population ≥3 months old had been vaccinated against rabies (Brooks, 1990). In 1994, Butler and Bingham found that 58.4% dogs in communal lands had been vaccinated against rabies (J. R. Butler & Bingham, 2000). More recently, in 2015, Hampson et al. estimated a country-wide vaccination coverage for Zimbabwe to be 11.15% (Hampson et al., 2015; *Zimbabwe | Global Alliance for Rabies Control*, 2021). All of these studies have reported vaccination levels well below the recommended 70% threshold to prevent a rabies outbreak (Coleman & Dye, 1996). Additionally, Bingham et al. (1999) observed a significant negative correlation between the number of vaccine doses administered and the number of rabies cases in dogs that lagged one year, suggesting that past levels of immunization affects disease incidence (Bingham et al., 1999a).

Zimbabwe can be divided into four different categories based on land use patterns: commercial farmland, communal farmland, urban areas, and protected areas. From 1950 until the early 1980s, most cases of rabies in dogs originated from commercial farming areas. However, from 1984-1996, Bingham et al. (1999) reported that most cases of rabies in dogs originated in communal farming areas, likely due to better surveillance of the disease and not to change in the prevalence of rabies (Bingham et al., 1999a). In contrast, few rabies cases are attributed to urban areas, except for the city of Mutare, or protected areas, where the few positive specimens were situated in lands near commercial farmland, communal farmland or near a river border crossing (Bingham et al., 1999a).

Areas of communal farmland are generally overpopulated and, while these areas have less suitable habitat for jackals, they typically have large free-roaming dog populations (Bingham et al., 1999a). The majority (71.3%) of Zimbabwe's dog population lives in these communal areas (Brooks, 1990). Communal land dogs could be classified as "neighborhood dogs" according to the WHO dog classification system, as these dogs are free-roaming, semi-dependent on people for their needs, and have free access to the rest of the dog population (J. R. Butler & Bingham, 2000). Butler and Bingham reported that, among dogs in communal lands, there is a rapid turnover in the dog population – resulting from a low life expectancy of dogs (1.1 years) and uncontrolled breeding. Despite a juvenile (dogs <1 years of age) mortality rate of 71.8%, the dog population grows at a rate of 6.52% every year (J. R. Butler & Bingham, 2000). In contrast, areas of commercial farmland have generally a low dog density, a low-to-moderate human population, and dense populations of jackals that results in the majority of jackal rabies cases (Bingham et al., 1999a; Bingham & Foggin, 1993). Between 1992-2003, 83.7% of positive rabies cases in wild animals were found in commercial lands in the northeast parts of Zimbabwe; jackals accounted for 90.9% of all positive wildlife cases, and 84.8% of jackal cases occurred in commercial lands (Pfukenyi et al., 2009).

Two biotypes (genetically distinct groups of viruses that are adapted to a specific set of host species) of rabies virus are recognized in southern Africa: the canid biotype, which circulates in dogs and other canids, and the viverrid biotype, which circulates in mongooses (Bishop et al., 2010). In southern Africa, rabies epizootics in jackals and dogs appear to be independent but are caused by closely related canid rabies virus variants (Bingham et al., 1999b; Sabeta et al., 2003; Zulu et al., 2009). Since the canid strain of rabies virus (canid biotype) has become well adapted to dogs and jackals, rabies infection is freely communicable between these species (Bishop et al., 2010; Swanepoel et al., 1993). Consequently, spillover infection from jackals to dogs, people, and livestock (especially cattle who are the main victim of jackal rabies) remains a serious threat (Bingham & Foggin, 1993).

Rabies occurs widespread throughout Zimbabwe, but cases in dogs have aggregated more frequently in the eastern regions, possibly due to migration of dogs from neighbouring Mozambique (Madzima, 1995). Notably, the Zambezi River, which flows on the northern border of the country, acts like a natural barrier in disrupting the free movement of dogs (Foggin, 1988).

From a public health point of view, dog rabies is of the greatest significance, as dogs have accounted for the most animal rabies cases and are the most common source of human infection in Zimbabwe. From 2017-2021, 589 rabies positive animal samples were recorded, with 64% (n=377) of these positive samples originating from dogs. Despite 18 human rabies cases being reported from 2017-2021, Hampson et al. estimated that 410 human rabies cases occur annually in Zimbabwe (Hampson et al., 2015; *Zimbabwe | Global Alliance for Rabies Control*, 2021).

1.6.2 Control of rabies in dogs in Zimbabwe

To control rabies in dogs, mass vaccination campaigns started in 1951 using the Flury LEP (low egg passage) vaccine. In the early 1980s, the use of the cell culture-based vaccine replaced the

Flury LEP vaccine (Bingham et al., 1999a). The Animal Health Act (Rabies Regulations) of 1966 provides details on rabies control and prevention legislation, which makes it compulsory for dogs to be vaccinated against rabies at 3 months of age, at 12 months of age, and every three years thereafter (Madzima, 1995). Most dog vaccinations in rural (commercial farming) areas are administered at a central point location, such as at dipping tanks, free of charge by the Zimbabwe Department of Veterinary Services during annual mass dog vaccination campaigns. In urban areas, dog vaccinations can be obtained from government or through a private veterinary practice. In addition, similar mass dog vaccination campaigns are carried out annually in urban areas at shopping centres. Annual vaccination campaigns are required because of the high turn-over in the dog population in rural settlements and among the urban poor (Madzima, 1995).

1.7 Victoria Falls, Zimbabwe as a study area

1.7.1 The Victoria Falls/Mosi-oa-Tunya

The Victoria Falls, also known as Mosi-oa-Tunya ("The Smoke that Thunders"), is the world's greatest sheet of falling water and is located on the Zambezi River bordering Zambia and Zimbabwe. The waterfall is approximately 1,700 meters wide and the water of the great Zambezi River plunges 108 meters down to a series of basalt gorges below, sending up a mist that can be visible for up to 20 kilometers (A. Moore & Cotterill, 2010). This Natural Wonder of the World is recognized for its impressive geological and geomorphological attributes, thus placing this site as an iconic, global tourist destination. The Victoria Falls/Mosi-oa-Tunya is also a UNESCO world heritage site, with important ecological, cultural, geological, and anthropological significance (A. Moore & Cotterill, 2010; *Mosi-Oa-Tunya / Victoria Falls - UNESCO World Heritage Centre*, 2022). The transboundary property of the Falls extends over 68.6 square kilometers and comprises parts of three protected land areas: Mosi-oa-Tunya National Park (Zambia), Victoria Falls National Park (Zimbabwe), and the Zambezi National Park (Zimbabwe). These areas provide habitat for a diverse and wide range of flora and fauna (*Mosi-Oa-Tunya / Victoria Falls - UNESCO World Heritage Centre*, 2022). In 2019, half-a-million (493,698) tourists from across the globe visited the Mosi-oa-Tunya/Victoria Falls World Heritage property (*THE MOSI-OA-TUNYA/VICTORIA FALLS WORLD HERITAGE PROPERTY STATE OF CONSERVATION REPORT 2019-2020*, 2020).

1.7.2 City of Victoria Falls, Zimbabwe

Located in the northwest corner of Zimbabwe is the city of Victoria Falls, a small resort town which lies in an ecologically sensitive location between the Zambezi and Victoria Falls National Parks (*City of Victoria Falls*, 2022; Dube & Nhamo, 2019). The climate is sub-tropical, with hot, muggy, and rainy summers, and mild dry winters (with cold nights). The main attraction of the city of Victoria Falls is the nearby waterfalls, which shares its name. However, tourists visiting the city of Victoria Falls can participate in a diverse range of activities, including nature and adventure activities such as hiking tours, a range of cultural activities, bungee jumping, mountain biking, sky diving, fly fishing, safari/game trips, swimming, and helicopter rides over the waterfalls (Dube & Nhamo, 2019). In Zimbabwe, the dry season is from April to October and the wet (rainy/green) season is from November through March (Wina & Money, 2021). During the wet season, the water is at its highest. Thus some activities, such as white-water rafting, are only available during the drier (low-water season) months. Tourism and immigration services are the primary economic activity of the city of Victoria Falls (Dube & Nhamo, 2019).

1.7.3 Rural communities of Victoria Falls, Zimbabwe

Outside the city of Victoria Falls are several rural communities, comprised mainly of agropastoral villages where the primary economic activities include growing crops and raising livestock. In addition, these rural areas have domestic animals, including dogs, which are considered of lesser value and so are often neglected (Moyo, 2020). The Victoria Falls Community Animal Health & Welfare Centre is in the rural village of Ntabayengwe, located 15 kilometers from the city of Victoria Falls. This Centre serves the rural community as a facility to treat their livestock and domestic animals (free of cost) and thus improve the general welfare of these animals and the livelihood of people living alongside wildlife. Transmission of diseases between domestic animals and wildlife, and human-wildlife conflicts remains an ongoing threat in these areas. This Centre also operates vaccination programs (e.g., rabies, distemper), conducted by veterinary staff, to protect the community from diseases that can be transmitted between animals (Moyo, 2020).

1.7.4 Rabies control in rural Victoria Falls, Zimbabwe

The diagnosis and control of rabies is the responsibility of the Zimbabwe Department of Veterinary Services; however, this organization lacks the resources to adequately apply dog vaccinations country-wide to control the disease in dogs and prevent human infections. Thus, non-governmental organizations are important partners to supplement the government's effort. Victoria Falls is one such area where the Victoria Falls Wildlife Trust and Veterinarians for Animal Welfare Zimbabwe, both operate and where they have partnered to conduct dog vaccinations in rural communities adjacent to The Victoria Falls and its extensive wildlife populations. Every year, between 1000 to 2000 rabies vaccinations are administered and certificates issued to dog owners. During the annual vaccination campaign of 2015, an unexpected outbreak of canine distemper occurred. In response, these two organizations provided vaccines to control this outbreak, which has reduced but not eliminated the distemper outbreak, which complicates the application of vaccine on a mass scale.

Rabies is not currently present in this vaccinated area, though it is at risk with a number of cases in neighbouring Zambia, as well as some 60 kilometers to the south-east, in communities bordering the Hwange National Park (C. Foggin, personal communication, 2020). At this time, it is uncertain if the annual vaccination coverage has been adequate to prevent incursion of the rabies into rural Victoria Falls, or whether this is due to its relative inaccessibility to wandering, rabies-infected dogs.

1.8 Knowledge, attitudes, and practices (KAP) survey related to rabies

A KAP survey is a method, commonly using predefined questions in a standardized questionnaire, that is used to obtain both qualitative and/or quantitative information (World Health Organization & Partnership, 2008). KAP surveys make use of the knowledge, attitudes and practices theory that posits that knowledge about a topic leads to an attitude which result in a different practice (World Health Organization & Partnership, 2008). The KAP theory proposes that the process of human behavioural change follows three successive steps: first is acquiring knowledge, second is generating attitudes, and third is forming behaviours or practices (Andrade et al., 2020; World Health Organization & Partnership, 2008). The KAP model has gained popularity for its use in cross-sectional surveys, which represents a common tool to collect information from participants on their health behaviours (Andrade et al., 2020). KAP surveys can be used to identify information that is commonly known, attitudes that are held, and investigate factors related to health behaviours in a population (World Health Organization & Partnership, 2008). The data collected from KAP surveys can assist in interventional public health planning and development of strategies that reflects local needs, wherein knowledge gaps can be targeted for change (World Health Organization & Partnership, 2008). The advantages of KAP surveys are that they are easy to conduct and can be implemented in a time-sensitive matter. However, KAP models have difficulty in properly incorporating and measuring cultural-specific knowledge (Schelling, 2022). Despite this limitation, KAP surveys remains an important tool to assess community knowledge of public health concepts, including the health behaviours associated with zoonotic diseases like rabies (Sambo et al., 2014). The rationale for using a KAP survey to collect information on the knowledge, attitudes and practices related to rabies is that, in respect to the KAP theory, the zoonotic transmission of rabies to humans is affected by the behavioural actions (i.e. health seeking action taken following an rabies exposure) of individuals, which are retroceded by existing rabies knowledge and attitudes (Sambo et al., 2014).

KAP surveys are especially necessary if no survey has been previously conducted in the population of interest(Andrade et al., 2020). Moreover, a KAP survey conducted in rural African settings can be useful when the objective is to obtain general information about community health knowledge related to the treatment and preventative health practices of individuals (Launiala, 2009). For example, a KAP study in rural Chad reported low (19%) awareness of appropriate post-bite treatment (wound washing) among respondents, and thus identified a knowledge gap that can be targeted by advocacy programs (Mbilo et al., 2017). As rabies disproportionality affects people living in rural and remote communities, information collected from a KAP survey can be used to help to inform public health interventions to empower communities with the life-saving knowledge and awareness to protect themselves against rabies (World Health Organization, 2018). In line with the One Health approach, important information on rabies prevention and treatment can be disseminated among all main stakeholders, using media (i.e. internet, radio, newspaper), at community meetings or through healthcare professionals (Sambo et al., 2014).

1.9 Overarching goal

This study contributes towards the overarching goal for the broader Victoria Falls community, which is to improve rabies control and reduce or eliminate the risk of zoonotic rabies in the human population, and thus promote Victoria Falls as a rabies-free tourist destination. First and foremost, this is important to improve animal and human health outcomes. Secondly, maintaining Victoria

Falls as a rabies free zone is important as it will protect the key economic activity in the area – tourism.

1.10 Specific study aims

1.10.1 Specific aim 1

Determine the level of protective rabies antibodies among a sample of rural dogs.

1.10.2 Hypothesis for specific aim 1

At least 70% of dogs have protective rabies antibodies during time of sampling.

1.10.3 Specific aim 2

Collect information and describe the knowledge, attitudes, practices related to rabies among dog owners.

2 A rabies serological survey of domestic dogs and Knowledge, Attitudes and Practices of rabies among dog owners in rural Victoria Falls, Zimbabwe

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Abstract

Introduction: Rabies is an acute, progressive viral encephalitis caused by lyssaviruses in the Rhabdoviridae family. Globally, dog bites are responsible for up to 99% of all rabies transmissions to humans. Rabies is widespread throughout Zimbabwe and human deaths are reported every year. To supplement the Government's effort on rabies control, Victoria Falls Wildlife Trust and Veterinarians for Animal Welfare Zimbabwe have joined forces to carry out dog rabies vaccinations in rural communities adjacent to the major tourist attraction of Victoria Falls (VF). This study aimed to determine the level of protective rabies antibodies among a sample of rural dogs, and collect information and describe the knowledge, attitudes, practices (KAP) related to rabies among dog owners to better understand behaviours and interactions between dogs, people, and wildlife in rural VF.

Methods: During an annual rabies vaccination campaign in rural areas in VF from October–November 2020, blood was collected from 500 dogs for rabies serology testing. 498 of the 500 dog sera samples were tested successfully for rabies antibodies at the Onderstepoort Veterinary

Institute, South Africa using the BioPro Rabies ELISA Ab kit. Sera samples with percentage of blocking ≥70% was defined as positive for protective rabies antibodies (PrAbs). Additionally, a KAP survey was implemented among dog owners (n=342) to collect information about the dog demographics, rabies vaccination history, and health behaviours in this community. KAP survey and serology data were merged for each dog sampled. Multivariable logistic regression was used to evaluate associations between rabies immune status and selected factors at time of sampling.

Results: In total, 500 surveys were completed by 342 dog owners. At time of sampling in 2020, 32.1% (160/498) of dogs had PrAbs. Among previously vaccinated dogs, 44.8% (133/297) had PrAbs. The proportion of dogs with PrAbs decreased with time since vaccination, with <1 year (87.5%), 1-2 years (45.1%) and >2 years (31.2%). The odds of having PrAbs at time of sampling was significantly associated with a dog being previously vaccinated against rabies (p<0.001, Odds ratio (OR) 5.1, OR 95% CI:3.2–8.4), being overweight (p=0.047, OR 8.4, OR 95% CI:1.0–68.7) and among dogs used for herding cattle (p=0.016, OR 0.55, OR 95% CI:0.34–0.89). KAP responses from dog owners indicated that most respondents knew that rabies is fatal to people (91.8%) and knew where to get post-exposure treatment for rabies (90.6%), with most (89.4%) dog owners responding that they would seek rabies treatment at a hospital or clinic. When dog owners were asked what they do immediately after being bitten by a dog, 87.4% responded that they would seek medical attention, but only 3.8% responded that they would wash the wound with soap and water.

Discussion: Evaluating the rabies antibody levels in the dog population in rural communities around VF is important to guide the process to re-vaccinate these animals. We found inadequate level of protective rabies antibodies among our sample of dogs and thus rabies vaccinations efforts in dogs should be increased in this area to protect and prevent rabies in dogs and thus prevent human infection and deaths. Information on bite wound management for rabies prevention in humans was a knowledge gap identified. Data from this study has been shared with key local stakeholders, thus data are being used to guide the decision making in the field when it comes to the prevention of rabies in dogs and people.

2.1 Introduction

Rabies, caused primarily by *Rabies lyssavirus*, is an ancient viral zoonotic disease acquired from the virus-laden saliva (usually via bites) of infected animals and results in fatal encephalomyelitis (Jackson, 2018a; Warrell & Warrell, 2004). All warm-blooded mammals, including people, are susceptible to rabies infection (Fisher et al., 2018). Every year, an estimated 59,000 people wordwide die of this disease, with rabid dog bites accounting for up to 99% of all human rabies deaths (World Health Organization, 2018). Rabies can be prevented following an exposure through prompt first-aid wound washing and timely administration of post-exposure prophylaxis (PEP), consisting of a series of rabies vaccines, and, if needed, injections of rabies immunoglobulin at the bite site (Jackson, 2018b). Globally, the domestic dog is the most important rabies reservoir, and the implementation of mass-dog vaccinations (MDV) is the most cost-effective way to achieve reduction in the number of human deaths (Cleaveland et al., 2006; Lembo et al., 2010). and has led to the successful elimination of dog rabies in many countries (Cleaveland et al., 2018; Vigilato et al., 2013). MDV, together with community awareness and accessibility of PEP, are the cornerstones of the internationally led “Zero by 30” rabies elimination strategy, that uses a “One Health” approach to bring together multiple stakeholders to achieve the target goal of zero human deaths from dog-mediated rabies by 2030 (World Health Organization et al., 2019).

The World Health Organization (WHO) recommends that at least 70% of the dog population needs to be vaccinated during annual campaigns to limit rabies transmission and thus achieve herd immunity (Coleman & Dye, 1996; World Health Organization, 2018). Although the WHO-recommended 70% coverage remains the target for campaigns in reaching long-term rabies elimination in an area, a study in Tanzania by Hampson and colleagues reported that 20-45% of dogs must always be immune for the duration of the interval between annual vaccination campaigns to maintain herd immunity (Hampson et al., 2009a). This coverage of 20-45% was termed the critical vaccination threshold (Hampson et al., 2009a). Monitoring the vaccination coverage of the dog population is an important step towards rabies elimination (Wallace et al., 2017). Rabies antibody neutralization assays are used to assess the rabies immune status of dogs following vaccination (Aubert, 1992). A rabies neutralization antibody titre ≥0.5 IU/ml is defined by the WHO and WOAH as a reliable indicator of an adequate immune response post-vaccination to guarantee protection (“Terrestrial Manual Online Access,” 2018; World Health Organization, 2018). Failure to vaccinate dogs leaves them susceptible to rabies infection, presenting a clear zoonotic risk. Rabies serology have been used to measure post-vaccination antibody levels in dogs, which can serve as an useful surveillance tool to monitor the success of vaccination campaigns (Cleaveland et al., 1999). Additionally, questionnaire surveys can collect valuable information among community members on the dog demographic and record the rabies knowledge of the community. When paired together, serological data and information gathered from surveys can then be used to guide the process to re-vaccinate dogs (to maintain ≥70% immunity) and help to inform public health campaigns.

In Zimbabwe, rabies is endemic in both dogs (canine rabies) and in wildlife (sylvatic rabies). Dogs (*Canis familiaris*) are the principal host for rabies and the most important vector for human infection (Bingham et al., 1999a). Jackals can also serve as hosts for rabies, maintain rabies cycles and sustain rabies virus transmission of canine rabies variants (Bingham, 2005; Sabeta et al., 2003). In Zimbabwe, there are two species of jackals, the black-backed jackal (*Lupulella mesomelas)* and the side-stripped jackal *(Lupulella adustus)*. Rabies epidemiological data collected from 1950-1996 shown that dogs made up the most animal rabies cases in Zimbabwe (45.7%), followed by jackals (25.2%), with most of the jackal-rabies being reported in side-stripped jackals, which mostly resides in the northern half of the country (Bingham et al., 1999a, 1999b; Bingham & Foggin, 1993; Foggin, 1988). Dog rabies was mostly found in rural (communal farming) areas, where 71.3% of the nation's dog population lives (Bingham et al., 1999a; Brooks, 1990). Jackal-rabies was almost exclusively found in commercial farming areas, where jackal population density is high (Bingham & Foggin, 1993). Spillover infection from jackals to dogs, people, and livestock (especially cattle which are the main victim of jackal rabies) remains a serious threat (Bingham & Foggin, 1993). Previous surveys of Zimbabwe's dog population have estimated that 40% of the dog population ≥3 months old was vaccinated against rabies in 1985 (Brooks, 1990) and 58.4% of communal land dogs had been vaccinated against rabies in 1994 (J. R. Butler & Bingham, 2000). More recently, Hampson and colleagues estimated a country-wide vaccination coverage for Zimbabwe to be 11.15% (Hampson et al., 2015; *Zimbabwe | Global Alliance for Rabies Control*, 2021).

At present, rabies is widespread throughout Zimbabwe, with an estimated 410 human rabies deaths occurring every year (Hampson et al., 2015), and the human rabies vaccine is not always readily available in rural areas. While the Zimbabwe Department of Veterinary Services is tasked with the diagnosis and control of rabies, this organization lacks adequate resources to conduct MDV nationwide. Thus, it often falls on non-governmental organizations (NGOs) to supplement the government's effort. Victoria Falls (VF) is one such area, where the Victoria Falls Wildlife Trust (VFWT) and Veterinarians for Animal Welfare Zimbabwe (VAWZ) have joined forces to carry out rabies vaccinations in rural communities adjacent to the major tourist attraction of The Victoria Falls. Every year, between 1,000-2,000 dog rabies vaccinations are administered and vaccinations certificates issued. There are no rabies cases currently in VF, but this vaccinated area is at risk with several rabies cases occurring in areas in Zambia adjacent to VF, as well as 60 km to the South-East, in communities bordering the Hwange National Park (C.Foggin, personal communication, 2020; Figure 2.1). It remains unclear whether the annual vaccination coverage has been sufficient to prevent incursion of rabies into rural VF. There is a critical need to evaluate if previous dog rabies vaccinations are being effective at conferring at least 70% immunity to the dog population. Therefore, we conducted this study in which we hypothesized that at least 70% of dogs sampled in this study area will have protective rabies antibodies. Hence the specific aims of this study were 1) to determine the level of protective rabies antibodies among a sample of rural dogs, and 2) collect information and describe the knowledge, attitudes, practices (KAP) related to rabies among dog owners in rural VF in Zimbabwe.

2.2 Materials & methods

2.2.1 Study type

This study is a cross sectional observational study.

2.2.2 Study area

The study was conducted in rural areas outside of the city of Victoria Falls, Zimbabwe during an annual dog rabies vaccination campaign during the months of October-November (dry season) 2020 (Figure 2.1).

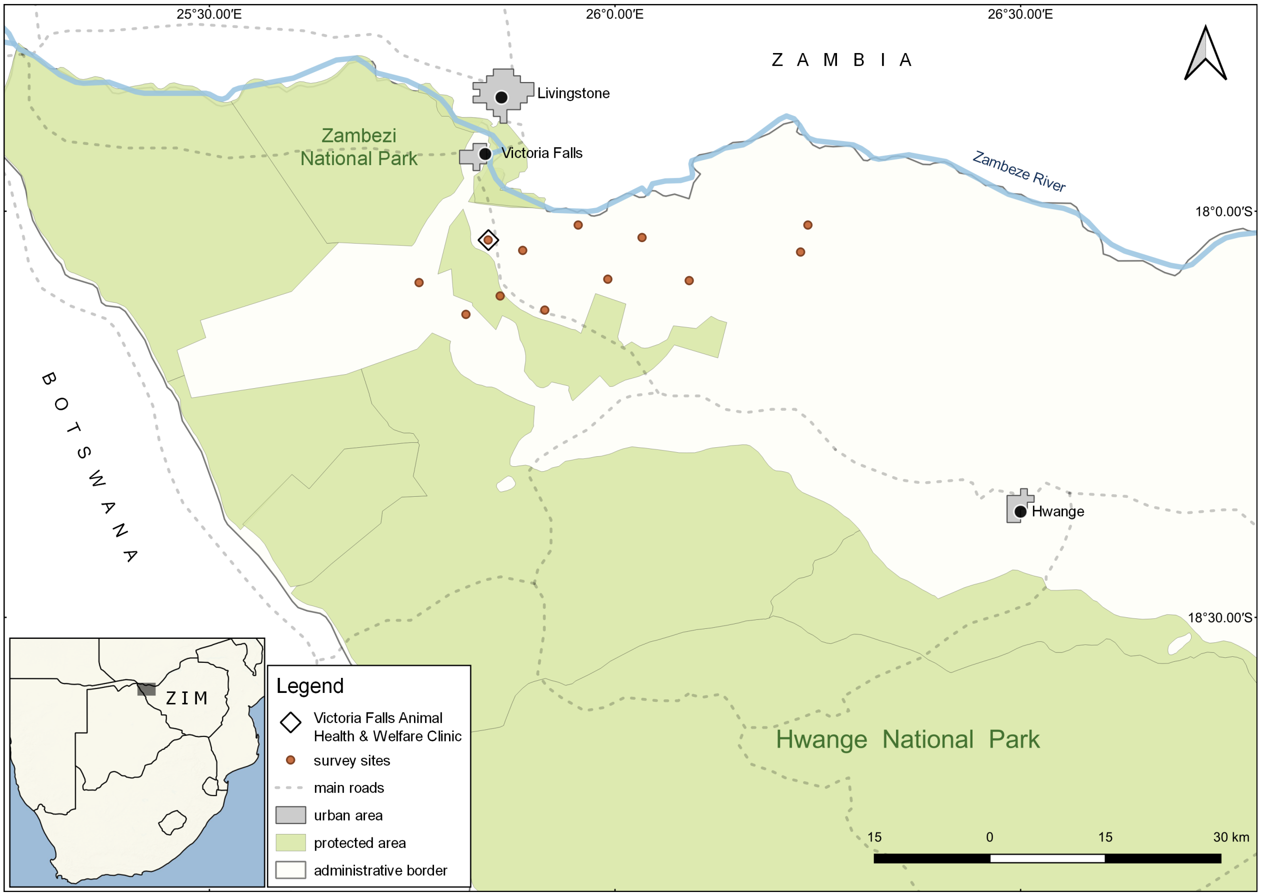

Figure 2.1: Study area of rural areas outside Victoria Falls, Zimbabwe

Spatial setting of study area in northwest Zimbabwe, southern Africa (insert) and the location of survey sites (orange dots) where dogs were bled for rabies serology testing and information on dogs and KAP related to rabies were collected from dog owners via survey during an annual dog rabies vaccination campaign in rural Victoria Falls between October-November 2020. ZIM=Zimbabwe.

2.2.3 Study rationale

Every year, dog rabies vaccinations are carried out by the Victoria Falls Wildlife Trust and Veterinarians for Animal Welfare Zimbabwe in communities adjacent to Victoria Falls to supplement the government's effort on rabies control. This study analyzed data collected in the field during the 2020 annual dog rabies vaccination campaign in rural areas outside of Victoria Falls, Zimbabwe to determine the level of protective rabies antibodies among a sample of rural dogs. Additionally, information on the knowledge, attitudes and practices related to rabies was collected among dog owners. This information was used to descriptively identity knowledge gaps to help guide future public health interventions in the Victoria Falls area, and to better understand behaviours and interactions between dogs, people, and wildlife. Thus, this study will contribute towards improving rabies control efforts in the local area, leading to better health outcomes in humans and animals.

2.2.4 Survey design

A semi-structured paper questionnaire was designed in collaboration with local partners in Victoria Falls, VFWT and VAWZ, to obtain information on dogs and their owners. The survey included 26 closed- and open-ended questions, with 5 sections: 1) responder's background, 2) dog's information, 3) dog's vaccination status, 4) dog's interaction with wildlife and 5) knowledge, attitude and practices related to rabies among dog owners. Survey responses were recorded as numerical (e.g., age of dog), categorical (e.g., sex of dog) or descriptive (e.g., what wild species have you seen interact with your dog). Dog owners present during the rabies vaccination campaign were recruited on site by a field veterinarian. After consent was obtained, the field veterinarian posed the questions to participants and recorded their responses. If needed, questions were translated from English to the responder's preferred language (e.g., Tonga, Ndebele, or Shona) for clarification. All responses were recorded in English. In addition, dogs enrolled in the study had their blood drawn for rabies serology testing to evaluate levels of rabies antibodies. Each completed survey corresponded to a sampled dog with their own unique study code identification number for data organization purposes. Only dogs above 1 years old were included in the study. A maximum of two dogs per owner were surveyed. Paper survey is presented in Appendix A1.

2.2.5 The KAP survey model

KAP surveys are popular research instruments for the investigation of health-related and health seeking behaviours, which aims to obtain information on what is known (knowledge), believed (attitudes) and done (practices) in relation to a specific health topic (World Health Organization & Partnership, 2008). In general, KAP surveys allow for the identification of baseline KAP, provide insights into barriers relating to developing a public health intervention strategy and be used to evaluate post-intervention changes (Andrade et al., 2020). KAP surveys have been used in many studies in the context of rabies control and prevention, where authors cite the importance of this method in identifying gaps, misconceptions and improper practices related to rabies such as the KAP pertaining to how rabies is transmitted, bite prevention, appropriate wound management, awareness of post-exposure vaccination and the importance of dog vaccinations (Ba et al., 2021; Mapatse et al., 2022; Mbilo et al., 2019; Ntampaka et al., 2019). These gaps represent opportunity for targeted public health intervention, with the goal of improving animal and human health outcomes.

2.2.6 Sample size determination for dogs

A post-hoc sample size analysis was performed to determine the minimum number of dogs to sample to test the hypothesis that at least 70% of the dog population has protective rabies antibodies. A minimum sample of 323 dogs was required to evaluate if 70% of the dogs have protective rabies antibodies, with a desired allowable error of 5% and 95% confidence. Therefore, the sample of 500 dogs included in this analysis is an ample size to test this hypothesis.

The formula for a 95% confidence interval, as discussed by Thrusfield (Thrusfield, 2007)

$$n = \frac{1.96^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

where:

n = minimum sample size;

P_{exp} = Expected proportion of dog population with protective rabies antibodies;

d = desired allowable error.

2.2.7 Rabies serology testing of dogs

Rabies antibodies were determined by testing dog sera using the BioPro Rabies ELISA Antibody Kit (Prague, Czech Republic). Blood samples were collected into a sterile tube from the cephalic vein and labeled with the dog's study code identification number (1-500). The blood samples were centrifuged to extract the sera. The sera were kept on a cold chain and transported to the Onderstepoort Veterinary Institute (OVI), Pretoria, South Africa and analyzed with the BioPro Rabies ELISA Antibody Kit according to the manufacturer's instructions. The percentage of blocking (PB) was calculated for each sample (PB% = [$OD_{negative\ control} - OD_{sample}$) / ($OD_{negative\ control} - OD_{positive\ control}$] x 100). Sera with PB ≥70% correlates with to a viral neutralization titre ≥0.5IU/ml, per the manufacturer's guidelines. Thus, we classified sera with PB ≥70% as having protective rabies antibodies and sera with PB <70% as having non-protective rabies antibodies for subsequent data analysis.

2.2.8 Data & statistical analysis

Responses, originally completed on paper surveys, were manually entered into Excel. Laboratory and survey datasets were transferred to Stata16 and merged for each sampled dog using the study code identification number (*Stata Statistical Software*, 2019). List of variables of the working dataset is presented in Appendix A3. Descriptive analyses were conducted to summarize the distribution of the data. The Shapiro-Wilk Test was used to assess for normality for continuous variables. Continuous data are presented as means, standard deviation, median, maximum, and minimum. Categorical data are presented as counts and percentage. Dogs were categorized as adults if they were >3 years and young adults if they were 1-3 years. Dog's body condition score was graded using a five category (1/5) classification system, where 1=emaciated, 2=underweight, 3=ideal weight, 4=overweight 5=obese. Descriptions of body condition scores: 1: "Ribs, backbone, pelvic bones visible from a distance. Obvious waist and abdominal tuck. No body fat" 2: "Ribs visible but no backbone visible. Some body fat present. Abdominal tuck evident. Waist visible from above" 3: "Ribs not visible even on close inspection. Waist visible from above. Abdominal tuck visible. Lower line of tummy slopes upwards from end of ribs to back leg" 4: "Waist barely visible from above. Abdomen slightly rounded, flanks concave. Lower line of tummy is horizontal from ribs to back leg. Moderate amount of fat – jiggle noted when walking" 5: "Waist absent. Abdomen rounded. Lower line of tummy bulges downward. Sway from side to side when walking" ("Dog Body Condition Scoring Training," 2022).

Dogs were classified as previously vaccinated based upon dog owner report. Initially, as an exploratory step (before using Logistic Regression), the Pearson's chi-squared test for used to test for statistical significance between previous vaccination status and whether dogs had protective rabies antibodies. For all previously vaccinated dogs, the date of their last rabies vaccination was recorded. "Time since vaccination" was calculated by subtracting the date of the last rabies vaccination from the date of blood collection, measured in days. The results were categorized as dogs vaccinated <1 year ago, dogs vaccinated 1-2 years ago, and dogs vaccinated >2 years ago. The temporal trend of the proportion of protective rabies antibodies over time in previously vaccinated dogs was described.

Univariate analysis was conducted to evaluate association between rabies immune status (protective/non-protective rabies antibodies) and predictor variables that might affect this outcome in dogs. Odd ratios and 95% confidence intervals were obtained. All predictor variables that had *p*-values ≤0.2 and considered biologically relevant were included into the multivariable model. A multivariable logistic regression model was used to evaluate associations between rabies immune status and selected risk factors at time of sampling to evaluate the combined effect of independent factors (variables) while controlling for potential confounding. The Hosmer-Lemeshow test was used to determine the goodness of fit of the model. A predicted probability cut-off value of 0.5 was used for model evaluation, the Receiving Operating Characteristic (ROC) curve obtained, as well as the model sensitivity and specificity.

A *p*-value of <0.05 was considered statistically significant. Analyses were performed using Stata16 (*Stata Statistical Software*, 2019). Figures were generated in Stata16, R 4.0.2 or Excel (*R: A Language and Environment for Statistical Computing*, 2021). Study area map was created using free and open sourced QGIS 3.16 (*QGIS Geographic Information System*, 2009).

2.2.9 Ethical statement

Written informed consent was obtained from all dog owners prior to their participation in this study. Canine blood sampling was authorized by the Zimbabwe Department of Veterinary Services. This study was approved by the Research Ethics Board at the University of Western Ontario, London, Ontario, Canada (No. 119480). See Appendix A2 for letter of approvement.

2.3 Results

A total of 500 surveys were completed on 500 dogs by 342 respondents (dog owners).

2.3.1 Descriptive results of dogs

The dog characteristics are presented in Table 2.1. The median dog age was 3 years (range = 1-16 years). When age was categorized into adult dogs and young adults, 296 (59.2%) dogs were young adults, and 204 dogs (40.8%) were adults. Of the dogs included in this study, 244 (48.8%) were male and 256 (51.2%) were female. For body condition, over half (60.2%) of dogs were classified as underweight. For the number of dogs in the household, we found that 109 (21.8%) dogs live alone, 167 (33.4%) dogs live with another dog, 123 dogs (24.6%) live with two other dogs and 101 (20.2%) dogs live with four or more other dogs. As reported by their owners, the majority of dogs included in this study sleep outdoors (96.4%), are never restrained (84.2%), eat home-prepared food (98.6%) and have never previously bitten a person (93%).

Table 2.1: Descriptive characteristics of 500 dogs surveyed in rural Victoria Falls, Zimbabwe, October-November 2020

Dog characteristic	Statistic
Continuous variables	**Mean ± SD (min, Q1, median, Q3, max)**
Age (in years)	3.41 ± 2.18 (1, 2, 3, 4, 16)
Mass (kilograms)	11.81 ± 2.17 (7, 11, 12, 13, 35)
Categorical variables	**Number of dogs (%)**
Age category	
Young adults (1-3 years)	296 (59.2)
Adults (>3 years)	204 (40.8)
Sex	
Male	244 (48.8)
Female	256 (51.2)
Body score condition	
Emaciated	14 (2.8)
Underweight	301 (60.2)
Ideal weight	174 (34.8)
Overweight	11 (2.2)
Obese	0 (0)

Presence of abnormal clinical findings	
Yes	22 (4.4)
No	478 (95.6)
Where does the dog sleep	
Indoors	18 (3.6)
Outdoors	482 (96.4)
Number of dogs in household	
One	109 (21.8)
Two	167 (33.4)
Three	123 (24.6)
Four or more	101 (20.2)
The dog is restrained	
Only Day	31 (6.2)
Only Night	38 (7.6)
Both Day and Night	10 (2)
Never	421 (84.2)
What type of food does the dog eat	
Dog food pellets	7 (1.4)
Home prepared food	493 (98.6)
Other	0 (0)
Has the dog ever bitten a person	
Yes	35 (7)
No	465 (93)

2.3.2 Rabies serology results

498 of 500 serum samples were successfully evaluated for rabies protective antibodies. Rabies ELISA serology testing revealed that 32.1% (160/498) of dogs had protective rabies antibodies and 67.9% (338/498) did not have protective rabies antibodies (Table 2.2).

Table 2.2: Rabies immune status of dogs sampled in rural Victoria Falls, Zimbabwe, October-November 2020 (n=498)

Rabies Immune Status	Number of dogs	Percentage (%)
Non-protective rabies antibodies	338	67.9
Protective rabies antibodies	160	32.1
Total	498	100.0

Note: Sera with PB ≥70% were categorized as having protective rabies antibodies. Sera with PB <70% were categorized as having non-protective rabies antibodies.

2.3.3 Temporal trend of the level of protective rabies antibodies in previously vaccinated dogs

Prior history of rabies vaccination in dogs was significantly associated with dogs having protective rabies antibodies using the chi-squared test (χ^2 (1) = 54, p <0.001) (Figure 2.2). Less than half (44.78%) of previously vaccinated dogs had protective rabies antibodies. Twenty-seven (13.43%) dogs who were not previously vaccinated had protective rabies antibodies (Figure 2.2).

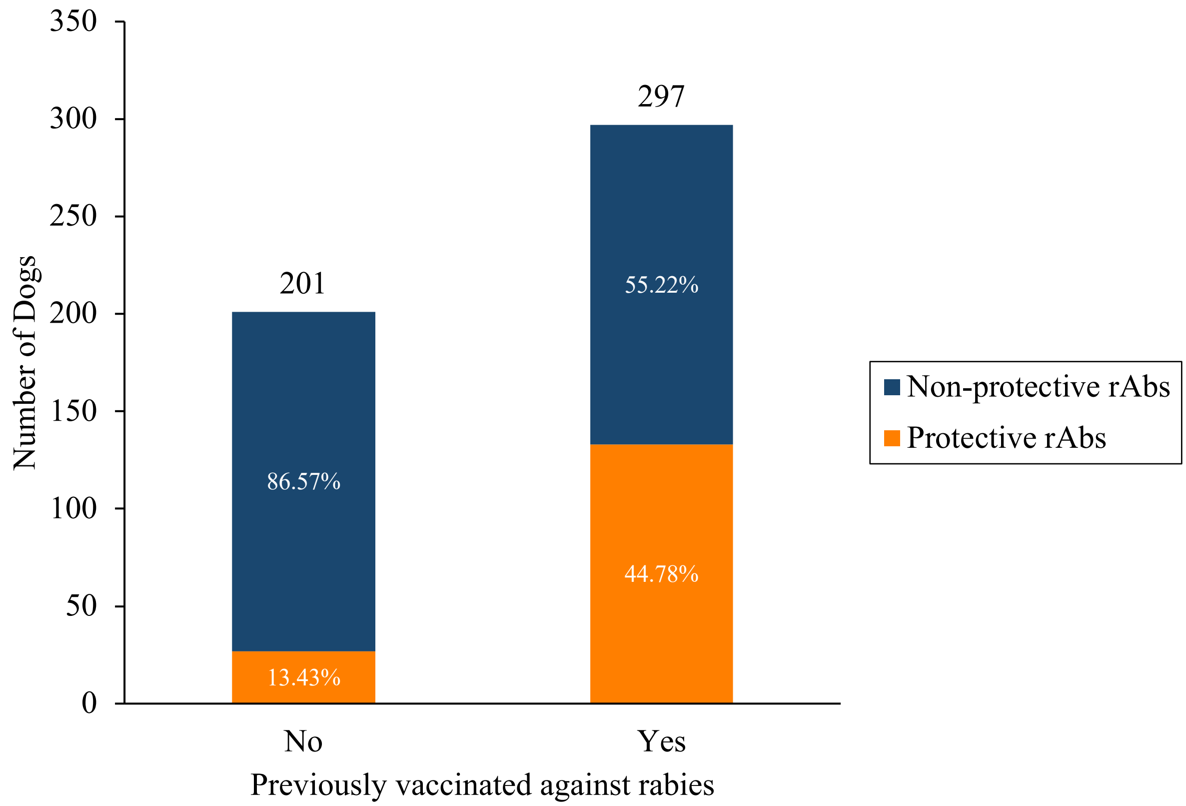

Figure 2.2: Number and proportion of dogs with protective rabies antibodies (rAbs) based on previous rabies vaccination history

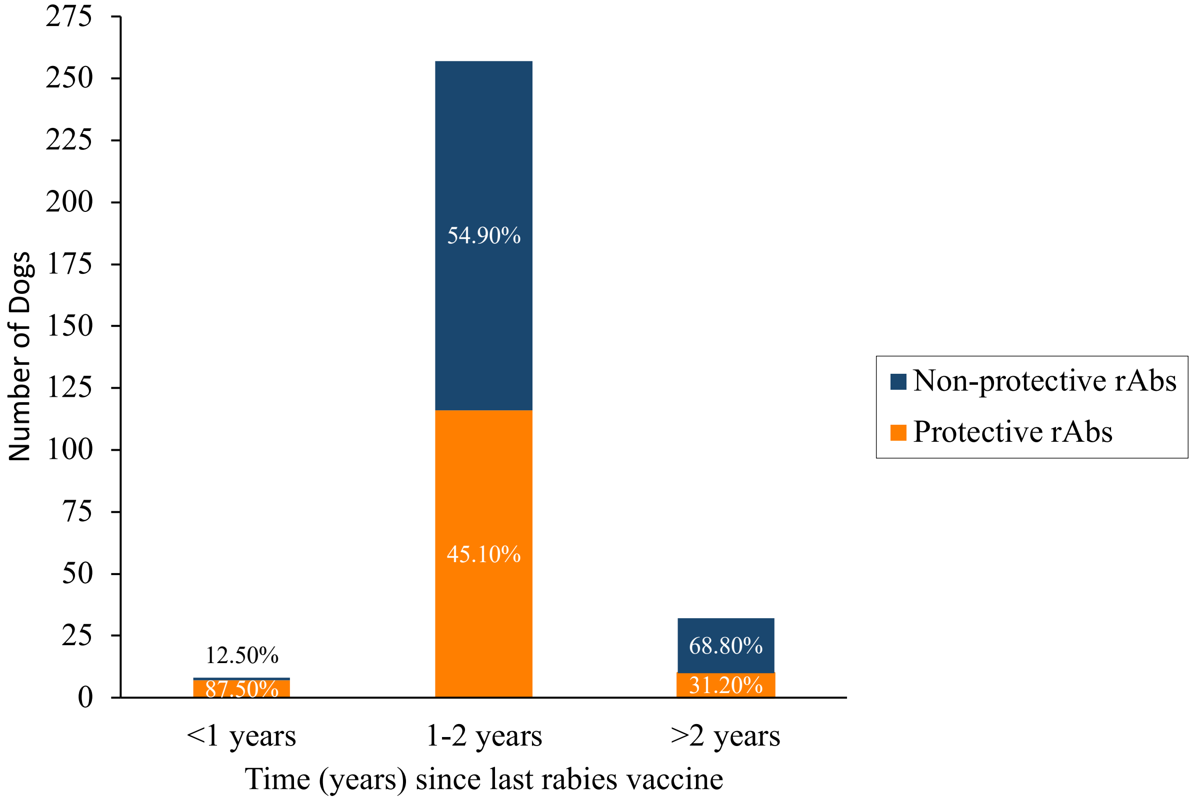

Figure 2.3: Number and proportion of previously vaccinated dogs with protective rabies antibodies (rAbs) since last rabies vaccination

Among previously vaccinated dogs, the proportion of dogs with protective rabies antibodies declined as the time since previous vaccination increased, with 87.5% (7/8) of dogs vaccinated less than one year ago having protective rabies antibodies compared to 45.1% (116/257) of dogs vaccinated 1-2 years ago and 31.2% (10/32) of dogs vaccinated more than 2 years ago (Figure 2.3, Figure 2.4).

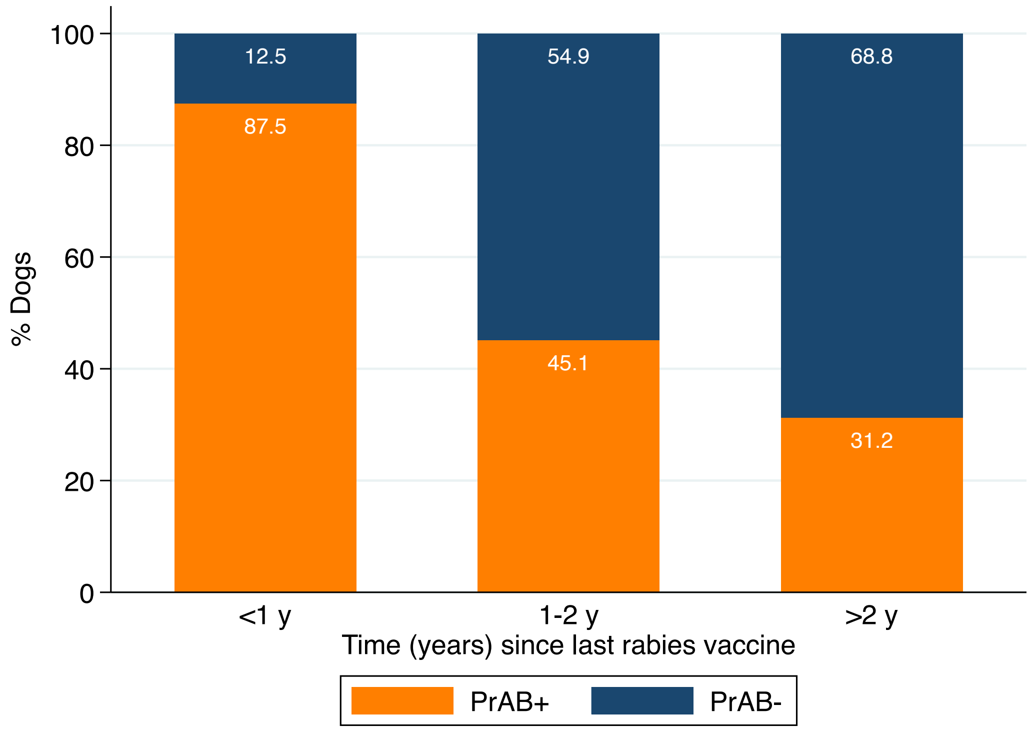

Figure 2.4: Decline in protection against rabies over time in previously vaccinated dogs

The proportion of vaccinated dogs with protective rabies antibodies decline as the time since last vaccination increase. PrAB+: protective rabies antibodies, PrAB-: non-protective rabies antibodies. y=years.

2.3.4 Vaccination history of all 500 dogs

Of the 500 dogs, 298 (59.6%) were reported by their owner to have been previously vaccinated against rabies (Table 2.3). A total of 290 of the 298 vaccinated dogs had a rabies vaccination certificate present.

Table 2.3: Rabies vaccination history of 500 dogs surveyed in rural Victoria Falls, Zimbabwe, October-November 2020

Characteristic	Number of dogs (%)
Previously vaccinated against rabies	
Yes	298 (59.6)
No	202 (40.4)

2.3.5 Dog's interaction with cattle and wildlife species

A total of 325 (65%) dogs were reported by their owners to have been seen interacting with a wild species (Table 2.4). Owners who reported seeing their dog interact with a wild animal were asked what wildlife species they saw their dog interact with. The number and type of wild species interactions with dogs is displayed in Figure 2.5. Dog owners reported seeing 19 different wild animal species interact with their dogs. The three most common wild animal species that have been seen interacting with dogs were antelopes (n=171), baboons (n=128) and hares (n=92). With regards to the dog's herding status, 384 (76.8%) dogs were reported by their owner to be used for herding cattle.

Table 2.4: Dog's interaction with wildlife and herding cattle status for dogs surveyed in rural Victoria Falls, Zimbabwe, October-November 2020

Characteristic	Number of dogs (%)
Have you seen your dog interact with a wild animal	
Yes	325 (65)
No	161 (32.2)
Uncertain	12 (2.4)
Bitten, scratched or no direct contact	2 (0.4)
Wild species seen interacting with dogs (n=325)	
Antelope	171 (52.6)
Baboon	128 (39.4)
Hare	92 (28.3)
Hyena	53 (16.3)
Jackal	47 (14.5)
Squirrel	27 (8.3)
Kudu	21 (6.5)
Warthog	21 (6.5)
Elephant	13 (4)
Skunk	13 (4)
Monkey	11 (3.4)
Wild Cats	11 (3.4)
Buffalo	7 (2.2)
Giraffe	3 (0.9)
Lion	3 (0.9)
Cheetah	1 (0.3)

Leopard	1 (0.3)
Porcupine	1 (0.3)
Zebra	1 (0.3)
Is the dog used for herding cattle	
Yes	384 (76.8)
No	116 (23.2)

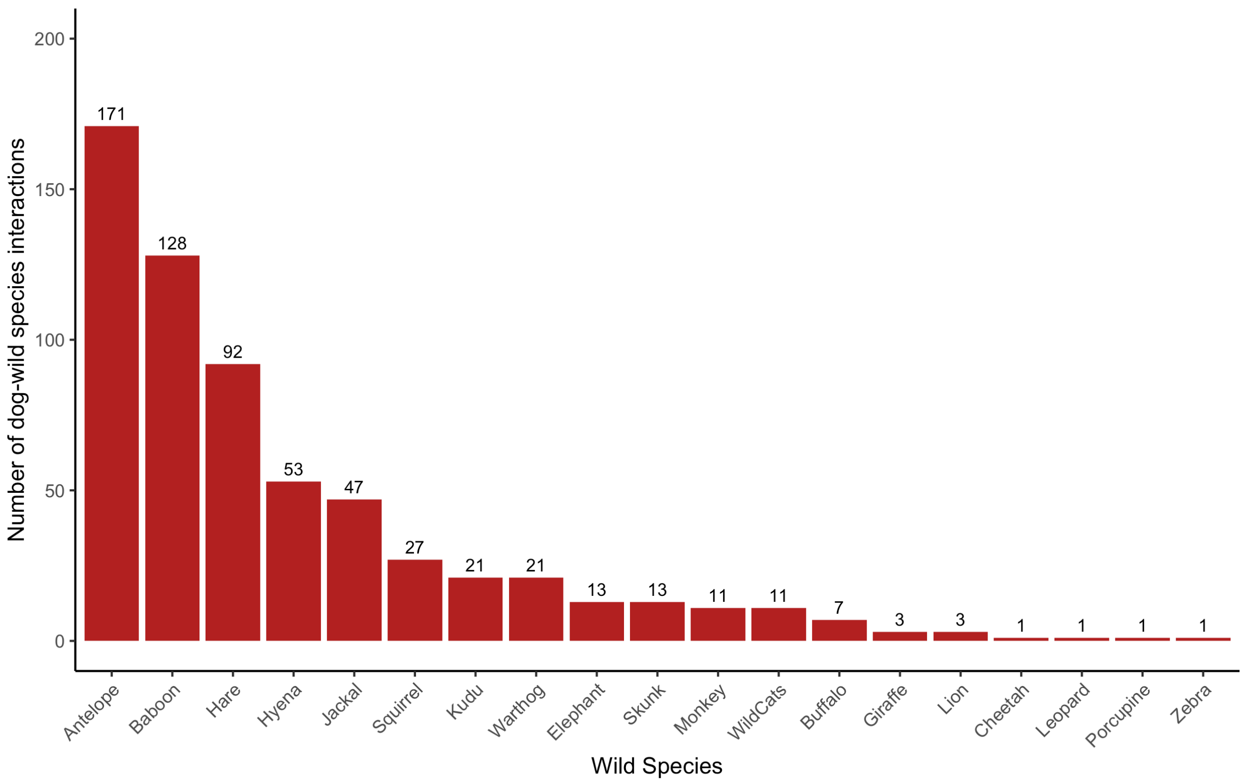

Figure 2.5: Number of wild species seen interacting with dogs in rural Victoria Falls

Bar graph displaying the number of dog-wild species interactions reported by dog owners surveyed in rural Victoria Falls, Zimbabwe, October-November 2020 (n=325). Wild species presented as common names.

2.3.6 Univariate logistic regression analysis of factors and their association with dogs having protective rabies antibodies in rural Victoria Falls, Zimbabwe, October-November 2020

In the univariable analysis, factors associated with having protective rabies antibodies in dogs are presented in Table 2.5. History of previous rabies vaccination was significantly associated with having protective rabies antibodies. The odds of having protective rabies antibodies were 5.2 times more in vaccinated dogs than unvaccinated dogs ($p<0.001$, 95% CI OR 3.28-8.32). Age of the dog was significantly associated with having protective rabies antibodies ($p=0.001$). Adult dogs (>3 years old) were 1.88 times significantly more likely to have protective rabies antibodies when compared to young adults ($p=0.001$, 95% CI OR 1.29-2.76). The association between sex of the dog and having protective rabies antibodies was not statistically significant ($p=0.145$). Compared to emaciated dogs, the odds of having protective rabies antibodies were 1.52 times more in underweight dogs, 1.96 times more in ideal weight dogs and 16.5 times more in overweight dogs, respectively. However, only the overweight dog category was significant as compared to emaciated dogs ($p= 0.006$). Dogs seen interacting with jackals had reduced odds (OR=0.47) of having protective rabies antibodies. The odds of having protective rabies antibodies were significantly associated with the herding status of the dog ($p= 0.027$), with the odds of having protective rabies antibodies being 0.62 times less in dogs used for herding cattle than dogs not used for herding cattle (95% CI OR 0.40-0.95).

Table 2.5: Univariate logistic regression analysis of factors associated with protective rabies antibodies in dogs sampled in rural Victoria Falls, Zimbabwe, October-November 2020

Predictor variables	Odds Ratio (OR)	95% Confidence interval (CI)	p-value
Age			
Young adult	1	ref.	
Adult	1.88	1.29-2.76	0.001
Sex			
Female	1	ref.	
Male	1.32	0.91- 1.93	0.145

*Body condition score**			
Emaciated	1	ref.	
Underweight	1.52	0.41-5.56	0.531
Ideal weight	1.96	0.53-7.31	0.314
Overweight	16.5	2.25-121.23	0.006
Previously vaccinated			
No	1	ref.	
Yes	5.23	3.28-8.32	<0.001
Interact with Jackal			
No	1	ref.	
Yes	0.47	0.22-0.98	0.045
Herding Cattle			
No	1	ref.	
Yes	0.62	0.40-0.95	0.027

Note: *Body condition score was scored on a 1-5 scale: 1 = emaciated, 2 = underweight, 3 = ideal weight, 4 = overweight, 5 = obese. ref.= reference group.

2.3.7 Multivariable logistic regression analysis of factors and their association with dogs having protective rabies antibodies in rural Victoria Falls, Zimbabwe, October-November 2020

All predictor variables with $p \leq 0.2$ from the univariate analysis were included to the final multivariable model. The final multivariable model is presented in Table 2.6. After adjusting for all variables included in the multivariable model, the following results were observed for the following risk factors. The odds of having protective rabies antibodies at time of sampling in October-November 2020 was 5.1 times greater if the dog was previously vaccinated against rabies when compared to a dog that was not ($p<0.001$, 95% OR CI 3.15-8.39). Adult dogs were 1.38 times more likely to have protective rabies antibodies compared to young adults, however this difference was not statistically significant ($p=0.131$). Emaciated dogs were used as the reference group. When compared to emaciated dogs, the odds of having protective rabies antibodies were 1.36 times greater in underweight dogs, 1.83 times greater in ideal weight dogs and 8.42 times greater in overweight dogs. However, underweight, and ideal weight dog categories were not statistically significant. Only overweight dogs shown a significant difference when compared to emaciated dogs (p= 0.047). The odds of having protective rabies antibodies for male dogs was 1.37 times greater than female dogs. However, this difference was not statistically significant. (p=0.140, 95% CI OR 0.90-2.07). The odds of having protective rabies antibodies for dogs not used for herding cattle were significantly lower (OR=0.55) than dogs used for herding cattle (p=0.016, 95% CI OR 0.34-0.89). The Hosmer-Lemeshow test was used to access the goodness-of-it of the model. The Hosmer-Lemeshow test was not statistically significant [χ^2 (8) = 8.36, p = 0.40], suggesting adequate fit. Using a predicted probability cut-off value of 0.5 for model evaluation, the area under the Receiving Operating Characteristic (ROC) curve was 0.73 (Appendix B1), with the model sensitivity of 27.5% and a specificity of 92.0% (Appendix B2).

Table 2.6: Multivariable logistic regression model to evaluate factors associated with protective rabies antibodies in dogs sampled in rural Victoria Falls, Zimbabwe in 2020

Factors	**OR (95% CI)**	***p-value***
Previously vaccinated		
No	ref.	
Yes	5.1 (3.15-8.39)	<0.001
Age		
Young adults	ref.	
Adults	1.37 (0.90-2.07)	0.131
Body condition score*		
emaciated	ref.	
underweight	1.36 (0.35-5.25)	0.653
Ideal weight	1.83 (0.47-7.18)	0.386
overweight	8.42 (1.03-68.68)	0.047

Sex		
Female	ref.	
Male	1.34 (0.90-2.07)	0.140
Interact with Jackal		
No	ref	
Yes	0.57 (0.25-1.27)	0.167
Herding Cattle		
No	ref.	
Yes	0.55 (0.34-0.89)	0.016

Note: *Body condition score was scored on a 1-5 scale: 1 = emaciated, 2 = underweight, 3 = ideal weight, 4 = overweight, 5 = obese. OR=Odds Ratio. ref.=Reference group. CI = Confidence Interval.

2.3.8 Dog owner characteristics

Dog owner characteristics are presented in Table 2.7. The average age of respondents was 35.84 years old (range 14-88 years). The median age of dog owners was 32 years old. Of the 342 respondents, 87.7% (n=300) were male and 12.3% (n=42) were female. The sex ratio (ratio of males to females) of the respondents was 7.14:1. Of the 342 respondents; 1.17% (n=4) had no formal education, 24.85% (n=85) had primary education, 72.51% (n=248) had secondary education and 1.46% (n=5) had tertiary education (individuals with a Certificate, Degree, or a Diploma).

Table 2.7: Dog owner characteristics collected from a survey in rural Victoria Falls, Zimbabwe, October-November 2020 (n=342)

Dog owner characteristics	Statistic
Continuous variables	Mean ± SD (min, Q1, median, Q3, max)
Age (years)	35.84 ± 6.74 (14, 21, 32, 46, 88)
Categorical variables	***Number of dog owners (%)***
Sex	
Male	300 (87.72)
Female	42 (12.28)
Education Status	
No formal education	4 (1.17)
Primary	85 (24.85)
Secondary	248 (72.51)
Tertiary	5 (1.46)
Ward (Administrative District)	
Chidobe	79 (23.10)
Chikandakubi	13 (3.8)
Hlanganani	4 (1.17)
Jambezi	77 (22.51)
Kachecheti	117 (34.21)
Matetsi	14 (4.09)
Mbizha	4 (1.17)
Nemananga	34 (9.94)

2.3.9 Rabies KAP responses of dog owners in rural Victoria Falls, Zimbabwe

A total of 342 dog owners responded to a series of questions associated with their knowledge, attitudes, and practices related to rabies. The list of questions and responses are presented in Table 2.8. When dog owners were asked the question of what they would do immediately after being bitten by a dog; 87.43 % choose to seek medical attention, 1.46% choose to wash the wound with water, 3.8% choose to wash the wound with soap and water, and 7.31% choose other. Description of responses from dog owners who choose "other" is listed in Table 2.9. When we asked if rabies is a fatal disease for people, most (91.8 %) respondents knew that rabies is fatal to people.

Most (90.6%) dog owners responded that they knew where to get post-exposure treatment for rabies following a dog bite. As a follow-up question to respondents who had knowledge of where to get post-exposure treatment for rabies after a dog bite, we then asked these individuals where they would seek post-exposure treatment for rabies. Among these 310 dog owners, 89.35% choose to go to the Hospital/Clinic and 10.65% choose "Other".

With regards to having seen an animal suspected of rabies, 23.68% (n=81) of dog owners have seen an animal suspected of rabies, 74.85% have not seen an animal suspected of rabies and 1.46% were uncertain. The 81 individuals who recalled seeing an animal suspected of rabies were asked two follow-up questions of what the identity of the suspect species was and the fate of this animal. Of the 81 respondents, 72 (88.89%) identified the species as a dog, six (7.41%) identified the species as a fox, two (2.47%) identified the species as a cow and one (1.23%) identified the species as a hyena. With regards to the fate of the suspect animal, 47 (58.02%) of the 81 dog owners reported that the animal was euthanized, 10 (12.35%) reported that nothing happened to the animal and 24 (29.63%) reported that they did not know the fate of the suspect animal.

Table 2.8: Knowledge, attitudes, and practices questions related to rabies among dog owners in rural areas in Victoria Falls, Zimbabwe, October-November 2020 (n=342)

Questions	Number of dog owners (%)
If Bitten by a dog, what do you do immediately after the bite happens?	
Seek medical attention	299 (87.43)
Wash bite wound with water	5 (1.46)
Wash bite wound with soap & water	13 (3.80)
Other	25 (7.31)
Is rabies a fatal disease for people?	
Yes	314 (91.81)
No	9 (2.63)
Uncertain	19 (5.56)
Do you know where to get "treatment" for rabies (PEP: Post-exposure prophylaxis) if bitten by a dog?	
Yes	310 (90.64)
No	32 (9.36)
Where would you seek "treatment" for rabies (PEP: Post-exposure prophylaxis)? n= 310	
Hospital/Clinic	277 (89.35)
Community Center	0 (0)
Native/Traditional Healer	0 (0)
Do Nothing	0 (0)
Other	33 (10.65)

Other location details	
(If answered "Other" to previous question) n=33	
Veterinary Clinic	32 (96.97)
Pharmacy	1 (3.03)
Have you seen an animal suspected of rabies	
Yes	81 (23.68)
No	256 (74.85)
Uncertain	5 (1.46)
What species?	
(If answered "Yes: to previous question) n=81	
Cow	2 (2.47)
Dog	72 (88.89)
Fox	6 (7.41)
Hyena	1 (1.23)
What happened to the suspected rabid animal?	
n=81	
Euthanized	47 (58.02)
Nothing	10 (12.35)
Uncertain	24 (29.63)

Table 2.9: Descriptions of actions from dog owners who choose "Other" in response to "If bitten by a dog, what would do immediately after the bite happens" (n=33)

Description of "Other" Specific Actions	Number of dog owners (%)
Apply Torniquet	1 (4)
Apply aloe vera on wound	1 (4)
Apply ashes of dog's burnt fur on wound	3 (12)
Apply herbal medicine on wound	1 (4)
Apply the herb Isihaqa and burnt fur of the dog's tail	1 (4)
Rub vigorously	1 (4)
Wash in hot water and use herbs	1 (4)
Wash in salty water	3 (12)
Wash with water and methylated spirit	1 (4)
Wash wound with dilute potassium chloride	2 (8)
Wash wound with salty water	5 (20)
Wash wound with warm salty water	3 (12)
Wash wound with water and methylated spirit	1 (4)
Wash with soap and salt and use herbs	1 (4)

2.4 Discussion

This study was part of ongoing field activities on rabies control in the VF area, whereby local agencies are working together towards maintaining the area as "rabies-free". This is important for protecting the health of animals and people, as well as protecting the key economic activity in the area – tourism. A part of this rabies control field activities, annual dog rabies vaccinations in rural communities surrounding VF occurs. A partnership was created between academics at Western University and local partners. The purpose of this study was to complement the field activities in VF by contributing towards the analysis of data collected in the field. The specific aims of this study were to determine the level of protective rabies antibodies among a sample of rural dogs, and to collect information and describe the knowledge, attitudes, practices (KAP) related to rabies among dog owners. We used these two aims in tandem because it was important for a comprehensive One Health approach that we combine rabies serology with gathering data on the characteristics and interactions of rabies reservoirs and socio-cultural practices that also contribute to rabies transmission.

In total, 498 of 500 sera samples were successfully processed for rabies antibodies. Two sera samples were not processed as they did not meet the laboratory quality standard. Rabies serology results revealed that 32.1% of all dogs sampled had protective rabies antibodies, and with only 44.8% of previously vaccinated dogs having protective rabies antibodies at time of sampling. These protective antibody levels did not support our hypothesis that at least 70% of dogs had protective rabies antibodies and these levels are lower than the WHO-recommended vaccination coverage of 70% to stop dog rabies transmissions (World Health Organization, 2018). However, these protective antibody levels would be sufficient in meeting the critical vaccination threshold of 20-45% to eliminate rabies in an area (Hampson et al., 2009b). The proportion of dogs with protective rabies antibodies (32.1%) in our study was lower compared to other rabies serology studies of dogs in southern Africa, where the proportion of dogs with protective rabies antibody levels of 42.2%, 54% and 80% were reported in studies in Zambia, Botswana, and Namibia, respectively (Canning et al., 2019; Hikufe, 2016; Kaneko et al., 2021). This lower proportion of protective rabies antibodies in our study could be explained by the inclusion of previously unvaccinated dogs (40% of dogs were not previously vaccinated against rabies).

Worth noting is that we found that less than half (44.8%) of previously vaccinated dogs had protective rabies antibodies. Plausible explanations to why vaccinated dogs failed to reach a protective rabies antibody level could be due to host factors of the dog (individual variation in immune response, genetics, immunosuppression), quality of the rabies vaccine used, delivery of the vaccine, handling of the vaccine, cold-chain transport, and storage. Counterintuitively, we found a small of proportion (13.4%) of unvaccinated dogs having protective rabies antibodies. It has been reported that some proportion of unvaccinated individuals that test positive for rabies antibodies could be explained by non-lethal exposure to rabies antigens, such as through the consumption of lyssavirus-infected carcasses (Berentsen et al., 2013). Although contradictory to the fact that rabies is invariably fatal, non-lethal rabies exposure in unvaccinated seropositive dogs is likely a result from a subclinical infection of the rabies virus, where the virus is cleared by the host's immune system before any clinical signs of the disease are detected (Gold et al., 2020). Moreover, this result could be due to misclassification of the rabies vaccination status of these dogs. However, it is unlikely that the previous vaccination status for these dogs were misclassified because in addition to using the dog owner's recall of the rabies vaccination history of the animal, we cross-verified this information with the presence of a rabies vaccination certificate.

The temporal description among previously vaccinated dogs indicates that the vast majority (87.5%) of dogs maintain protective rabies antibodies one-year post-vaccination. This level of protection is above the WHO-recommended threshold of 70% coverage. The protective rabies antibody levels of dogs vaccinated 1-2 years ago (45.1%) were below the 70% threshold but met the critical vaccination threshold of 20-45%. Moreover, the protective antibody levels of dogs vaccinated more than 2 years ago (31.2%) did not meet the 70% threshold but reached the critical vaccination threshold. The linear decline in the proportion of dogs with protective antibodies between dogs vaccinated <1 year ago, 1-2 years ago and >2 years ago could be a result of naturally waning rabies antibody levels in these animals in the absence of a booster vaccination. Antibody decline over time among single-vaccinated dogs was also observed in Lusaka district, Zambia where 89.3% of dogs seroconverted one year post-vaccination (Kaneko et al., 2021). In our study, we did not record information about all previous rabies vaccinations among dogs (i.e., multi-vaccinated dogs), therefore it is unknown whether multi-vaccinated dogs developed protective rabies antibodies at a higher rate compared to single-vaccinated dogs.

The purpose of building a multivariable model was to investigate the combined effect of all relevant factors that could contribute to the development of protective rabies antibodies in all dogs in our sample. Although previously vaccination is the main risk factor associated with having protective rabies antibodies, other factors also play a role as recent studies have provided evidence for the phenomenon of non-lethal rabies exposure as a method of developing protective rabies antibodies.

Our multivariable logistic model results indicate that vaccinated dogs, overweight dogs, and dogs not used for herding cattle were all significantly associated with having protective rabies antibodies during sampling time of October-November 2020. As expected, vaccinated dogs had higher odds (OR=5.1) of having protective rabies antibodies compared to unvaccinated dogs (despite the fact that less than half of all vaccinated dogs had protective rabies antibodies in our study sample). The body condition score of dogs appears to contribute to the immune response following rabies vaccination, as dogs with a body condition score of 1-3 were associated with having a lower leukocyte count compared to a body condition score of 4+ (Morters et al., 2014). We found that overweight dogs were significantly more likely (OR=8.4) to have protective rabies antibodies when compared to emaciated dogs. However, we observed a very wide confidence interval likely due to a small sample size for this body condition category (n=11). From our model, dogs used herding cattle were less likely (OR=0.55) to have protective rabies antibodies. It was unexpected that dogs used for herding cattle had lower odds of having protective rabies antibodies. Initially, based on information from local partners in VF, herding cattle status was added to our analysis because herding dogs were thought to be at an increased level of rabies exposure due to the potential closer proximity to rabies and rabies vectors. Therefore, this factor requires further analysis. The variable related dogs having contact with jackals was non-significant when accounting for other factors in the multivariable model.

Although related to body condition score, one possible factor could be the nutrition in dogs (i.e., food source, caloric intake) as malnutrition can lead to immunosuppression, as seen with declines in immunoglobulin and lymphocyte numbers and counts (Morters et al., 2014). Most dogs included in the study were categorized as being underweight which could be due to a lack of nutrition rather than disease as only 4.4% of dogs presented with an abnormal clinical finding. Home-prepared food was the main source of diet for most of these dogs; however, this study did not ask dog owners for the composition of the dietary intake. A dietary assessment study of free-roaming dogs in rural

Zimbabwe found that dogs' most important food sources were sadza (thick porridge, made from white maize), cow carrion and human feces (J. R. A. Butler & du Toit, 2002), A diet comprising predominantly from these aforementioned food items may lack the necessary nutrients to maintain a functional immune system.

Based on ROC results, our model has a moderate predictive power, therefore, indicating that data on other factors (not included in this study) are needed to better predict rabies protective antibodies levels in dogs. In addition, our model reported very low sensitivity (27.5%) but high specificity (92.0%), indicating that the model was better at correctly classifying dogs with non-protective rabies antibodies than correctly classifying dogs with protective rabies antibodies. Thus, other factors that were not included in this study could be contributing to the presence of protective rabies antibodies among dogs in VF.

Our study found that 59.6% of dogs were previously vaccinated against rabies. This coverage was higher than previous reported percentages by historic surveys of the dog population in Zimbabwe; 40% in 1985 and 58.4% in 1994 (Brooks, 1990; J. R. Butler & Bingham, 2000). Vaccination coverage reported in this study is not representative of the nation's vaccination coverage because of the sampling procedure in our study (dogs were surveyed during a vaccination campaign) and inclusion criteria (only dogs >1 years of age were included in the study). Dog characteristic results in this study were consistent with dog demographic and ecology data of the dog population in communal lands presented in the 1994 survey, which found that all communal dogs were owned (no presence of a stray dog population), mostly unrestricted (free roaming) and only semi-dependent on humans (J. R. Butler & Bingham, 2000). The free movement of dogs has important implications for the local spread of canine infectious diseases such as rabies and the long-range spread to maintain rabies persistence at the country level (Colombi et al., 2020). Although only 7% of dogs were reported to have bitten a person, this finding is concerning as dog bites are the most important source of rabies transmissions to humans. In addition, this result further stresses the importance of keeping dogs vaccinated to protect the community.

The high frequency of dog-wildlife interactions reported in this study is concerning as infectious disease may easily spread between species, with rabies virus being the most concerning infectious agent (Hughes & Macdonald, 2013; Stuchin et al., 2018). The rabies pathogen has been linked with the population decline and local extinctions of the endangered wild dog (*Lycaon pictus*), whose home range extends into protected land and reserve areas surrounding Victoria Falls (Canning et al., 2019; Woodroffe & Sillero-Zubiri, 2020). Rabies-infected dogs present a risk towards conservation efforts aimed at restoring wild dog numbers, thus highlighting the urgent need to vaccinate dogs to prevent spillover to sensitive wildlife species. This study did not specifically investigate the situational description of the dog-wild species interaction, nonetheless dogs may have been seen interacting with wild species through scavenging for food sources (as it is often the case for free roaming dog populations). Using experimental carcasses to study scavenging behaviour of communal dogs in rural Zimbabwe, it found that dogs were the dominant scavenger of domestic animal carrion and outcompeted wild scavengers. Dogs' main competitors for carcasses were vultures who they outcompeted, probably due to their physical size, high density, great tolerance to human activity, nocturnal and diurnal activity (J. R. A. Butler & du Toit, 2002). Another potential dog-wildlife interaction is the attempted predation of rabies-infected dogs by large carnivores (dog-wildlife conflicts) which provides ideal conditions for rabies virus transmission. A study observing a population of 236 free-ranging dogs at the interface between communal land and wildlife reserves in northwest Zimbabwe found that lions, leopards and spotted hyenas preyed on dogs, removing ≥6% of the dog population in a communal land study area (J. R. A. Butler et al., 2004). All wild species reported to be interacting with dogs were mammals, thus are all potentially susceptible to rabies infection. The most interesting dog-wild species finding was that 47 dogs were seen by their owner to be interacting with jackals, an important wildlife rabies reservoir in Zimbabwe (Bingham et al., 1999b). In southern Africa, rabies epizootics in jackals and dogs appear to be independent but are caused by closely related canid virus variants (Bingham et al., 1999b; Sabeta et al., 2003; Zulu et al., 2009). Thus, dog-jackal interactions present a potential opportunity for cross-species rabies transmission if one of these species were rabid as canid variants are freely communicable between these species. Using dog bite case data, a study in Murewa district (northeast Zimbabwe) found that spatial overlap of jackal presence and unvaccinated dogs was a risk factor for contracting rabies in people, as rabies can be transmitted from jackals to dogs, and then subsequently to humans (Chikanya et al., 2021).

When it came to the responses of the knowledge, attitudes and practices related to rabies among dog owners, we found that overall, most dog owners were knowledgeable about rabies. The study reported that most dog owners knew that rabies is fatal to people, which perhaps is a contributing factor to why owners are vaccinating their dog(s). However, this study did not explicitly ask respondents the reason for voluntarily attending a vaccination campaign. While most dog owners would seek medical attention immediately if bitten by a dog, only 3.8% would wash the wound with soap and water, which is the recommended first-aid measure by the WHO following animal bites, prior to seeking medical attention to determine the exposure risk severity and whether the event signals for PEP administration (World Health Organization, 2018). This finding was in agreement with other KAP surveys that found similar concerning results of low awareness of this critical first step for post-bite care (Hergert & Nel, 2013; Mbilo et al., 2017; Sambo et al., 2014). This knowledge gap needs to be assessed otherwise it will decrease the possibility of achieving the "Zeroby30" initiative. Information on proper wound care and management should be communicated with key stakeholders or to public health strategists in high-risk areas to empower communities (especially children) with this knowledge. Thirty-three respondents choose "other" specific actions if bitten by a dog. Responses included the application of salt, herbs, animal, and plant-based products (*Isihaqa*; wild garlic). These items most likely have some connection to local home remedies or traditional medicine for the treatment of dog bites. The use of traditional healing methods have no scientific or medical basis for rabies prevention from dog bites and these techniques should not be used in replacement of effective rabies treatment through with PEP (Beasley et al., 2022).

Most respondents were knowledgeable of where to assess rabies treatment (PEP) if bitten by a dog, with most choosing to seek PEP at a clinic or hospital. Knowing where to seek PEP is "half the challenge" in receiving life-saving treatment following a dog bite. A significant proportion of bite victims who sought medical aid in two rural districts in Tanzania did not receive PEP because of high cost and low PEP availability. In addition, low socioeconomic class and distance to medical facilities increase delays prior to PEP administration (Hampson et al., 2008). Although we asked dog owners what their immediate action would be after a dog bite, a rabies KAP survey conducted in Zimbabwe's capital city of Harare found that 88% of respondents could correctly list the correct course of action (i.e. wound washing, seek primary healthcare) to take following exposure to a suspect animal (Spargo et al., 2021). Roughly a quarter of respondents had previously seen a rabid animal, with canids being the principal species involved. This observation is not surprising as dogs have accounted for most animal rabies cases in Zimbabwe (Bingham et al., 1999a).

This study was limited by evaluating dogs' serology at only one time point. The KAP survey was also limited in its focus on gathering existing knowledge, attitudes, and practices, without exploring the sources of information or experience informing these. The data source of the dog demographic and KAP of rabies among dog owners, obtained via survey, is subject to potential information bias as it relied on the ability of the respondent (dog owner) to recall information. Despite these limitations, these data provide vital information on the rabies immune status of dogs and KAP of rabies among dog owners in rural Victoria Falls that can be used to guide the decision-making process in the field when it comes to the prevention of rabies in dogs and people. The KAP related to rabies data will serve as a baseline to measure the effectiveness of a future public health intervention program. Wound management as a first step for post-bite care was a key knowledge gap we identified and should be targeted by rabies advocacy and educational groups in the area. Future assessment of rabies antibodies of dogs in this area should be focused on sampling at multiple time points (i.e., one-year post-vaccination) to evaluate dog's long-term protective rabies immune status). Additionally, a study assessing the differences in the level of protective rabies antibodies in dogs between two areas – one area where routine vaccinations occurs and another area where routine vaccinations do not occur – could be examined. Moving forward, oral rabies vaccinations in dogs could be explored to complement annual parenteral rabies vaccination in the study area to help reach the 70% vaccination coverage. A field trial in Namibia which evaluated the uptake of oral rabies vaccination baits among 1,115 dogs found that 90% consumed the bait and approximately 73% were observed as being vaccinated (Freuling et al., 2022). Thus, the addition of oral rabies vaccines for dogs as a potential future tool for the rabies control program in rural VF should be kept into consideration.

2.5 Conclusion

Evaluating the rabies antibody levels in the dog population in rural communities around Victoria Falls, Zimbabwe is important to guide the process to re-vaccinate these animals, thus protecting dogs, people, and sensitive wildlife species at the interface from contracting this deadly disease. A multi-disciplinary One Health approach should continue to be utilized for rabies control, which prioritizes dog vaccinations, increasing rabies knowledge (including bite prevention and wound management) and having PEP accessible to bite victims. This study found that dogs in rural Victoria Falls had an inadequate level of protective antibodies against rabies during the sampling period of October-November 2020. However, dogs vaccinated within the last year reached an adequate level of protection against rabies (≥ 70% immunity), hence this finding demonstrates that annual rabies vaccinations in this area can achieve the recommended 70% coverage to protect and prevent rabies in dogs and thus prevent human deaths. These rabies antibody levels found among dogs in this study should stress the urgent need for veterinary authorities to vigorously increase rabies vaccination efforts in this area.

3 Overall conclusions

3.1 One Health relevance

As a student using the One Health approach, my thesis allows me to integrate knowledge, skills, and approaches from immunology, virology, epidemiology, data analysis, animal health, public health and zoonoses. The need to apply a One Health approach for rabies control stems from its ability to be transmitted across the dogs-people-wildlife interface. The control and prevention of rabies requires a robust understanding of the unique socio-cultural environment. An inter-disciplinary One Health approach can serve this need by bringing together various stakeholders from academia, non-governmental organizations, government, scientists, veterinarians, researchers, and the local community (i.e., dog owners) to address the challenges rabies pose to dogs, people, and wildlife in this specific ecosystem of rural Victoria Falls (VF). Thus, this work relied heavily upon multi-disciplinary collaboration among various stakeholders, including NGOs actively working at the local level in VF on rabies control in dogs and prevention in humans (VFWT and VAWZ) and a World Organization for Animal Health (WOAH) rabies reference laboratory, with expertise on laboratory techniques for rabies research (Onderstepoort Veterinary Institute). Working closely with these partners, we were able to collect and analyze data that can assist in the decision making in the field when it comes to the control of rabies in dogs and the prevention of rabies in people in rural VF. Therefore, the focus of this thesis aligns perfectly with One Health as rabies affects dogs and people who live in a shared environment like in rural communities of VF.

The global health initiative for the elimination of human deaths from canine mediated rabies, spearheaded by the WHO and other international colleagues, uses the One Health approach. For rabies, One Health is required and key for rabies control and elimination programs. This work presented here provides a template for One Health rabies research which could be applied in other areas when it comes to the control of rabies in dogs and preventing the risk of zoonotic transmission to people, however each area should keep into consideration the local, unique physical, social, and political environments, to find local strategies that can be applied to address the challenges posed by rabies in each specific environment. With the tools for control and elimination of rabies known, a One Health approach will be required to support collaboration and communication among stakeholders working across multiple disciplines as well as securing a significant level of political commitment and investment in both capital and human resources in order to achieve the goal of zero human deaths from dog-mediated rabies by 2030.

3.2 Significance & impact

The work presented in Chapter 2 of this thesis had direct benefit to study participants. Following blood collection of dogs, rabies vaccines were administered to animals with certification of proof also provided. The rabies vaccine applied to dogs is known to be an effective and safe vaccine. Thus, it is expected that vaccinated dogs will be protected against rabies. For this reason, it is also expected that the owners of these dogs will have a dog with a considerable reduced risk to acquire rabies, and thus owning healthier animals having a benefit to their own health by reducing the risk of zoonotic transmission of the rabies virus from their dog. In addition, this work has a positive impact to the inhabitants in rural VF. Vaccinations of dogs will have a direct benefit on protecting dogs against rabies and thus contributing to herd immunity in this population, thus also helping to reduce the risk of zoonotic transmission to humans. The information collected from dog owners from the completion of survey will play a role in generating preliminary information to inform decision makers when designing strategies to improve rabies control in this area. Results from this work will be disseminated to participants and the extended rural communities in VF through direct communication between the local veterinarian and dog owners, the local news, and meeting held by local authorities. For example, future rabies vaccinations campaigns for dogs can provide a good opportunity for results of this thesis to be shared with the community members of rural VF.

Our findings will contribute towards enhancing rabies prevention measures in dogs and people, and to improve the care and treatment provided to individuals bitten by rabid animals in rural Victoria Falls. For example, the survey data obtained will help local health leaders to develop and implement an evidence-informed public health campaign in VF, placing children at the center of this effort. Considering that from our KAP responses, only 3.8% of dog owners would wash the wound with soap and water immediately after bite occurs, public health messaging should emphasize the need to disseminate important information regarding rabies awareness and bite prevention among community members in VF, which can dramatically reduce this zoonotic risk that rabies poses to people.

The area of VF represents an ecological sensitive area and relies on nature-based tourism as a main economic diver. VF lies at the heart of the Kavango-Zambezi Transfrontier Conservation Area, a conservation region comprising five countries and 36 protected areas, and spans of an area of around 520 000km^2 (*Kavango Zambezi Home*, 2019). Moreover, the free roaming dog population in these rural areas of VF are closely associated with people, and many dogs have encounters with wildlife species. The annual vaccination of dogs against rabies in rural VF will thus contribute to reducing the risk of spillover to wildlife species. In turn, this project will contribute towards the overarching goal of promoting VF as a rabies-free tourist destination, as well as protecting sensitive wildlife species at the interface such as the highly susceptible wild dogs.

This study has contributed to capacity building among stakeholders and institutions and serves as a proof-of-concept for future collaborative project focused on rabies and other zoonotic diseases that impact people and animals. In recent developments in the year 2022, this study is now being used as a model by the Asia Pacific Alliance for Health and Development and the Asia-Pacific branch of the World Organization for Animal Health for the implementation of a rabies project, focusing on a large-scale mass rabies vaccination campaign of approximately 250,000 dogs in Bali, Indonesia. Thus, this work has triggered commencement of an international rabies project that uses the One Health approach for rabies but fine-tuning the work to meet the area's own unique socio-cultural, and economic challenges.

3.3 Future directions

To build from this thesis work, it is envisaged that more rabies serology data of dogs in rural VF be collected and analyzed, to access the level of immunity in the dog population. A follow-up rabies KAP survey could be implemented after a public health campaign to gauge the change in health behaviours among dog owners in rural VF. Such a second KAP survey could be compared with results presented in this thesis, and thus measure the effectiveness of the public health intervention at changing rabies-related knowledge, attitudes, and practices.

From the VAWZ Victoria Falls Community Animal Health & Welfare Centre Annual Report 2021, a total of 1770 dogs were vaccinated against rabies throughout the year and no rabies cases in either wildlife or domestic animals occurred in their area (Moyo, 2021). The continuation of annual dog vaccinations and promotion of rabies awareness through public health campaigns among communities of rural VF are recommended. The work presented in this thesis re-emphasizes the need to continue to implement sustainable and community based One Health rabies projects in the VF area to safeguard communities by protecting dogs from rabies through vaccinations, which also improve their general welfare, and consequently lowers the risk of rabies transmission to people, livestock, and wildlife. In conclusion, I present here my contributions to rabies using the One Health approach, and I am confident this work has fostered future One Health rabies projects and activities, aiming towards the control and elimination of this disease.

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Appendices

Appendix A: study items

Appendix A contains items relevant to the completion of the research study, presented in Chapter 2 of the thesis.

Appendix A1: paper survey

VICTORIA FALLS WILDLIFE TRUST

VAWZ Veterinarians For Animal Welfare Zimbabwe

Questionnaire for Rabies Vaccination in Dogs

Consent: My name is **Isaac Moyo**, a veterinarian at Veterinarians for Animal Welfare Zimbabwe (VAWZ), Zimbabwe. This Research is to help us determine rabies immune status in the dog population in rural Zimbabwe. The information you provide will be kept confidential. You are free not to participate in the study if you so wish.

Would you like to proceed? i) Yes ☐ ii) No ☐

Signature/ thumb print of the interviewee...Date..................................

Telephone number...Study code.........................

Section A. RESPONDER BACKGROUND

1. Name of Respondent____

2. Sex of the respondent: i) Female ☐ ii) Male ☐

3. How old are you? ....

4. What is the highest level of formal education you attained?

i) No formal education ☐	iv) Certificate ☐
ii) Primary school ☐	v) Diploma ☐
iii) Secondary ☐	vi) Degree ☐

5. Where do you currently live? Please mark the appropriate region and provide more specific location in the adjacent space.

i) Village: ☐ ....

ii) Ward: ☐ ....

iii) Nearest Business Centre: ☐ ....

iv) GPS Location (S – DD.ddddd; E – DD.ddddd) ☐ ....

Section B.1 DOG'S INFORMATION

6. Microchip ID Number (if applicable): ……………………………………………..

7. Name of the dog: ……………………………………………..

8. Age of Dog: ……………………………………………..

9. Sex of Dog: ……………………………………………..

10. Approximate mass of dog (kg): ……………………………………………..

11. Body condition score of the dog (1 to 5; 1 = emaciated, 5 = obese):

i) 1 ☐ ii) 2 ☐ iii) 3 ☐ iv) 4 ☐ v) 5 ☐

11b.) Other abnormal clinical features: ……………………………………………..

12. Reproductive status of dog

i) Intact ☐ ii) Neutered ☐ iii) Spayed ☐

13. Description of Fur Color: ……………………………………………..

14. Where does the dog sleep?

i) Inside Home ☐ ii) Sleeps outdoors ☐

15. Number of dogs in household

i) 1 ☐ ii) 2 ☐ iii) 3 ☐ iv) 4+ ☐

16. The dog is restrained:

i) Only during the day ☐ ii) Only during the night ☐

iii) Both day and night ☐ iv) Never, the dog is not restrained ☐

17. What type of food the dog eats?

i) Dog food pellets ☐ ii) Home prepared food ☐ iii) Other ☐

18. Has this dog ever bitten a person?

i) Yes. ☐ ii) No ☐

Section B.2 DOG'S RABIES VACCINATION STATUS

19.a Has this dog been previously vaccinated against Rabies?

i) Yes. ☐ ii) No ☐

19.b Does this dog has Rabies vaccination certificate? i) Yes☐ ii) No ☐

If answered **Yes** to question 19.b, provide the Rabies vaccination certificate serial number

YY/MM/DD/Number 001→ ……………………………………

If answered **Yes** to question 19.b, when and where (geographical location) was the dog last vaccinated?

Enter date: …………………………………………………….

Geographic location: …………………………………………………….

If answered **Yes** to question 19.b, has the dog a rabies collar?

i) Yes. ☐ ii) No ☐

Section B.3: DOG'S INTERACTION WITH WILDLIFE

20. Have you seen your dog interact with a wild animal?

i) Yes. ☐ ii) No ☐ iii) Uncertain ☐ iv) Bitten scratched or no direct contact ☐

If answered **Yes** to question 20, what wild animals?

……………………………….. ……………………………….. ………………………………..

……………………………….. ……………………………….. ………………………………..

21. Is the dog used for herding cattle?

i) Yes. ☐ ii) No ☐

Section C: KNOWLEDGE ATTITUDE AND PRACTICES RELATED TO RABIES

22. If bitten by a dog, what do you do immediately after the bite happened?

i) Seek medical attention ☐

ii) Wash the bite wound with water ☐

iii) Wash the bite wound with soap and water ☐

iv) Other ☐

Specify ………………………………….

23. Is Rabies a fatal disease for people?

i) Yes. ☐ ii) No ☐ iii) Uncertain ☐

24. Do you know where to get 'treatment' for rabies (Post Exposure Prophylaxis: PEP) if bitten by a dog?

i) Yes. ☐ ii) No ☐

If answered **Yes** to question 24, where would you seek 'treatment' for rabies (Post Exposure Prophylaxis: PEP treatment)?

i) Hospital/Clinic ☐ ii) Community Center ☐ iii) Native/ Traditional Healer ☐

iv) Do Nothing ☐ v) Other ☐

25. Have you ever seen an animal suspected of rabies?

i) Yes. ☐ ii) No ☐ iii) Uncertain ☐

If answered **Yes** to Question 25, respond to the following set of questions.

a.) What species? ………………………………………………..

b.) What happened to the animal?

i) Euthanized ☐ ii) Nothing ☐ iii) Uncertain ☐

c.) Where did the event take place? ………………………………………………..

d.) When did the event take place? _………………………………………………..

e.) Brief description of the incident (Optional):

……

……

……

26. Have you ever seen a dog with canine distemper?

i) Yes. ☐ ii) No ☐ iii) Uncertain ☐

THE END - THANK YOU

Appendix A2: western research ethics approval

Western Research

Date: 28 July 2021

To: Dr Francisco Olea Popelka

Project ID: 119480

Study Title: ASSESSMENT OF RABIES CONTROL IN RURAL VICTORIA FALLS, ZIMBABWE

Short Title: Rabies Zimbabwe Secondary

Application Type: NMREB Initial Application

Review Type: Delegated

Full Board Reporting Date: 06/Aug/2021

Date Approval Issued: 28/Jul/2021 16:31

REB Approval Expiry Date: 28/Jul/2022

Dear Dr Francisco Olea Popelka

The Western University Non-Medical Research Ethics Board (NMREB) has reviewed and approved the WREM application form for the above mentioned study, as of the date noted above. NMREB approval for this study remains valid until the expiry date noted above, conditional to timely submission and acceptance of NMREB Continuing Ethics Review.

This research study is to be conducted by the investigator noted above. **All other required institutional approvals and mandated training must also be obtained prior to the conduct of the study.**

Documents Approved:

Document Name	Document Type	Document Date	Document Version
Research Plan, 2020	Protocol	25/Jun/2021	001
De-identied List of Variables Received, 2021	Paper Survey	26/Jul/2021	001

No deviations from, or changes to the protocol should be initiated without prior written approval from the NMREB, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

The Western University NMREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the Ontario Personal Health Information Protection Act (PHIPA, 2004), and the applicable laws and regulations of Ontario. Members of the NMREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB. The NMREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000941.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Ms. Katelyn Harris , Research Ethics Officer on behalf of Dr. Randal Graham, NMREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Page 1 of 1

Appendix A3: list of variables in dataset

Variable Name	**Description of variable**
Date	Date when questionnaire was done
Study Code	Study Code number
Sex	Sex of the respondent
Age	Age of the respondent
Education	Highest Education level of respondent
Village	Village name of respondent
Ward	Ward name of respondent
Nearest Business Centre	Nearest Business Centre of respondent
GPS	GPS coordinates of rabies vaccination site
Microchip	Microchip information of the dog
Ageofdog	Age of the dog
Sexofdog	Sex of the dog
Massofdog	Mass of the dog
Bodycondition	Body score condition of the dog
Abnormalcf	Abnormal clinical features of the dog
Reprostatus	Reproductive status of the dog
Furcolor	Fur color of the dog
Sleeploc	Location where the dog sleeps
Dogsinhouse	Number of dogs in household

Dogrestrained	Is the dog restrained
Fooddogeats	Type of food the dog eats
Otherfooddogeats	Other types of food the dog eats
Dogbite	Has the dog ever bitten a person
Rvaccpreviously	Has the dog been previously vaccinated against rabies
Rcertpresent	Does the owner have a rabies vaccination certificate present
Rcertnumber	What is the rabies certificate number
Dateofvacc	What date was the dog previously vaccinated against rabies
Loccofrvacc	Where was the dog previously vaccinated against rabies
Rcollar	Does the dog have a rabies collar
Wildlife	Has the respondent seen the dog interact with a wild animal
Jackal	Has the dog ever interacted with a jackal
Baboon	Has the dog ever interacted with a baboon
Antilopes	Has the dog ever interacted with an antilope
Buffalo	Has the dog ever interacted with a buffalo
Warthog	Has the dog ever interacted with a warthog
Squirrel	Has the dog ever interacted with a squirrel

Skunk	Has the dog ever interacted with a skunk
Hyena	Has the dog ever interacted with a hyena
Leopard	Has the dog ever interacted with a leopard
Girraffe	Has the dog ever interacted with a giraffe
Kudu	Has the dog ever interacted with a kudu
Cheetah	Has the dog ever interacted with a cheetah
Zebra	Has the dog ever interacted with a zebra
Rabbit	Has the dog ever interacted with a rabbit
Monkey	Has the dog ever interacted with a monkey
Porcupine	Has the dog ever interacted with a porcupine
WildCats	Has the dog ever interacted with a wildcat
Elephants	Has the dog ever interacted with an elephant
Lions	Has the dog ever interacted with a lion
Herdingcattle	Is the dog used for herding cattle
Actionafterbite	What action would the respondent take if bitten by a dog
Otherspecificaction	Other actions the respondent would take if bitten by a dog
Isrfatal	Is rabies a fatal disease
Knowledgeoftreatment	Do you know where to get treatment (PEP) if you were bitten by a dog

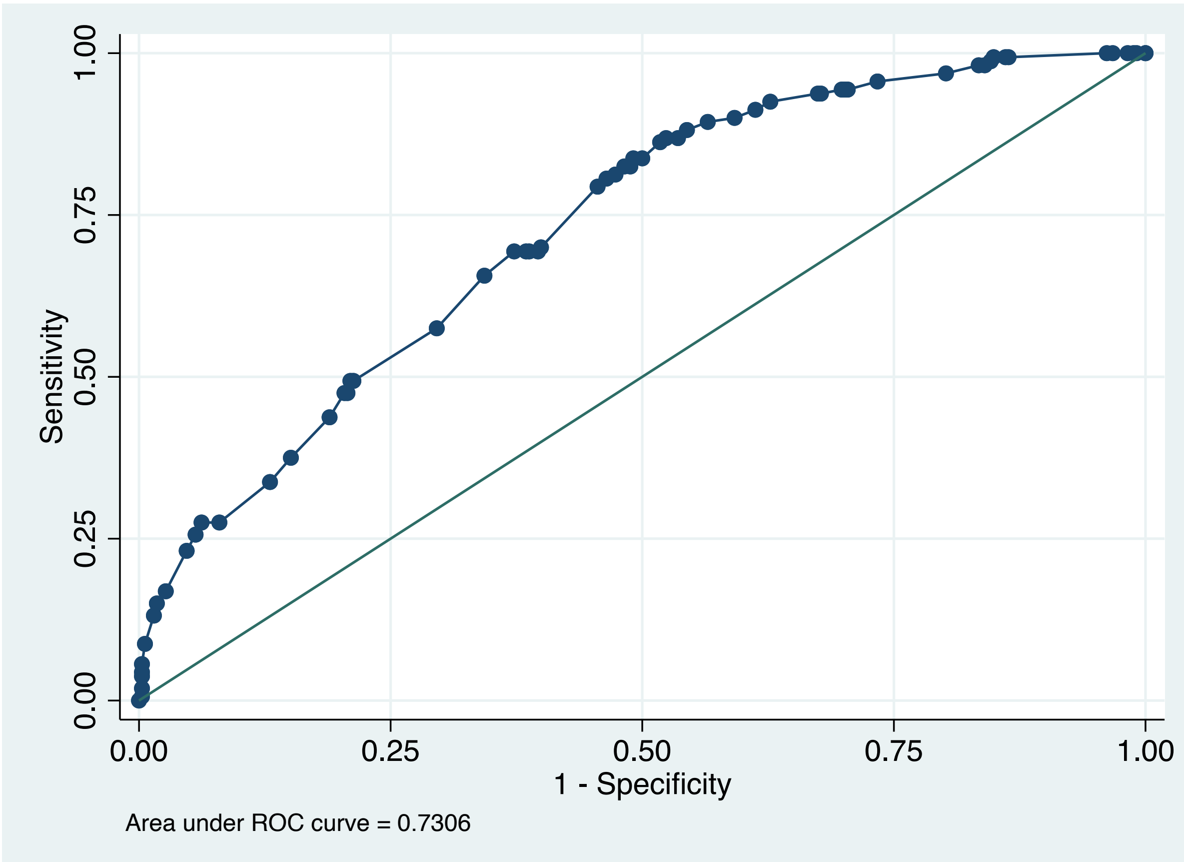
Treatmentloc	Where would you go to get treatment (PEP)
Otherlocdet	Other locations to get treatment (PEP)
Seenanimalwithr	Have you ever seen an animal with rabies
Species	What animal did you see that was rabid
AnimalEnd	What happened to the animal
Where	Where did the event take place
When	When did the event take place
Description	Brief description of the incident
Distemper	Have you ever seen a dog with canine distemper
PercentageBlockingPB	Percentage of blocking result
RabiesAb_IU	**Does the dog have protective rabies antibodies**
TimefromV	**Time elapsed between dogs' previous rabies vaccine and date of blood collection**
TimesinceV	**Time elapsed between dogs' previous rabies vaccine and date of blood collection, for dogs last vaccinated against rabies <1 year ago, 1-2 years ago, and >3 years ago**
Ageofdog_binary	**Ageofdog coded as a binary variable. Two age groups: Young adults (dogs 1-3 years old) and Adults (dogs >3 years old)**
Rvaccpreviously_recoded	**Recode of Rvaccpreviously**

Herdingcattle_recoded	**Recode of Herdingcattle**

Bolded variables: indicate "dummy variables" created from original dataset

Appendix B: supplementary materials for the multivariable logistic regression model

Appendix B1: receiver-operating characteristic (ROC) analysis



ROC Curve

Appendix B2: sensitivity & specificity analysis

Logistic model for RabiesAb_IU

Classified	True D	True ~D	Total
+	**44**	**27**	**71**
-	**116**	**311**	**427**
Total	**160**	**338**	**498**

Classified + if predicted Pr(D) >= .5
True D defined as RabiesAb_IU != 0

Sensitivity	Pr(+| D)	**27.50%**
Specificity	Pr(-|~D)	**92.01%**
Positive predictive value	Pr(D| +)	**61.97%**
Negative predictive value	Pr(~D| -)	**72.83%**
False + rate for true ~D	Pr(+|~D)	**7.99%**
False - rate for true D	Pr(-| D)	**72.50%**
False + rate for classified +	Pr(~D| +)	**38.03%**
False - rate for classified -	Pr(D| -)	**27.17%**
Correctly classified		**71.29%**

Sensitivity & specificity analysis: classification table for the multivariable logistic regression model

Curriculum Vitae

Name:	Ryan LaPenna
Post-secondary Education and Degrees:	University of Western Ontario London, Ontario, Canada 2015-2020 BMSc.
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