Mortality rates and survival analysis of a cohort of owned adult dogs and puppies in Hluvukani, Bushbuckridge, South Africa

by

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DECLARATION

I declare that this dissertation, which I hereby submit for the Master of Science degree in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, is my original work and has not been submitted by me for a degree to any other university.

SIGNED-------------------------- DATE-------------------------

FRANCIS BABAMAN KOLO

This dissertation emanates from Project V033/11 approved by the Research Committee of the University of Pretoria on 17th June 2011 and the Animal Use and Care Committee of the University of Pretoria on 29th June 2011.

DEDICATION

To the Lord God Almighty, the beginning and the end, the protector of my soul, who has kept me and has given me the opportunity to achieve this milestone; to you I dedicate this work.

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LIST OF ABBREVIATIONS

RABV	Rabies virus
OS	Organ system
PP	Pathophysiology
W.H.O	World Health Organization
PEP	Post-exposure prophylaxis
DALYs	Disability-adjusted life years
YLL	Years of life lost
YLD	years of life lived with disability
KZN	KwaZulu-Natal
HDSS	Health and demographic surveillance system
DSA	Demographic surveillance area
EMH	Extramedullary haematopoiesis
MIN	Minimum
MAX	Maximum
IQR	Interquartile range
N	Number

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SUMMARY

Rabies is an acute, fatal, progressive, incurable viral encephalitis affecting all warm-blooded animals, including humans. Dogs are the primary reservoir of rabies virus (RABV) in Africa for both humans and animals. Although rabies can be successfully controlled through vaccination, high rates of dog population turnover through births and deaths make the maintenance of herd immunity through vaccination challenging in populations of free-roaming dogs in low-resource settings. Understanding these demographic processes may help find solutions to create stable, vaccinated populations. The primary aim of this study was to determine the rates and causes of mortality in owned, mostly free-roaming dogs in Hluvukani village of the Mnisi community, in Bushbuckridge Municipality, Ehlanzeni District, Mpumalanga Province. The study enrolled a cohort of adult dogs (one year and older) in May and June, 2014, and followed them for 12 months. Litters of puppies were also enrolled at birth and followed for 120 days each. Outcomes were recorded during frequent follow-up visits,

and causes of mortality were determined through owner interview (‘verbal autopsy’) and post-mortem examination.

A cohort of 367 adult dogs was enrolled in the study (203 males and164 females). Of these, 27 (7.4%) died during the follow-up period, and seven (1.9%) were lost to follow-up. We observed a mortality rate of 77.6 per 1,000 dog-years in the cohort (50.8 per 1,000 dog-years in males and 112.7 per 1,000 dog-years in females). There was very high mortality in female dogs, especially from the age of 5 years and above (305.8 per 1,000 dog-years). Female dogs had a shorter survival time (mean = 341.7 days) compared to the male dogs (mean = 355.8 days); this difference was significant with the log-rank test (p = 0.04) and by Cox regression (p = 0.05). Adult dogs of age 5 years and above had shorter survival time (mean = 338.5) than dogs of ages 3-4 years, which in turn had higher survival times (mean = 358.4) than dogs of ages between 1-2 years old (mean = 348.5). The chi-square test gave a p-value of p = 0.06 and the Cox proportional hazard a p-value of p=0.3. Thus, there is a marginally significant difference in survival between the age categories of adult dogs. Twenty-seven ‘verbal autopsy’ results were collated including 15 natural deaths, 7 deliberate deaths, 3 accidental deaths and 2 euthanasia’s. Of the 15 deaths classified as ‘natural’, two were considered to be due to snake envenomation. The remaining 13 were considered to be due to infectious and/or parasitic causes. Of the seven deaths classified as ‘deliberate’, four were considered to have been caused by poisoning and three by trauma. The two accidental, non-anthropogenic deaths were due to animal bite wounds, and the single accidental, anthropogenic death was caused by a motor vehicle accident. Two dogs were euthanized because they were unwanted by the owner.

Of the 164 females enrolled in the study, 57(34.8%) had at least one litter of puppies during the study period. The total number of puppies enrolled at birth for the study was 329, comprising 152 (46.8%) males, 148 (44.4%) females, and 29(8.8%) puppies of unknown sex. Of the 329 puppies enrolled, 135 (36.6%) died during the follow-up period, and 126 were lost to follow-up. The mortality rate for puppies was 2,389.3 deaths per 1,000 dog-years. Sex-specific mortality rates were 1,811.5 deaths per 1,000 dog-years for male puppies and 2,172.2 deaths per 1,000 dog-years for female puppies. The survival rates were not significantly different between male and female puppies (p=0.3).

One hundred and thirty five verbal autopsy results were collated and analyzed from all dead puppies. Of the 54 puppy deaths classified as 'natural', 53 were considered to be due to infectious and/or parasitic causes. Of the 23 deaths classified as 'deliberate', six were considered to have been caused by poisoning and 17 by trauma. The 47 accidental, non-anthropogenic deaths were considered to be due to the following causes: eaten by mother (n = 18), starvation (n = 8), laid on by mother (n = 7), animal bite (n = 5), drowning (n = 4), suffocation (n = 3) and crushed by a brick (n = 2). The single accidental, anthropogenic death was caused by a motor vehicle accident. Five puppies were euthanized because they were unwanted.

In conclusion, free-roaming dogs in this study were owned, and provided for by their owners without reliance on environmental resources. Mortality rate was low in the adult dog cohort. Birth rate in the female cohort was high and the mortality rate was also high in the puppies before they reached 120 days of life. Natural causes were the highest causes of death in puppies, but accidental and deliberate causes were also frequent. This study recommends future validation studies of methods of verbal autopsy in determining causes of death of dogs in underserved, resource-constrained communities like the Hluvukani village in the Mnisi community area of Mpumalanga Province, South Africa.

CHAPTER 1: INTRODUCTION

Canine rabies in dogs and humans can be controlled and in certain instances eradicated by the mass vaccination of dogs against the virus. The control of infectious diseases in populations through mass vaccination is based on the concept of herd immunity, when the vaccination of a significant proportion of the population (or 'herd') provides a measure of protection for those individuals who are not vaccinated. Although only low levels of vaccination coverage are theoretically required to control dog rabies, in practice the achievement of this goal is hampered by factors responsible for declines in the proportion of the vaccinated population over time. Host demography has a major influence on the long-term effectiveness of vaccination efforts; because coverage levels decline as vaccinated dogs die and new susceptible dogs are born. The movement of dogs by people similarly affects coverage, particularly if large numbers of unvaccinated dogs are brought into an area. In most dog rabies-endemic areas, dog birth rates are high and therefore coverage levels decline rapidly. If coverage falls below the target threshold of 20-40%, rabies transmission can be sustained and outbreaks can persist. This phenomenon is particularly acute when mass dog vaccination is conducted as 'pulsed' campaigns (rather than through the ongoing vaccination of new dogs born into the population), and is influenced by the interval between campaigns.

The birth rate in dog populations can be reduced by sterilization, which is the permanent or temporary removal of the ability of individuals to reproduce. Sterilization can be surgical (ovariohysterectomy/orchidectomy) or chemical (hormone administration or immunocontraceptive vaccine). Surgical sterilization of a significant proportion of the population is cost prohibitive in most cases, and the repeated use of hormones to prevent breeding may be detrimental to the health of animals, and therefore ethically unacceptable. Immunocontraceptive vaccines may offer an alternative, affordable and effective method of sterilization that, if used in conjunction with mass vaccination of dogs, may provide an effective tool for the management of rabies.

When mortality rates remain unchanged, decreasing birth rates through immunocontraception will lead to a reduction in the rate of natural increase of a population. If mortality rates are higher than birth rates, there will be a natural population decrease. There is evidence that in communities where there is a sustained demand for dogs (for the protection of households, property, and livestock), a natural population decrease (where mortality rates exceed birth rates) may be compensated for by an increase in human-mediated in-migration of dogs into the population, to maintain the supply. If these new dogs are not vaccinated against rabies, this influx of susceptible dogs into the population may offset any benefit to herd immunity brought about by a reduction in the birth rate through sterilization. Cost-effective rabies control in dogs through mass vaccination may be best achieved by encouraging stable, healthy populations of vaccinated dogs. This can be achieved by reducing the turnover rate of populations (loss of vaccinated dogs through mortality or out-migration, and influx of unvaccinated dogs through birth or in-migration), which in turn relies on an understanding of demographic rates (birth, death and migration), and factors that affect those rates, in dog populations in rabies-endemic areas.

The ongoing Health and Demographic Surveillance System in Dogs (HDSS-Dogs) is collecting data on rates of birth, mortality and migration in a population of owned, free-roaming dogs in a rabies-affected, resource-constrained community in Bushbuckridge

Local Municipality, Mpumalanga Province, South Africa. Data from this project shows a relatively high mortality rate in the population. Owner reports on causes of death suggest that the majority of deaths (67%) are due to disease, while only 8% of deaths are due to accidents. The current study was therefore developed to collect in-depth data on age and sex-specific rates and causes of mortality in dogs enrolled in the HDSS-Dogs.

CHAPTER 2 : LITERATURE REVIEW

2.1 Rabies

Rabies is an acute, fatal, progressive, incurable viral encephalitis affecting all warm-blooded animals (mammals), including humans (Rupprecht et al., 2002). It is caused by neurotropic RNA viruses in the family Rhabdoviridae, genus Lyssavirus (Rupprecht et al., 2002). Fourteen lyssaviruses are currently recognized within the genus including the prototype species, rabies virus (RABV) and thirteen 'rabies-related viruses' (Wunner and Conzelmann, 2013). These share certain morphological and structural characteristics, and most have been associated with a rabies-like encephalomyelitis in humans or other animals. RABV is distributed worldwide among specific mammalian reservoir hosts comprising various terrestrial carnivores and bat species. Dogs are the primary reservoir host of RABV in Africa, transmitting the virus to both humans and animals (Talbi et al., 2009). In Africa and Asia the disease burden is very high, owing to the fact that dog rabies control is generally nonexistent or inadequate (Knobel et al., 2005, Dodet, 2009). Dog-associated rabies in most sub-Saharan African countries has been on the increase over the last seventy years (Cleaveland, 1998). Over 90% of reported and confirmed human rabies cases or exposures in the developing world are from dogs (WHO, 1999). Free-roaming dogs either owned or not, may be allowed to roam the communities (Slater, 2001). Free-roaming dogs and their population dynamics influence the occurrence of rabies in communities, and so it is important to understand these dynamics (Slater, 2001). The need to address the minimum pre-requisites in planning a study, including but not limited to the dynamics, size and demography of a given population, addressing the variability of rabies epidemiology in different locations, is imperative (Wandeler et al., 1988, Meslin et al., 1994)

2.2 Rabies in the developing and the developed world

In many developing countries, where dogs are the main reservoirs of RABV, canine rabies remains a significant problem. In these areas, transmission to humans remains a problem (Knobel et al., 2005). It is estimated that every ten minutes, someone dies of this disease in the developing world, and over 7 million people receive post-exposure prophylaxis (PEP) annually (Knobel et al., 2005). Dog-transmitted rabies is estimated to kill approximately 24,000 and 31,000 people annually in Africa and Asia respectively (Knobel et al., 2005). Disability-adjusted life years (DALYs) are a standardized, comparative measure of the burden of disease(Murray, 1994). For any particular condition, it is the composite score of the years of life lost (YLL) due to premature mortality and the years of life lived with a disability (YLD) due to the condition (Murray, 1994). Deaths due to rabies are responsible for an estimated 1.74 million DALYs lost per annum. Furthermore, following the side effects of rabies nerve tissue vaccines, DALYs lost through morbidity and mortality are estimated at an additional 0.04million (Knobel et al., 2005). The bulk of expenditure, accounting for nearly half of the total PEP costs of rabies on, is borne by patients. A study of the burden of rabies in Africa and Asia showed that there was five times more rabies death in rural areas than in urban areas (Knobel et al., 2005). Children in particular are at higher risks of exposure to rabies from dogs, and are also more likely to suffer multiple bites to the face or the head - exposures associated with a higher risk of developing the disease (Knobel et al., 2005).

Whereas in developed countries, cases of dog-transmitted human rabies have been eliminated or greatly reduced (Velasco-Villa et al., 2008), in most developing countries, rabies remains a neglected disease. Although effective and economical control measures are available, low levels of political commitment to rabies control can be attributed to a lack of accurate data on the true public health impact of the disease (Knobel et al., 2005).

2.3 Rabies in South Africa

In South Africa, rabies may have existed for more than a century, with the mongoose variant of rabies virus implicated in early accounts of the disease (Snyman, 1940). There is anecdotal evidence of human disease thought to be due to wildlife exposure in the 19thand 20th centuries (Weyer et al., 2011). Rabies in dogs in South Africa was first confirmed in a rabbit inoculation test in 1893 (Du Toit, 1930). This small outbreak in the Eastern Cape was thought to have been due to an infected owned dog imported from England. The disease was eradicated by the introduction of movement controls, destruction of stray dogs and muzzling of dogs. Human rabies in South Africa was first confirmed in 1928, following the death of two children bitten by a yellow mongoose in the Wolmaranstad district of the North West Province (Herzenberg, 1928). The occurrence of endemic “herpestid” (previously referred to as “viverrid”) mongoose rabies is closely associated with the distribution of yellow mongooses (*Cynictus penicillata*) (Neitz and Marais, 1932). This species occurs widely on the central plateau of the country (Swanepoel et al., 1993). Although dogs were thought to be occasionally infected through contact with rabid yellow mongooses, there is no evidence that canid rabies was present in South Africa before the late 1940s (Swanepoel et al., 1993).

Veterinary authorities in South Africa were aware of this strain of rabies virus (the canid strain) that existed in Namibia and Botswana prior to 1950 (Swanepoel et al., 1993); however, dog rabies was first confirmed in South Africa in 1950 in the Limpopo Province, and reached Zimbabwe later that year. It then spread through Mozambique in 1952, and dog rabies was confirmed in KwaZulu-Natal (KZN) in 1961. Rabies was eliminated by 1968 in KZN due to vigorous vaccination and dog elimination. The disease reappeared again in KZN in 1976, likely due to the migration of refugees from Mozambique. Evidence shows that rabies is moving northwards from KZN into south-eastern Mpumalanga, and southwards into areas of the Eastern Cape Province, likely due to the movement of people and their pets (Coetzee and Nel, 2007). Canine rabies is currently endemic in the north-eastern regions of the Eastern Cape, the entire KZN and the eastern and south-eastern areas of Mpumalanga Province adjoining Swaziland. It has moved eastward through Lesotho to parts of the eastern Free State. More recently, increasing numbers of rabies cases have been reported in the Limpopo Province, especially in areas where it was formally controlled such as the Vhembe district of Limpopo Province (Cohen et al., 2007). The entire country of South Africa was declared rabies-endemic in 1999. Johannesburg in the Gauteng Province reported its first local transmission of rabies virus in 2010 (Sabeta et al., 2013).

2.4 Dog rabies control

The control of rabies in domestic dogs is important for public health, especially in Africa and Asia where canine rabies is endemic and control has been severely neglected (Rupprecht et al., 2002). Elimination of rabies in dogs through animal vaccination is unarguably the most cost-effective strategy for controlling and preventing rabies in humans (Knobel et al., 2005). Throughout Africa and Asia, domestic dogs are the major reservoir of RABV. The relationship between human rabies and the bite of a "mad" dog has been recognized a long time ago (Neville et al., 2004). Measures such as the muzzling of dogs, and movement restrictions successfully eliminated dog rabies in parts of Europe in the 19th century. Furthermore, interventions such as culling or sterilization programmes targeting free-roaming domestic dogs, aiming to stabilize or reduce population size or density, adjust population structure, improve overall health, or alter the behaviour of the dog population have been practiced. These methods are sometimes combined with rabies vaccination to manage dog populations and rabies (Reece and Chawla, 2006, Totton et al., 2010b, Totton, 2009). It is important to note that dog vaccination programs alone can effectively control canine rabies in most circumstances (Knobel et al., 2005). In the early 1900s, Japan began the development of animal rabies vaccines, with the first mass vaccination in dogs in 1921 (Umeno, 1921). Over the second half of the century, successes in rabies elimination which were associated with mass vaccination were reported in Western Europe (King et al., 2004), Japan (Shimada, 1971), and the USA (Belotto et al., 2005). Between 1993 and 2002, cases of human and canine rabies fell by 80% in many countries in the Latin America (Belotto et al., 2005). Current practices like mass vaccination of dogs, improved implementation of post exposure prophylaxis in people, rabies monitoring, notification of suspected rabid animals, viral circulation surveillance, quantification of dog population and population control of stray dogs are responsible for this decrease (Schneider et al., 2007). Similar success in the control of rabies in some countries in Africa was recorded during the colonial era. This was achieved through mass vaccination and also through measures involving strict adherence to 'tie-up' orders of dogs (Shone, 1962); however, the disease re-emerged as these control measures lapsed. The lack of awareness about the true burden of rabies disease has compounded this issue by the perception that rabies is of less importance than other infectious diseases, and also attributable to less funds allocated to the control of rabies in Africa (Kaare et al., 2009).

2.5 Herd immunity and dog vaccination coverage

Herd immunity is the phenomenon that occurs when the vaccination of a significant portion of a population or herd provides a measure of protection for individuals who have not developed immunity (John and Samuel, 2000). The term herd immunity was first used in 1923 in a paper titled “The spread of Bacteria infection: the problem of Herd immunity” (Topley and Wilson, 1923). Herd immunity occurs through the protection of a population by the presence of immune individuals. Herd immunity can be defined as “the resistance of a group to attack by a disease to which a large proportion of the members are immune, thus lessening the likelihood of a patient with a disease coming into contact with a susceptible individual"(Fox et al., 1971).

Canine rabies in dogs and humans can be controlled and in certain instances eradicated by the mass vaccination of dogs against the virus. Mass vaccination based on the concept of herd immunity is used in the control of infectious diseases in populations. The maintenance population must be targeted to control the disease and to eliminate infection (Lembo et al., 2010). The long-term effectiveness of vaccination efforts is influenced by host demography, because as vaccinated dogs die and new susceptible dogs are born, vaccination coverage levels decline. Furthermore, the movement or migration of dogs by people affects coverage, particularly if large numbers of unvaccinated dogs are brought into an area. In Africa, over 30% of the dog population is made up of young puppies (Barrat et al., 2001). There is a perception by dog owners and veterinary authorities that puppies should not be vaccinated; with consequent insufficient coverage (Kaare et al., 2009). Evidence shows that rabies vaccine can be administered to puppies less than three months of age (Barrat et al., 2001). Although some laboratories have indicated that maternally-derived antibodies (MDAs) may adversely affect vaccine-induced active immunity in puppies less than 120 days of age (Precausta et al., 1985), other recent studies have countered that MDAs do not significantly influence puppies from responding to vaccination (Chappuis, 1998, Barrat et al., 2001). This is very important given that birth rates in most dog rabies endemic areas are high and therefore coverage levels decline rapidly. Including puppies in the vaccination rounds is imperative to the maintenance of sufficient herd immunity in the dog population for adequate canine rabies control, but most countries have adopted the three months cut off age as a policy (Beran and Frith, 1988). Reducing the rate of turnover in dog populations helps to maintain herd immunity in the period between vaccination campaigns. This is also true when the mortality of dogs in the maintenance population is taken into consideration. Hence to achieve stability in the maintenance population, a critical factor will be to understand the rates at which dogs give birth and the rates at which dogs die. The rate at which dogs migrate into and out of the population is important, to understand its dynamics, helps for a successful control of canine rabies. Interventions that lead to permanent or temporary inability of individuals to reproduce in a dog population affect the birth rate. Such practices may include surgical sterilization (orchidectomy/ovariohysterectomy), and immunocontraception. However, when birth rate is reduced but mortality rate is unchanged, this will lead to a reduction in the rate of natural population increase. In communities where there is a sustained demand for dogs, used for protection of livestock, property and household, a natural population decrease may be compensated for by an increase in the migration of dogs (mediated by humans) into the population to maintain the supply. Many of these migrating dogs may not be vaccinated, and their influx as susceptible dogs into the population may remove any benefit from herd immunity obtained by the reduction in the birth rates through fertility control (Conan et al., 2015). The World Health Organization (WHO) recommends that, to eliminate or prevent outbreaks of rabies, 70% of dogs in a population should be vaccinated during annual campaigns(WHO, 2013). In practice the achievement of this goal is hampered by factors responsible for declines in the proportion of the vaccinated population over time. This phenomenon of decrease in herd immunity is particularly acute when mass dog vaccination is conducted as pulsed campaigns (rather than through ongoing vaccination of new dogs born into a population), and is influenced by the interval between campaigns. This demographic factor influencing the rapid turnover of domestic dog populations is a challenge to the success of vaccination in the developing countries, thus regular pulse vaccinations will be required to maintain the immune level of the population between campaigns (Hampson et al., 2009). The critical threshold vaccination coverage in a study in Tanzania for rabies was in the range of 20% to 40%, this threshold can be evaluated from the basic reproductive number, which is the average number of secondary infections caused by an infected individual in a susceptible population (Anderson and May, 1991). If coverage falls below the critical threshold, rabies transmission can be sustained and outbreaks can persist. This study shows that herd immunity declined rapidly between vaccination campaign due to births and deaths in the domestic dog population; therefore to maintain the herd immunity above the critical point of between 20% to 40%; the target vaccination coverage to be achieved in annual campaigns was 60% (Hampson et al., 2009). The success of this concept demonstrated the elimination of dog rabies through vaccination in many countries (Hampson et al., 2007). Mass vaccination of dogs as a cost-effective rabies control will be optimally achieved when a stable, healthy population of vaccinated dogs is encouraged. This will also be achieved when the turnover rate of population is reduced, that is; loss of vaccinated dogs through mortality or out-migration, and influx of unvaccinated dogs through birth or in- migration is reduced. All these will rely on an understanding of demographic rates (birth, death and migration), including factors that may affect those rates, in dog populations in rabies- endemic areas. An important aspect is to understand the factors responsible for mortality in dog populations and ways to mitigate them. Cost-effective reduction in mortality rates, in conjunction with fertility control, will lead to stable, healthy populations of domestic dogs, thereby enhancing the benefit of herd immunity to rabies through vaccination (Hampson et al., 2009, Conan et al., 2015)

2.7 Causes of mortality

Understanding rates and causes of mortality in dog populations gives understanding of population turnover, and can inform the design of interventions to create stable, healthy population of dogs. Vital information can be obtained from population data on the rates and causes of death in dogs. Ongoing or current causes of health problems in a breed can be identified by looking at breed-specific mortality rates and estimates of the proportion of deaths due to a particular cause (Bonnett et al., 2005).This can be used to inform and evaluate health promotion strategies. The age pattern of death, especially estimates of survival to certain ages, is informative for current and prospective owners of a breed, and for veterinarians and researchers (Bonnett et al., 2005). Understanding causes of death (disease aetiologies), and how these vary with age, sex, breed and other factors (e.g. management, lifestyle) is also important (Bonnett et al., 2005). There are few studies on canine longevity and cause of death in dog populations. A high proportion of such studies have used veterinary teaching hospital populations, which invariably include a high population of referral cases (Adams et al., 2010). A study of purebred dogs in the UK collected information on the causes of death and longevity of dogs owned by members of the numerically-largest breed clubs of 169 UK Kennel Club-recognized breeds. Using 58,363 questionnaires and sent to breed club members in 2004 (Adams et al., 2010). Owner-reported age at death and causes of death for all dogs that had died within the previous ten years were recorded. A total of 13,741 questionnaires (24% response rate) containing information on 15,881 deaths was included in the analyses (Adams et al., 2010). The median age at death was reported at 11 years 3 months (range: 2 months -23 years and 5 months); this varied by breed. The most common causes of death were cancer (n = 4,282, 27%), old age (n = 2,830, 18%), and cardiac conditions (n = 1,770, 11%) (Adams et al., 2010). Breed difference in life span and causes of death was shown in this survey, and this survey supports previous evidence that smaller breeds tend to have longer life span compared to larger breeds (Adams et al., 2010). Another study, into the mortality of dogs in North America from 1994 to 2004, showed that not all causes of death contribute equally to mortality within age, size, or breed cohorts (Fleming et al., 2011). The total number of dogs in the dataset from 1984 to 2004 that met the inclusion criteria was 80,306. This study showed that younger dogs die most commonly from trauma, congenital disease, or infectious causes, while older dogs to a large extent die of cancer, peaking within the 10-year-old age range and decreasing in frequency thereafter. In older dogs, complications of the nervous system are also implicated as a major cause of death (Fleming et al., 2011).

Another study into the mortality of purebred and mixed breed dogs in Denmark (Proschowsky et al., 2003), recorded causes of death and age at death of 2,928 dogs from a questionnaire study among members of the Danish Kennel Club (DKC) in 1997.Twenty-two different causes of death and a miscellaneous group were included in the questionnaire. The median age at death for all dogs in the study was 10.0 years. Old age was the most frequent reported cause of death (20.8%) followed by cancer (14.5%), behavioural problems (6.4%) accidents (6.1%), hip dysplasia (4.6%), heart diseases (4.6%) and spinal diseases (3.9%) (Proschowsky et al., 2003). Similar data on over 350,000 insured Swedish dogs up to 10 years of age, during 1995 to 2000, was presented in a study by Bonnett et al. (2005).A total of 43,172 dogs died or were euthanized; of these, 72% had a diagnosis for the cause of death including tumour (18%), trauma (17%), locomotor (13%), heart (8%), and neurological conditions (6%)among others (Bonnett et al., 2005).

Whereas the above studies have focused on the situation in the developed world, a study of canine life expectancy and causes of deaths in dogs in a metropolitan area in Sao Paulo, Brazil was conducted on 2,011 dogs attending a university veterinary hospital or collected from veterinary clinics, kennels and private owners. The median age of dogs at death due to all causes was 36 months (Bentubo et al., 2007), and contrary to the results of the studies in the developed world, dogs of medium, large and giant breeds live longer than small breeds. Female and neutered dogs lived longer than male and intact dogs, and there was no difference in life expectancy of pure bred or mixed indigenous breeds (Bentubo et al., 2007). In addition, mortality was due to infectious diseases, neoplasms, and traumatic injuries in decreasing order and canine life expectancy was shorter than that observed in other countries (Bentubo et al., 2007).

Apart from this study, there appears to be lack of peer-reviewed data from most developing countries and resource-poor settings.

2.8 Verbal autopsy

Verbal autopsy is a research method that helps determine probable causes of death through the gathering of health information in cases where there are no medical records (Fortney et al., 1986, Gray et al., 1990, Bang and Bang, 1992, Ross, 1992, Snow et al., 1992, Snow et al., 1993, Chandramohan et al., 1994). The concept is that a trained interviewer uses a questionnaire to collect information from a relative or close care giver or another person familiar with a deceased individual; the collected information is about the signs, symptoms and demographic characteristics before death. The method is based on the assumption that most aetiologies of death can be distinguished by their signs and symptoms, and that these can be accurately recognized, recalled and reported by a lay respondent (Snow et al., 1992). To enhance good health planning and policy, verbal autopsy is now adopted especially in resource-constrained communities, by researchers to achieve evidence-based data in such places where individuals die at home and where no civil registration system is in place. Studies in South Africa have shown that the use of verbal autopsy in human populations is reliable and cost-effective (Botha and Bradshaw, 1985, Kahn et al., 1999). This method serves as an essential tool for research and public health studies, in that it gives some structural mortality estimates at a population or community level. While this offsets the limitation of lack of records of mortalities occurring away from health facilities and certified cause of death in such areas, its accuracy for attributing causes of death at an individual level is questioned by some practitioners (Zahr, 2007).

Verbal autopsy methods are evolving in areas such as the development of questionnaires and the use of reliable statistical methods that reduce human bias in assigning cause of death. Existing practices include:

Physician review: In this method, questionnaires are reviewed and cause of death is assigned by two or three physicians (Todd et al., 1994). There is often a conflict of opinions between physicians with this method, it is time consuming and expensive with little reliability, and cannot be used for comparisons across populations (Murray et al., 2007).

Expert system*:* In this system, a decision tree is constructed, to obtain the best physicians judgment; it is more reliable, but when symptoms are measured in error, the method can be highly inaccurate (Chandramohan et al., 1998, Coldham et al., 2000).

Statistical classification: This approach requires additional sample or deaths from a medical facility where each cause is known and symptoms are collected from relatives (Quigley et al., 1996)

Verbal autopsy in people has provided vital information on causes of death in developing countries and in resource-constrained areas. Verbal autopsy methods have been used in human studies in South Africa, especially where the quality of the statistical data collected routinely is poor, showing marked under-reporting of deaths and misclassification of the causes of these mortalities (Botha and Bradshaw, 1985). Kahn et al., (1999) have also used verbal autopsy as a tool in the Agincourt field site in Bushbuckridge, South Africa as early as 1992. In this case, deaths were recorded and follow-up interviews were conducted by trained field workers with closest caregivers. It was demonstrated that a single verbal autopsy tool can be used effectively and satisfactorily validated across all age groups and in areas without vital registration, to prioritize public health problems, inform resource allocation, and target and evaluate community-based interventions (Kahn et al., 2000).

In the current study, we used a semi-structured questionnaire to conduct verbal autopsies to gain information on causes of death in adult dogs and puppies that died during the course of the study.

2.9 Aim and objectives of the study

The aim of this study was to determine the rates and causes of mortality in owned dogs enrolled in the ongoing Health and Demographic Surveillance System in Dogs, through frequent follow-up visits, post-mortem examination, and owner interview ('verbal autopsy').

The objectives of the study were to:

1. Determine the rates and causes of mortality over a 12-month period in an age-stratified cohort of 200 adult (≥ 1 year) female dogs.
2. Determine the rates and causes of mortality over a 12-month period in an age-stratified cohort of 200 adult (≥ 1 year) male dogs.
3. Determine the sex-specific rates and causes of mortality from birth to three months of age, for litters of puppies born over a 12-month period to females in the above cohort.
4. Test for the effect of age (for adults) and sex on mortality rates in the adult cohorts and puppies.
5. Describe and categorise causes of death determined through verbal autopsy

CHAPTER 3 : MATERIALS AND METHODS

3.1 Study area

The study area is located in South Africa, which stretches from 22°S to 35°S and from 17°E to 33°E, with a surface area of 1,219,602 km^2. The specific study location was Hluvukani village of the Mnisi community, in the Bushbuckridge Municipality, Ehlanzeni District, Mpumalanga Province. A map of the location of the study area is shown in Figure 3.1. The global positioning system coordinates in the approximate centre of the study site are 24^{o}39'S, 31^{o}20'E.

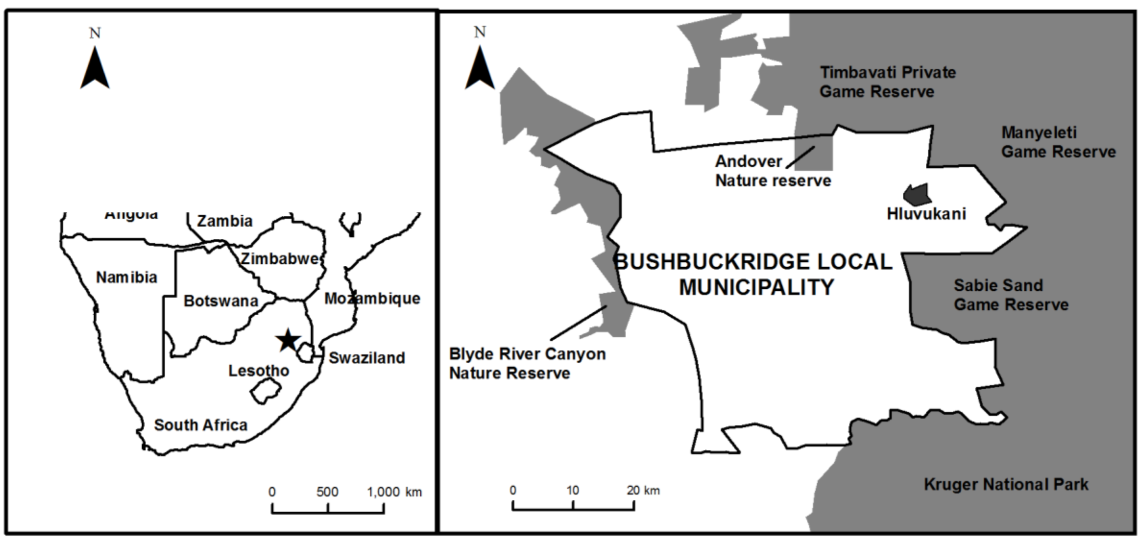

Figure 3.1: Location of Hluvukani village in Mpumalanga Province, South Africa

The mean annual rainfall of Bushbuckridge is about 600mm. The average maximum temperature is 29^{o}C in the summer and the average minimum temperature is 12^{o}Cin winter. Annually, the mean minimum and maximum temperatures ranges from; 6^{o}C to

$23^{o}C$. This area was purposively selected as the site for this study because of its importance as a domestic animal-human-wildlife interface area, and the prior presence of a Health and Demographic Surveillance System in Dogs (HDSS-Dogs) (Conan et al., 2015).

A Health and Demographic Surveillance System collects demographic data in a geographically-defined population on a longitudinal basis. Core parameters such as social units, births, migrations, mortality and causes of mortality, are measured in HDSS sites, with the purpose of informing rational strategic health planning and meaningful health programmes for people in developing countries, particularly those countries without systems for registration of vital events (e.g. births and deaths).

The Health and Demographic Surveillance System in Dogs (HDSS-Dog; University of Pretoria protocol V022/11) allows the collection of data on rates of birth, mortality and migration in the population of owned, mostly free-roaming dogs in Hluvukani. The HDSS-Dogs has been established in Hluvukani village since 2011. All dogs enrolled in the HDSS-Dogs and which can be readily handled have a microchip subcutaneously implanted, for unique and permanent identification. All households in the study area (known as the Demographic Surveillance Area or DSA), and all owned dogs in households, are uniquely identified and recorded in the HDSS-Dogs database, which is updated during follow-up visits to all households every 4-6 months. The HDSS-dogs covers a population of approximately 10,000 people in 2,000 households. At the time of enrolment, the owned dog population was estimated at 755dogs.

3.2 Study design and enrollment of dogs

For the adult mortality study, the aim was to enroll a cohort of 200 adult (one year of age and older) male dogs and 200 adult female dogs, and to follow each dog in the cohort for a period of 12 months. It was intended to randomly select eligible male dogs from the HDSS-Dogs database, and enroll these and any eligible female dogs present in the same households. Enrolment took place in May and June, 2014. In the event, all adult female dogs present in the study area were enrolled (n = 164), along with 76 male dogs living in the same households. An additional 127 male dogs were randomly selected and enrolled from the HDSS-Dog database. The selection of all dogs (female and males) was stratified by age into three categories: 1–2 years, 3-4 years and 5 years and above. After selection of a dog, enrollment was done by locating the household using the municipal stand number, and by identifying the dog through its microchip number. Dogs without microchips or those whose microchips numbers could not be read were given a unique identification, using the dog’s name, sex, and stand number of the household. In addition, the research team took photographs of the study dogs as an additional identification method. For the puppy mortality study, the aim was to enroll all litters born to the cohort of adult female dogs over the 12 months of observation of that cohort, and to follow individual puppies for a period of 120 days.

3.3 Ethics

An informed consent document (Appendix 1) was prepared in English and translated into the local language of Xitsonga by an indigenous field research assistant. This consent form was tested for accuracy of translation, by back-translation to English by a second indigenous field research assistant. The form was then read out to each selected dog owner, who then signed the document to give their informed consent to participate in the study. Each consent form had on it the stand number, the respondent's or owner's name, phone number, owner's signature, and identification number and name of the dogs. A copy of the consent form was left with each owner.

3.4 Communication of event (births and deaths)

The consent form included the dedicated phone number for communication between dog owners and the study team, to report events of interest (births or deaths) within the study cohorts. The study team also contacted the owners to make enquiries on the cohort. Periodic reminders in form of bulk SMS were sent to each of the households, to facilitate the reception of information from them regularly. The participants were reimbursed any costs incurred in contacting the research team by means of phone calls or SMS, to encourage respondents to notify the study team of events in the participating dog cohorts. Follow-up visits to the enrolled households with litters were conducted every week, in addition to which a reminder to report puppy deaths was sent to the phone numbers of these respondents every month. Households with litters were visited as soon as a birth report came, to ascertain the litter size and sex ratio of the litter. Dogs in the adult cohorts were visited every month, and a reminder SMS was sent to the phone numbers of these owners every two weeks.

3.5 Data collection

3.5.1 Births

A questionnaire (Appendix 2) was used to record all the information observed by the research team, about the litter and the identity of the female dog on a longitudinal basis. Data collected on this form included the stand number of the household, microchip number or identification code of the bitch, number of puppies born, the sex of puppies, the date of birth, and the number and sex of still-born puppies, if any. A second form recorded the events that took place per week for a period of 120 days for the litters.

3.5.2 Verbal autopsy

A verbal autopsy questionnaire (Appendix 3) was developed by the team to interview owners in the case of death of one of the study dogs. We adopted and modified the standard questionnaire used by the WHO in a study of a standard verbal autopsy method for investigating causes of death in infants and children (Anker et al., 1999). The modification of the WHO standard questionnaire was to meet the needs of our study, as it affects mortality in dogs and to accommodate diseases that affect dogs in this rural area. The questionnaire was translated to Xitsonga and pre-tested in the field by the research team. We classified the verbal autopsy results into three main categories of causes of death: (i) natural causes (e.g. infectious diseases or envenomation), (ii) non-natural causes (accidental, deliberate and undetermined) and (iii) euthanasia. The accidental subgroup of non-natural was further classified into anthropogenic and non-anthropogenic causes (i.e. with or without human agency).

This verbal autopsy questionnaire had eight sections as shown below (see Appendix 3 for more details):

1. Background information on dog and household
    - Name
    - ID no. of dog/home address
2. Background information about the interview
    - Language
    - Interviewer name
    - Date of interview
    - Date of entry into the computer
3. Background information about owner/care giver/respondent
4. Information about the dog
    - Age of the dog
    - Date of death
    - Where the dog died
5. Open history questions
    - Description of illness in caregivers own words (no specific prompting, and it's a detailed narrative)
    - Length of illness
    - Type of care given---hospital----home----elsewhere

6a. Accidents (puppy)

- Injuries
- Accidents
- Poisoning

- Bites
- Burns
- Drowning

6b. Puppy death (Puppies<120days)

7a. Accidents (Adult)

- Injuries
- Accidents
- Poisoning
- Bites
- Burns
- Drowning

7b. Adult death (Adults≥12 months)

8. Treatment

3.5.3 Data on management practices in household

A questionnaire (Appendix 4) was developed to ask about the type of dog management practiced in the household; this was to give a clear picture of factors that could influence the wellbeing of the dogs. The questions included confinement practices, the type of food and how many times dogs are fed, sources of water, rabies vaccination status, how many dogs in each household at the start of the study and at the end of the study.

3.6 Post-mortem examination

In the event of a dog's death, owners were requested to inform the research team within 24 hours by phone call, SMS or visitation to the Animal Clinic at Hluvukani. The research team equipped with carcass collection materials visited the stand and collected the dead dog. A verbal autopsy interview with the owner or a close caregiver was conducted by the research team. The carcass was put in a sealed plastic bag and transported by the research team to the post-mortem room. An equipped post-mortem room located at the Hans Hoheisen Wildlife Research Station, at the Orpen gate of Kruger National Park (approximately 35 km from the study site) was dedicated to the dog mortality study; this was operated in conjunction with the Veterinary Pathology section of the University of Pretoria.

A post-mortem examination was conducted by the principal researcher or final year students of the Faculty of Veterinary Science at the University of Pretoria. Organs were systematically harvested from the thoracic and abdominal cavities, and long bones samples were taken. These samples were fixed in 10% formalin sample bottles. Brain samples were collected and fixed in glycerin saline, ocular fluid was taken from the carcass using a 2cc gauge syringe and needle, and simple blood smears were conducted and fixed on slides using the Diff-Quik fixing solution. Faecal examination using the faecal floatation method was also conducted and slides with positive parasitic eggs were recorded and preserved for further examination. All of these samples were taken for further histopathology and immunochemistry examinations at the Veterinary Pathology section of the University of Pretoria.

A master transport permit was obtained from the State Veterinary Officer at the Mpumalanga Area Veterinary Office, Department of Agriculture, Forestry and Fisheries, to transport obtained samples to the Pathology Section at the Faculty of Veterinary Science, University of Pretoria. The formalin-fixed samples were sectioned on tissue cassettes within five days after arrival. Multiple tissue sections per case slide were examined, using a light microscope. Histopathological evaluations were supported by histological check-lists by the pathologist; the combination of macro- and histopathology examinations, as well as immunohistochemistry provided an indication of the diseases underpinning the cause of death, or the decision to euthanize the dog. Fresh tissues were not available for confirmation or exclusion of a diagnosis by means of bacterial or fungal culture, and virus isolation and PCR could not be conducted due to the location of the target population combined with transport restrictions. The only additional diagnostic tests used included immunohistochemistry and special staining techniques. Therefore it was not consistently possible to determine an exact aetiology for all examined cases. The final outcome of each case was in a written report format. Master problem lists reported by the pathologist, which enumerated the disease profile from most to least important, were used to classify causes of death.

3.7 Data management and analysis

3.7.1 Microsoft Access database

Data collected was entered on spreadsheets and managed using Microsoft Access 2007 version. The database comprised the following tables, which were linked to each other on the basis of the relationship between the entries.

- **Household table:** this consists of entries such as the owners' names, the phone numbers, and consent given, the date of consent by the owner, stand number, and date of first visit.

-**Dog list table**: the entries on this table describe the particulars of dogs that were enrolled, such as the identification number, dog name, sex of dog, age of dog, stand number of the household, and the date of first visit to the stand.

-**Female dog list**: the entries on this table included the dog identification number link, name of dog, number of litters, number of puppies born alive, sex of puppies born alive, number of stillborn puppies, sex of stillborn puppies.

-**Dead dog table**: entries included the identification link of dogs, identification link of puppy to its dam, dead adult or dead puppy entry, date of exit by events such as death, stolen or missing, sex of dead dog.

-**Post-mortem table**: entries include the identification link of dead dog, date of post mortem, date of death, post-mortem diagnosis.

3.7.2 Epidata software 2010 version

Data collected on the verbal autopsy questionnaire for the causes of mortality study was transformed and managed with Epidata software 2010 version. The data was entered into the software, by two individuals independently and checked for consistency and other data entry errors.

3.7.3 Mortality rates and survival analysis

Mortality rates were calculated for the cohorts and then stratified by sex and age (adults only), after which the sum of days of follow-up was calculated to determine the number of days each dog contributed to the follow-up days. The number of dog-years was calculated by dividing the sum of dog-days by 365.25 days. The mortality rate was derived by multiplying the number of dead dogs by 1,000, and then dividing it by the number of dog-years lived. This calculation was applied for monthly crude mortality rates for both the adult cohort and the puppies, to derive the monthly crude mortality rates per 1,000 dog-years.

The software R (RCoreTeam, 2013) was used to analyze the data. The package *survival* (Therneau, 2014) was used to create a survival object for both the adult cohort and for the puppies born to the female cohort. Kaplan-Meier analysis was performed for both the adult and the puppies, and a survival curve was plotted for the adults and puppies; this was further stratified by age and sex for the adult dogs and by sex for the puppies. Semi-parametric Cox regression analysis was performed for both populations from enrolment (adults) and from birth (puppies). The log-rank test and chi-square test were used to test for the effects of age and sex on survival.

CHAPTER 4 : RESULTS

4.1 Participating households

4.1.1 Number of households

A total of 399 households with eligible dogs were identified from the HDSS-Dogs database. Eligible dogs were assigned a random number and then sorted from lowest to highest random number, together with their associated households. Households were then visited in the order in which they had been sorted. At first, all eligible dogs (male and female) in visited households were enrolled, until the full quota of male dogs was reached (n = 203). Thereafter, remaining households were visited to enroll female dogs. In the end, all 399 households were visited, and all eligible female dogs enrolled, resulting in a final cohort of only 164 female dogs. Of the 399 eligible households identified from the HDSS-Dogs database, 34 were found at the time of the visit to contain no eligible dogs, as these had died or disappeared since the HDSS-Dogs data had been collected. In summary, 217 households were enrolled, containing the cohort of adult male dogs (n = 203), adult female dogs (n = 164), as well as 125 dogs not enrolled (surplus adult males as well as sub-adults 0-11 months who were not eligible for enrollment). Figure 4.1 shows the location of these enrolled households in the Demographic Surveillance Area (DSA) in Hluvukani.

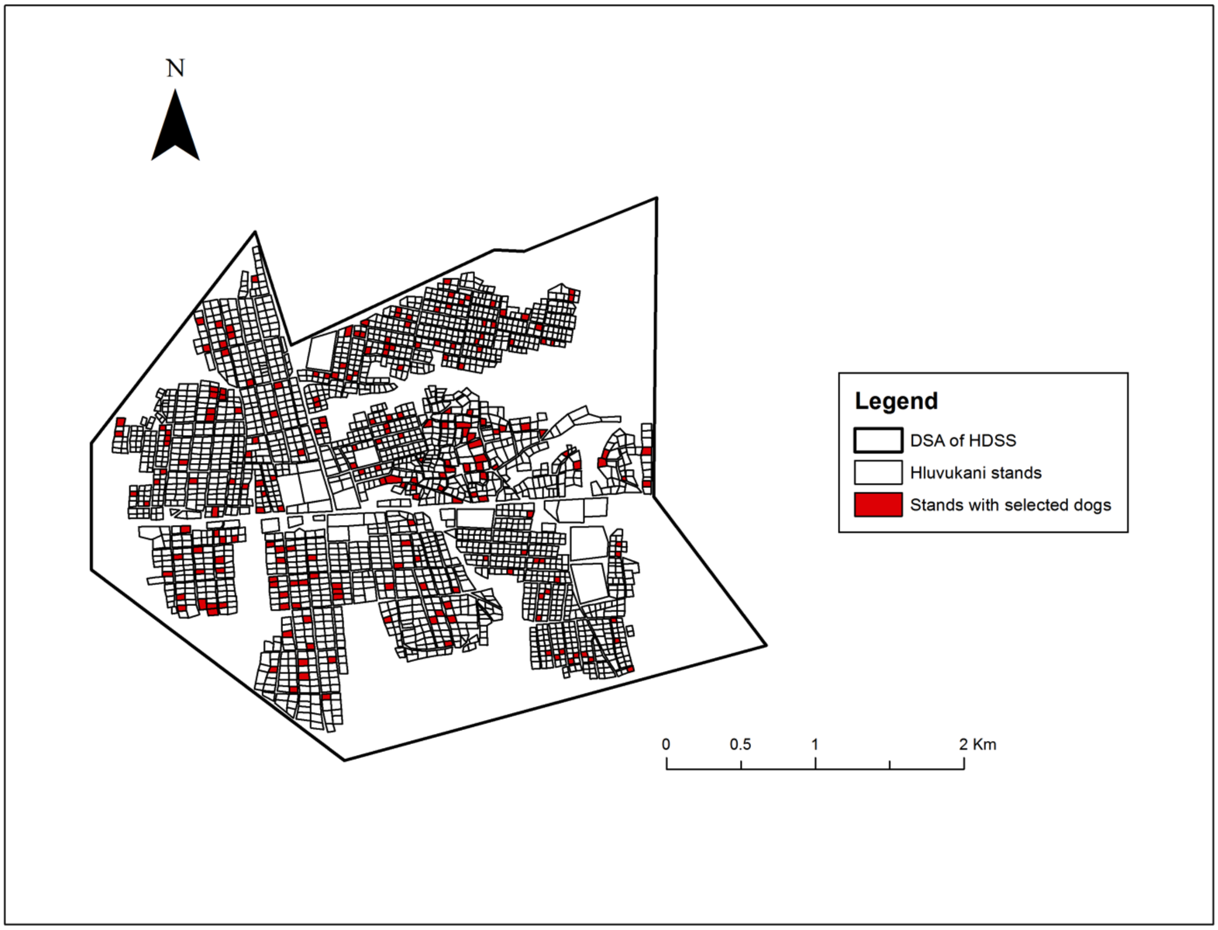

Figure 4.1: The map of households in the study area of Hluvukani, showing in red those selected for the study.

4.1.2 Household-level dog management characteristics

Among the 217 enrolled households, the reasons given for ownership of dogs were as follows: for security (81.6%, n =177), as pets (7.8%, n =17), both for security and as a pet (3.7%, n = 8), for cultural reasons (2.8%, n = 6), for security and hunting 1.8% (n = 4), and for hunting only (0.5%, n = 1). Four households (1.8%) did not provide a reason for keeping dogs. The cultural reason given was said to be ancestral obligation to have a dog that is passed down through generations, especially in families with *sangomas* (traditional healers).

The household-level management practices in terms of feeding show 83.9% (n = 182) feed their dogs two or more times a day, 14.3% (n = 31) feed their dogs once a day, while 1.8% (n = 4) cannot say how many times per day their dogs are feed. For the type of food, 80.4% (n = 194) of the owners provide *pap* (maize meal) and soup or bones, 4.6% (n = 10) leftovers, 5.1% (n = 11) commercial dog food, 0.5% (n = 1) milk and 0.5% (n = 1) meat. The majority of households provided drinking water from a borehole (97.2%, n=211). Two households (1%) provided water from other sources (such as rainwater), while four households (1.8%) did not report providing any water for their dogs.

4.2 Adult cohort

4.2.1 Description of cohort at enrolment

Enrolment of the adult cohort took place from 9th May through4thJune, 2014. The total number of dogs enrolled was 367. Stratification of dogs by age and sex is shown in Table 4.1.

Table 4.1: Description of the adult dog cohort at enrolment, by age and sex.

		Total	**Male (%)**	**Female (%)**
Total		367	203 (55.3%)	164 (44.7%)
Age categories	1-2 yrs	129	71 (55%)	58 (45%)
	3-4 yrs	141	74 (52.5%)	67 (47.5%)
	≥5 yrs	97	58 (59.8%)	39 (40.2%)

4.2.2 Dog-level management characteristics

Among the 367 enrolled dogs, 316 (86.1%) were vaccinated against rabies. In all cases, vaccination was reported to have occurred during the previous year. Table 4.2 shows vaccination status by age and sex. Dogs were classified as confined (caged or restricted to the household) or unconfined (households with partial or no fencing, in which dogs were free to roam without restrictions).Table 4.3 shows confinement status by age and sex. Of the 84 dogs classified as confined, only six (7.1%) were caged.

Table 4.2: Data on rabies vaccination status of 367 dogs stratified by age and sex at enrollment, Hluvukani, May/June 2014.

	Vaccinated	**Not vaccinated**	**Unknown**
Total (n=367)	316 (86.1%)	35 (9.5%)	16 (4.4%)
<u>Sex</u>			
Male (n=203)	176 (86.7%)	20 (9.9%)	7 (3.4%)
Female (n=164)	140 (85.4%)	15 (9.1%)	9 (5.5%)
<u>Age</u>			
1-2 years (n=129)	115 (89.1%)	8 (6.2%)	6 (4.7%)
3-4 years (n=141)	118 (83.7%)	17 (12.0%)	6 (4.3%)
≥ 5 years (n=97)	83 (85.6%)	10 (10.3%)	4 (4.1%)

Table 4.3: Data on confinement status of the cohort of 367 dogs (confined vs. unconfined) stratified by age and sex, Hluvukani, May/June 2015.

	Confined	**Unconfined**
Total (n=367)	**84 (22.9%)**	**283 (77.1%)**
<u>Sex</u>		
Male (n=203)	49 (24.1 %)	154 (75.9%)
Female (n=164)	35 (21.3%)	129 (78.6%)
<u>Age</u>		
1-2 years (n=129)	31 (24.0%)	98 (76.0%)
3-4 years (n=141)	35 (24.8%)	106 (75.2%)
≥5 years (n=97)	18 (18.6%)	79 (81.4%)

4.2.3 Mortalities and loss to follow-up

Of the 367 adult dogs enrolled, 27 (7.4%; 10 males and 17 females) died during the follow-up period, and seven (two males and five females) were lost to follow-up. All seven were lost to follow-up due to disappearance from the household. The characteristics of the dogs lost to follow-up are given in Table 4.4. The median follow-up time for these dogs was 169 days (interquartile range 72-233 days).

Table 4.4: Description of adult dogs that were lost to follow-up during the 12-month follow-up period

Dogs	Date enrolled	Date of exit	Follow-up days	Exit	Sex	Age
1	22-May-14	05-Jul-14	45	lost	Female	3-4
2	22-May-14	05-Jul-14	45	lost	Female	3-4
3	19-May-14	25-Aug-14	99	lost	Male	3-4
4	19-May-14	03-Nov-14	169	lost	Female	1-2
5	21-May-14	05-Jan-15	230	lost	Female	1-2
6	16-May-14	05-Jan-15	235	lost	Male	1-2
7	21-May-14	05-Jan-15	261	lost	Female	≥5

The number of deaths, dog-years lived and mortality rate (per 1,000 dog-years lived) is given in Table 4.5 by age category and sex. Figure 4.2 shows the mortality rates by age category and sex.

Table 4.5: The number of dog deaths, dog-years lived and mortality rates, stratified by sex and age in the adult dog cohort during 12 months of follow-up

	Dogs enrolled	**Deaths**	**Dog-days lived**	**Dog-years lived**	**Mortality rate /1000 dog-years**
Total	367	27	126,938	347.5	77.6
Male	203	10	71,852	196.7	50.8
Female	164	17	55,086	150.8	112.7
1-2 years	129	9	44,540	121.9	73.8
Male	71	5	24,825	67.9	73.6
Female	58	4	19,715	53.9	74.2
3-4 years	141	6	49,645	135.9	44.1
Male	74	3	26,244	71,8	41.7
Female	67	3	23,401	64.1	46.8
≥5years	97	12	32,753	89.6	133.9
Male	58	2	20,783	56.9	35.1
Female	39	10	11,970	32.7	305.8

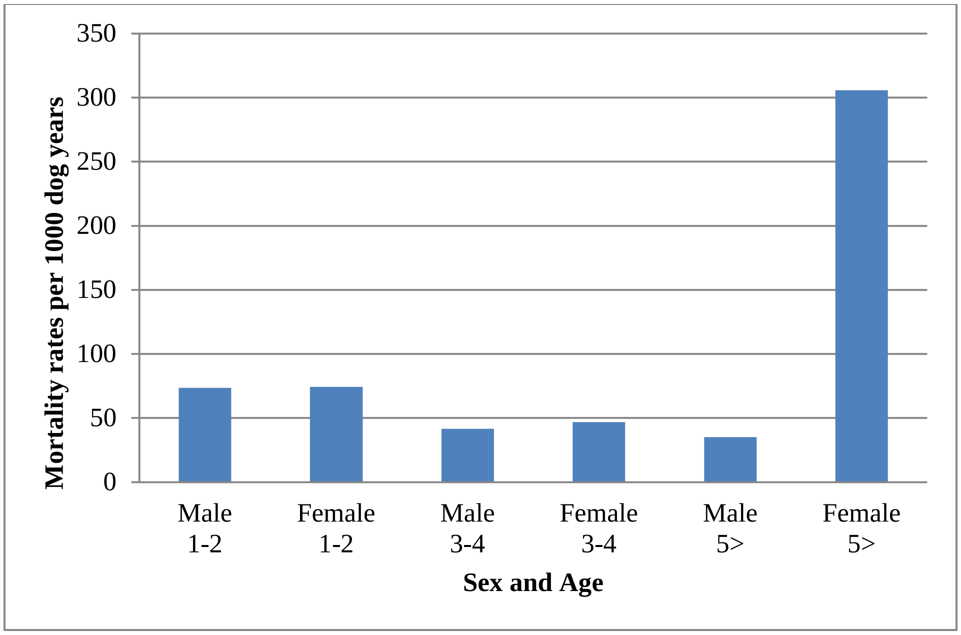

Figure 4.2: A bar plot showing the mortality rate of adults dogs over 12 months, by sex and age.

The crude mortality rates from May 2014, through June 2015 is shown in Table 4.6, and the mortality curve over the same period is shown in Figure 4.3.

Table 4.6: Mortality table by months for the cohort of adult dogs in Hluvukani from May 2014 through June 2015

Month	Number of death	Number of dog days	Number of dog-years	Crude mortality rate (per 1000 dog-years)
May	0	4,036	11.0	0.00
June	5	10,813	29.6	168.89
July	1	11,106	30.4	32.89
August	6	11,043	30.2	198.45
September	1	10,541	28.9	34.65
October	3	10,500	28.7	104.36
November	1	10,413	28.5	35.08
December	3	10,660	29.2	102.79
January	2	10,544	28.9	69.28
February	5	9,386	25.7	194.57
March	0	10,447	28.6	0.00
April	0	9,990	27.4	0.00
May	0	7,054	19.3	0.00
June	0	49	0.13	0.00

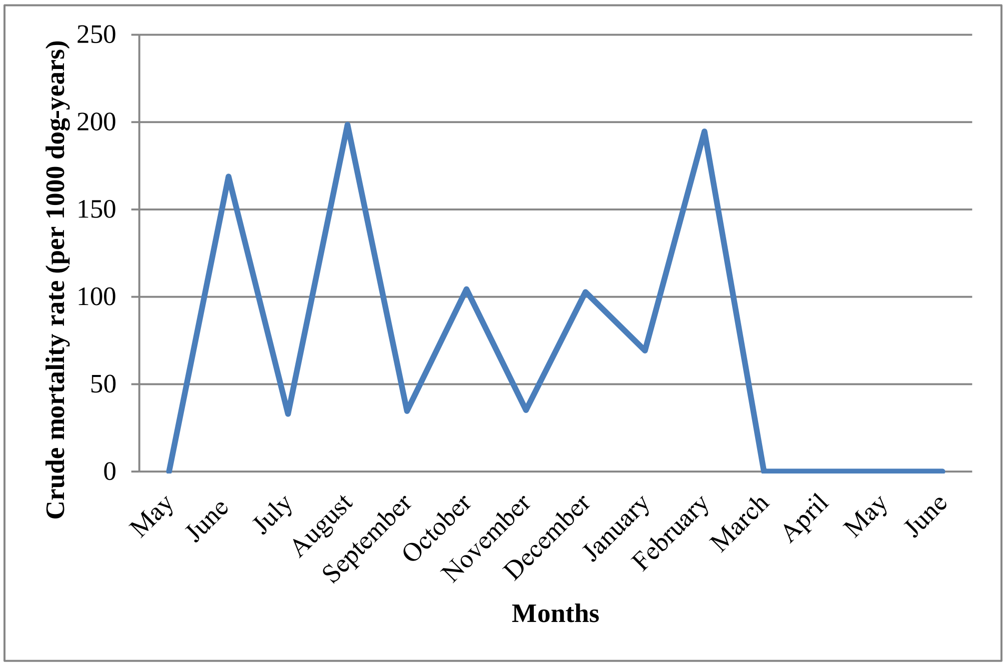

Figure 4.3: A line graph of adult mortality rates by month from May 2014 to June 2015

4.2.4 Kaplan-Meier survival analysis

All the seven dogs lost to follow up were excluded from the survival analysis, due to the fact that in survival analysis the assumption is that the outcome (death) and reason for censoring and loss to follow-up are not related. These dogs may have been reported as lost but it is likely that they may have in fact died or been killed. Figure 4.4 shows the survival curve for the entire adult dog cohort in the study.

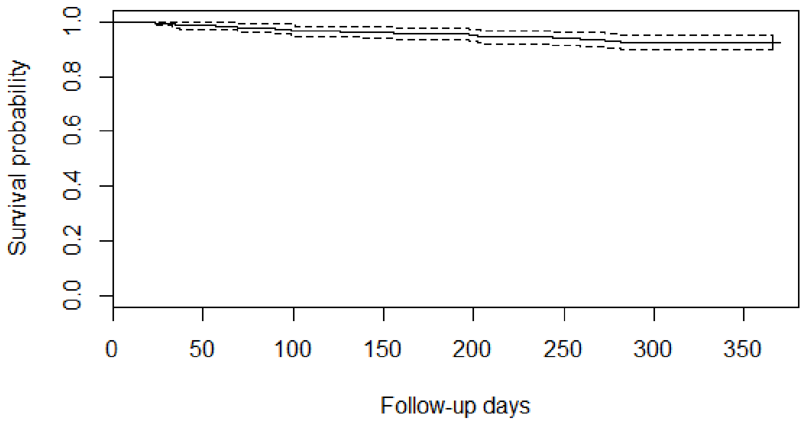

Figure 4.4: Survival curve for enrolled adult dogs during one year. Dotted lines show 95% confidence intervals.

Figure 4.5 shows the survival curves by sex. It shows the probability of the two sexes dying at each time interval.

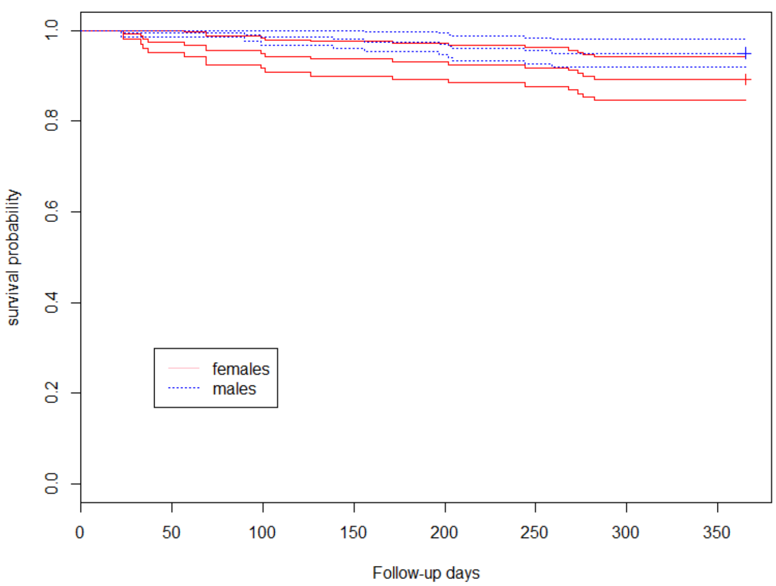

Figure 4.5: Survival curve for comparison between male (blue) and female (red) adult dogs. Dotted lines show 95% confidence intervals.

Female dogs had a shorter survival time (mean = 341.7) compared to male dogs (mean=355.8) (Table 4.7). The difference was significant with the log-rank test (p =0.04) and by Cox regression (p = 0.05).

Table 4.7: Summary of Cox regression and log-rank test for the effect of sex on survival of adult dogs

Sex	N (number of dogs)	Number of deaths	Min	Median	Mean	IQR	Max	Log-rank p-value	Cox regression p-value
Male	203	10	22	366	355.8	366-366	366	0.04	0.05
Female	164	17	23	366	341.7	366-366	366		

Figure 4.6 shows the survival curves for the adult dog cohort by age. It shows the probability of the three ages dying at each time interval.

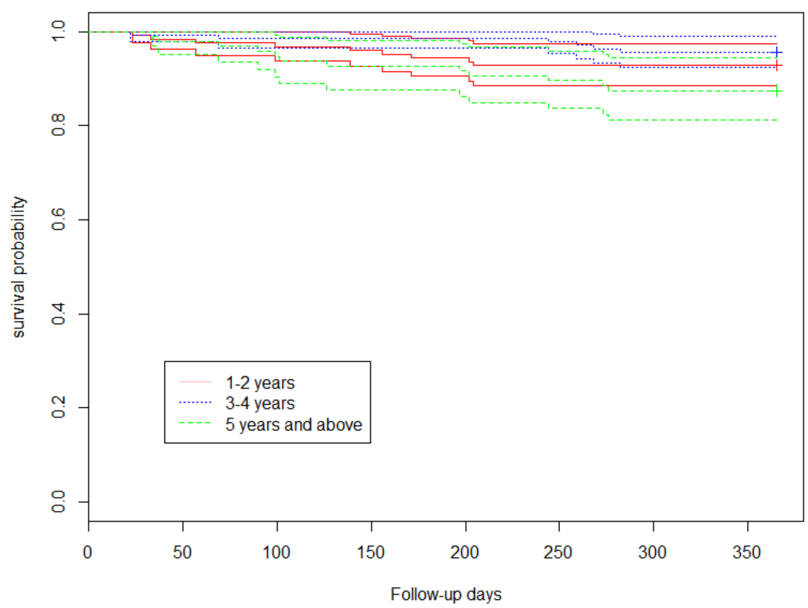

Figure 4.6: Survival curve for adult dogs by age categories. Dotted lines show 95% confidence intervals.

Adult dogs of age 5 years and above had shorter survival time (mean = 338.5), dogs of ages 3-4 years also had survival time (mean = 358.4) more than dogs of ages between 1-2 years old (mean = 348.5). The chi-square test gave a p-value of p = 0.06 and the Cox proportional hazard a p-value of p=0.3as seen in Table 4.8.Thus, there is a marginally significant difference in survival between the age categories of adult dogs.

Table 4.8: Summary of Cox regression and log rank test for the age category of the dog cohort in Hluvukani.

Sex	**N (number of dogs)**	**Number of deaths**	**Min**	**Median**	**Mean**	**IQR**	**Max**	**Log-rank P value**	**Cox regression p-value**
1-2 yrs	129	9	23	366	348.5	366-366	366	0.06	0.3
3-4 yrs	141	6	22	366	358.4	366-366	366		
≥5 yrs	97	12	34	366	338.5	366-366	366		

4.2.5 Causes of death

4.2.5.1 Verbal autopsy results

Verbal autopsy results were collated and analyzed for the 27 adult dog deaths. Figure 4.7 shows the results of the classification of causes of death in adults, determined by verbal autopsy.

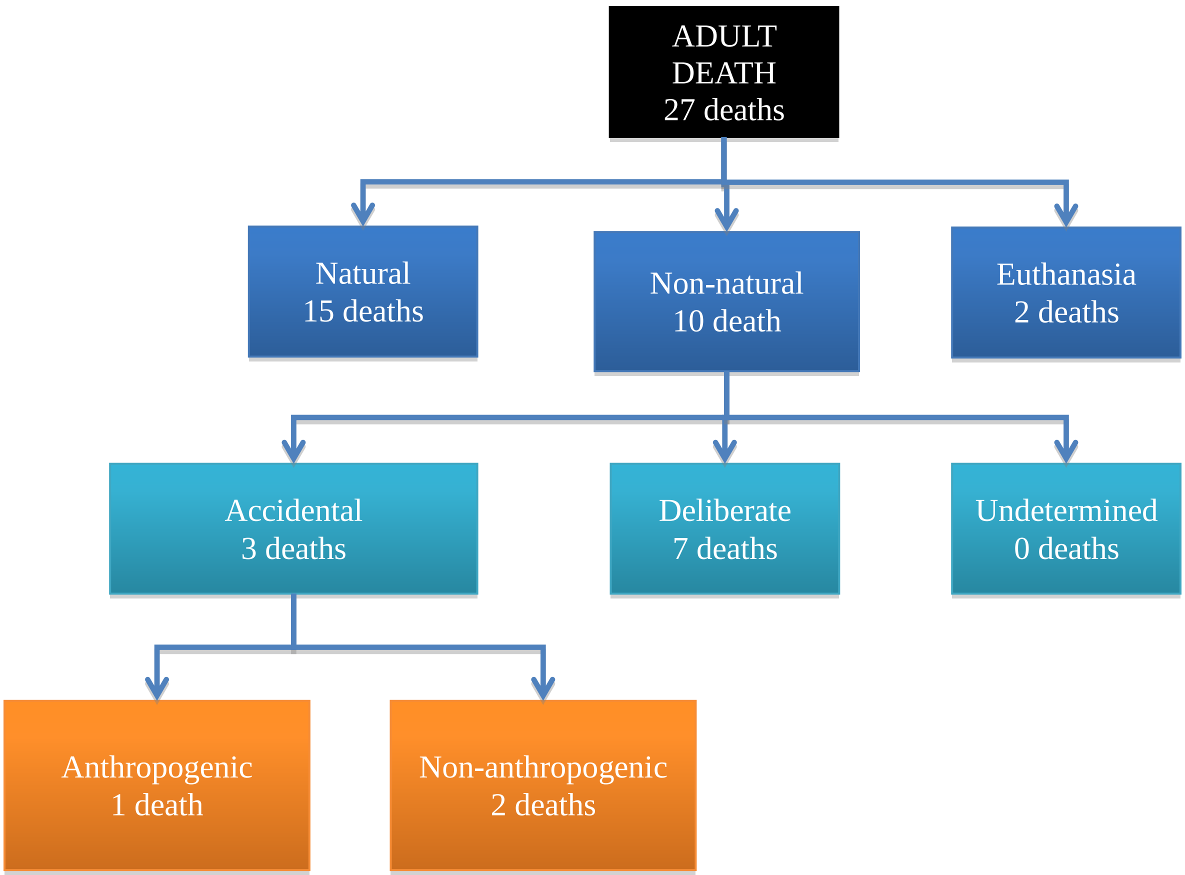

Figure 4.7: Flow chart showing the classification of causes of death in adults using the verbal autopsy method.

Of the 15 deaths classified as 'natural' using the verbal autopsy method, two were considered to be due to snake envenomation. The remaining 13 were considered to be due to infectious and/or parasitic causes, with reported signs indicating one or more organ systems affected (digestive n = 7, respiratory n = 5, nervous n = 4, circulatory n = 1). Of the seven deaths classified as ‘deliberate’, four were considered to have been caused by poisoning and three by trauma. The two accidental, non-anthropogenic deaths were due to animal bite wounds, and the single accidental, anthropogenic death was caused by a motor vehicle accident. One dog was euthanized because it was unwanted by the owner, while a second dog was killed because it was sick.

4.2.5.2 Necropsy results

Of the 27 adult dogs that died, 18 (67%) were submitted for necropsy. For the 9 other dead dogs, owners did not inform the study team early enough for carcass collection, or did not call the study team at all. Of the 18 submitted for postmortem, ten carcasses were in an advanced stage of autolysis and did not undergo necropsy. Of the eight carcasses examined, three had advanced post-mortem changes that prevented proper examination. A diagnosis on post-mortem examination was therefore only made for five of the 27 dead adult dogs as indicated in Table 4.9.

Table 4.9 : Comparison of cause of death for five adult dogs, determined by post-mortem examination and verbal autopsy in the cohort of adult dogs in Hluvukani

Dogs	Morphological/ Histopathologicaldiagnosis	Verbal autopsydiagnosis	Verbal autopsy classification of cause of death
1	Hepatic/pulmonary cause suspected	Accident (poison)	Non-natural / Deliberate
2	Multifocal spongiosis ofcerebellum	Infectious (respiratory)	Natural
3	Pulmonaryedema, spirocercosis with aortic aneurysm, myolysis	Infectious (circulatory)	Natural
4	Trauma and severe generalized carcass congestion	Accident (bite)	Non-natural /Accident /Non-anthropogenic
5	Spirocercosis, hyperadrenocortism, regenerative anaemia	Euthanasia	Euthanasia

4.3 Puppies

4.3.1 Description of puppies at enrollment

The total number of adult females enrolled in the study was 164. Of these, 57(34.8%) had at least one litter of puppies during the study period; among this number 52 (91.2%) littered only once, and five (8.8%) littered twice, giving a total of 62 litters. Figure 4.8 shows the number of litters per month during the study period. The mean number of litters per enrolled female was 0.38.

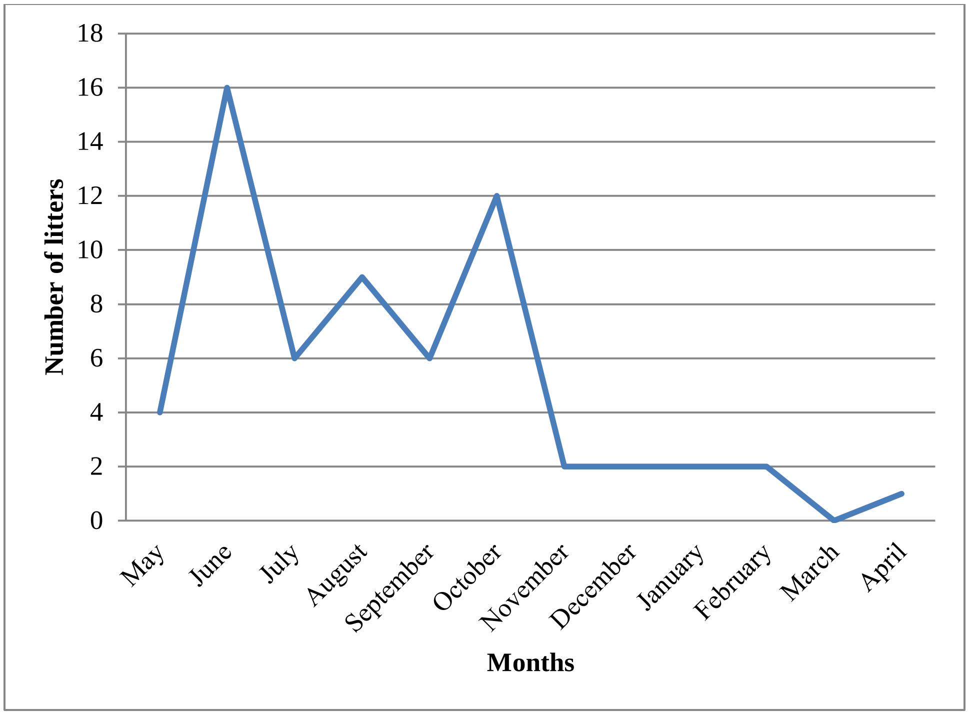

Figure 4.8: Number of litters born to the enrolled female cohort per month.

The total number of puppies enrolled at birth for the study was 329, comprising 152 (46.8%) males, 148 (44.4%) females, and 29 (8.8%) puppies of unknown sex, with a mean litter size of 5.6 (IQR 2-7), (minimum = 1 and a maximum = 16puppies). The sex ratio of puppies at birth was 1.03 males per female. The crude birth rate was 896.5 per 1,000 dogs and the crude rate of natural increase is 528.6 per 1,000 dogs.

4.3.2 Mortalities and loss to follow-up

Of the 369 puppies enrolled at birth, 135 (36.6%; 50 males, 58 females, and 27 of unknown sex) died during the follow-up period from birth to 120 days, and 126 (34.1%; 66 males, 58 females and two of unknown sex) were lost to follow-up. The characteristics of puppies lost to follow-up and the summary of the follow-up days are shown in Tables 4.10 and 4.11 respectively.

Table 4.10: Puppies lost to follow-up from birth to 120 days, from the enrolled female cohort that littered in Hluvukani between May 2014 and April 2015.

Exit type	No. of puppies	Male	Female	Unknown sex
Lost	8	0	6	2
Stolen	15	10	5	0
Given out	101	55	46	0
Sold	2	1	1	0
Total	126	66	58	2

Table 4.11: Summary of the follow-up days of puppies lost to follow-up from the enrolled female cohort that littered in Hluvukani between May 2014 and April 2015.

Exit type	Time of follow-up (days)					
	Min.	**1st Quart**	**Median**	**Mean**	**3rd Quart**	**Max.**
Lost	1	15	50	45	76.	88
Stolen	16	23	35	46	61	89
Given out	4	36	56	53	66	104
Sold	72	72	72	72	72	72

The mortality rate for puppies was 2,389.3 deaths per 1,000 dog-years. Sex-specific mortality rates for puppies were 1,811.5 deaths per 1,000 dog-years for males and 2,172.2deaths per 1,000 dog-years for females. The mortality rate for puppies with unknown sex is 12,857.1 deaths per 1,000 dog-years (Table 4.12). The mortality rate by months for puppies born to the cohort of female dogs that littered is shown in Table 4.13 and illustrated with the mortality curve in Figure 4.9.

Table 4.12: Mortality and dog years lived table for puppies born to the female cohort in Hluvukani from May2014 to June 2015.

	Puppies born alive	**Number of deaths**	**Number of dog-days**	**Number of dog-years**	**Mortality rates/1,000 dog-years**
Total	329	135	20,638	56.5	2,389.3
Male	152	50	10,100	27.6	1,811.5
Female	148	58	9,760	26.7	2,172.2
Unknown sex	29	27	777	2.1	12,857.1

Table 4.13:Mortality data by month for puppies born to the cohort of female dogs that littered in Hluvukani from June 2014 to August 2015.

Year	Month	Number of deaths	Number of dog days	Number of dog-years	Crude mortality rate (per 1,000 dog-years)
2014	June	27	1,868	5.11	5,279.31
2014	July	12	3,243	8.89	1,351.53
2014	August	37	2,775	7.60	4,870.00
2014	September	13	3,195	8.75	1,486.15
2014	October	14	3,134	8.58	1,631.62
2014	November	10	2,133	5.84	1,712.38
2015	December	4	1,142	3.13	1,279.33
2015	January	3	906	2.48	1,209.44
2015	February	4	570	1.56	2,563.16
2015	March	3	461	1.26	2,376.90
2015	April	1	352	0.96	1,037.64
2015	May	1	305	0.85	1,197.54
2015	June	5	165	0.45	11,068.18
2015	July	1	53	0.145	6,891.51

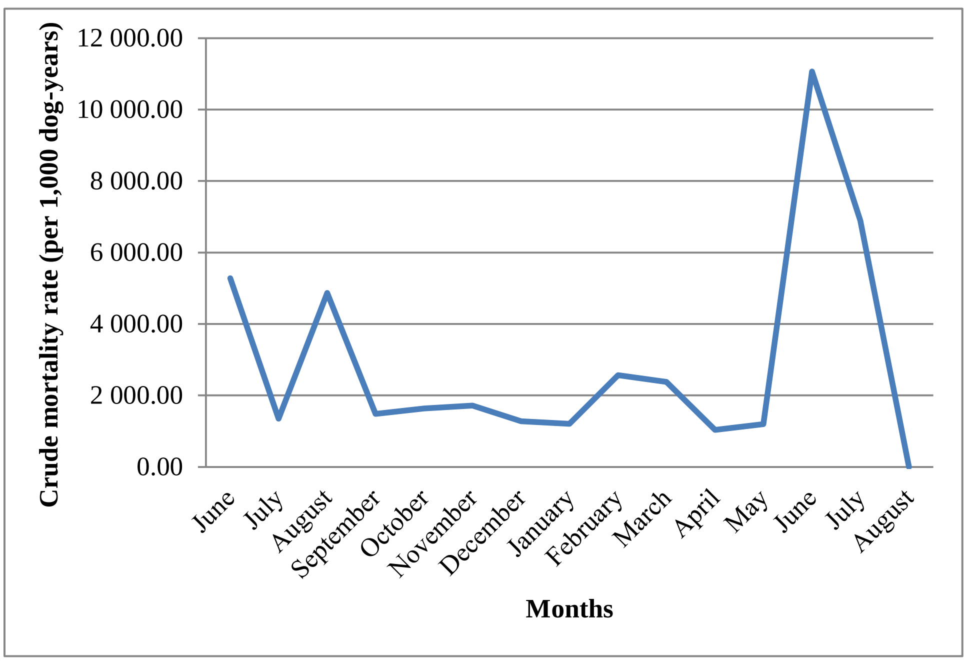

Figure 4.9: Crude mortality rate of dead puppies by month from May 2014 to August 2015.

4.3.4 Kaplan-Meier survival analysis

The total number of puppies lost to follow-up was 126. For the survival analysis, the puppies sold or given out (n = 103) were included in the analyses as right-censored observations, while the puppies reported as lost and stolen (n = 23) were excluded from the analyses. For these latter dogs, we cannot be sure that they had in fact not died, and for survival analysis the assumption is that the outcome (death) and reason for death and loss to follow-up are not related. Figures 4.10 and 4.11 show the survival curves of the puppies and survival curve stratified by sex respectively.

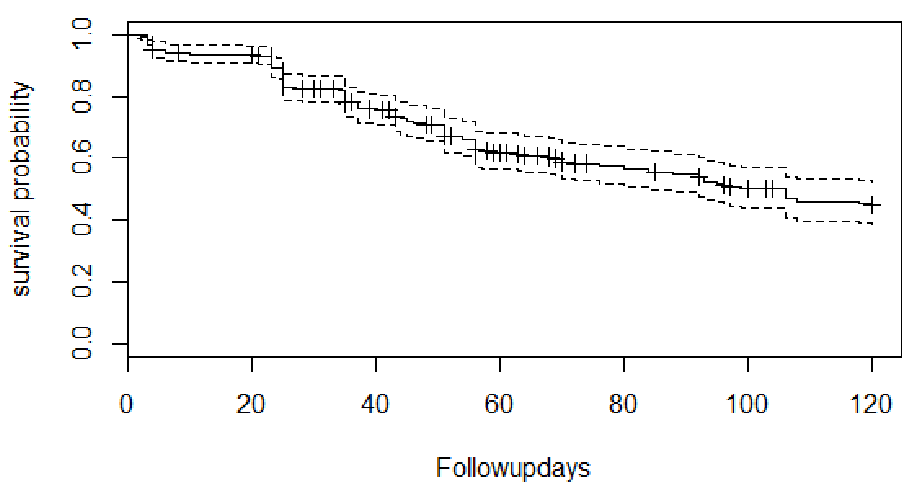

Figure 4.10: Survival curve during 120 days for puppies born from the female cohort in Hluvukani from May 2014 to August 2015.

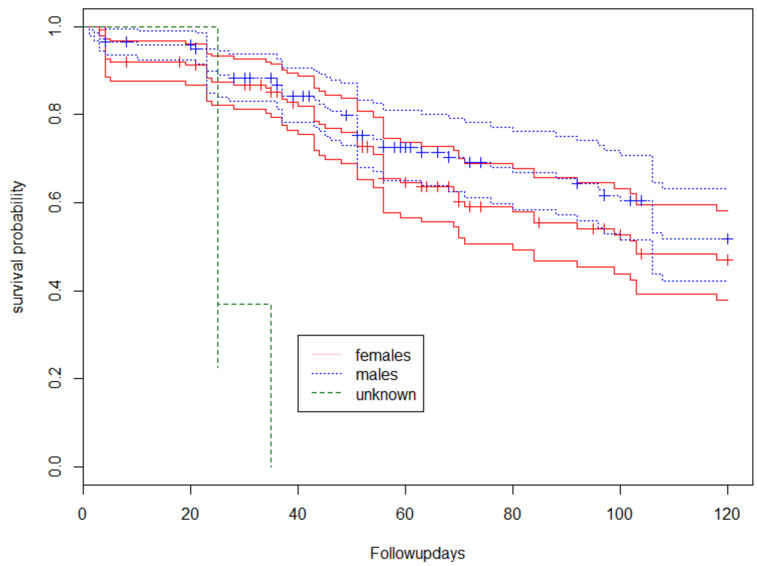

Figure 4.11: Survival curve of puppies stratified by sex males, females and unknown sex).

The dead puppies with unknown sex were excluded from the comparison test. The Cox proportional hazard model with chi square analysis indicated a p-value of 0.3 and the log rank test with p-value of p = 0.3. This shows there was no significant difference between the male and the female puppies' survival, as seen in Table 4.14

Table 4.14: Summary of Cox regression and log-rank test for the male and female dog cohort in Hluvukani.

Sex	**N (number of puppies)**	**Number of deaths**	**Min**	**Median**	**Mean**	**IQR**	**Max**	**Log-rank p-value**	**Cox regression p-value**
Male	152	50	1	60.5	67.9	36-117	120	0.3	0.3
Female	148	58	3	60	66.93	38-104	120		

4.3.5 Causes of death

4.3.5.1 Verbal autopsy results

One hundred and thirty-five verbal autopsy results were collated and analyzed. Figure 4.12 shows the results of the classification of causes of death in puppies, determined by verbal autopsy.

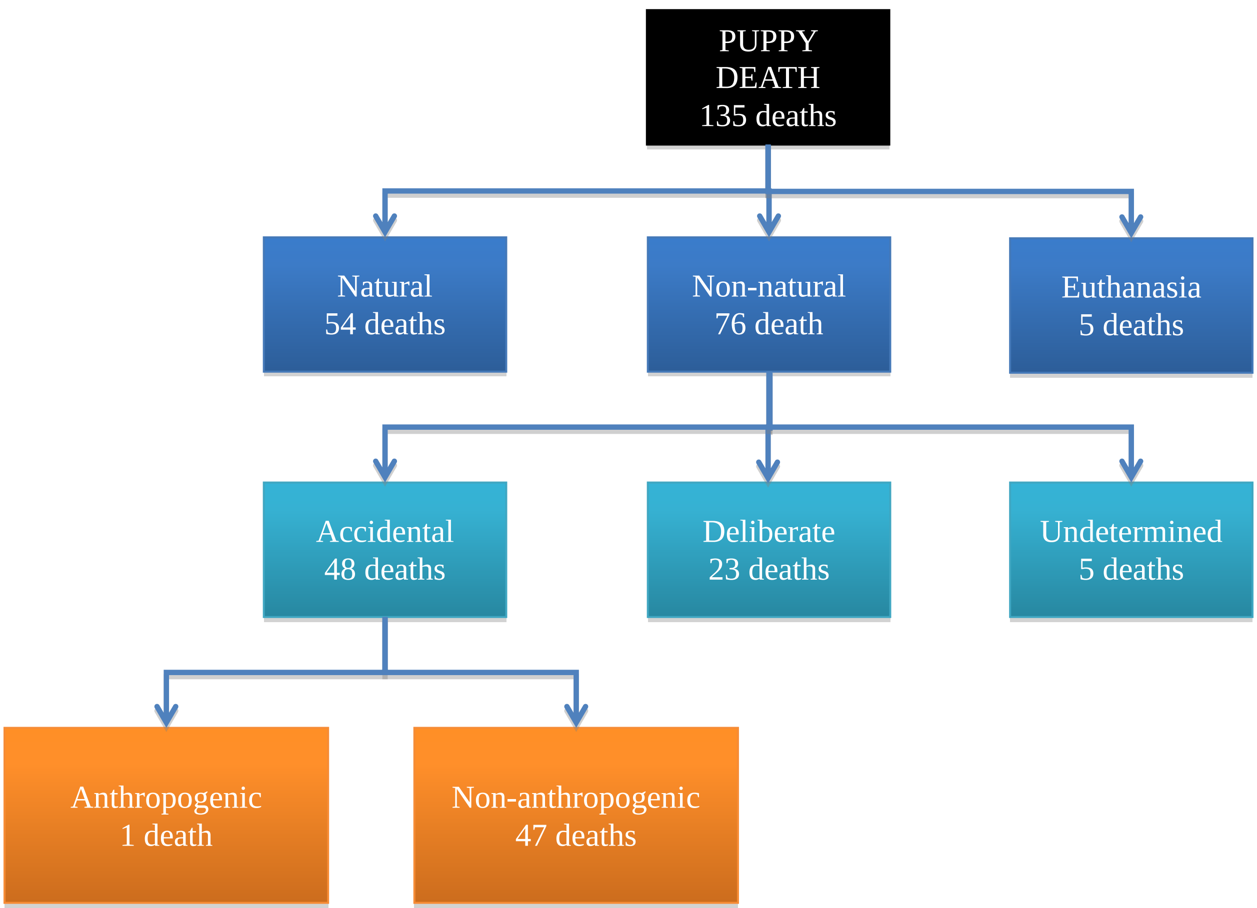

Figure 4.12: Flow chart showing the classification of causes of death in puppies using the verbal autopsy method.

Of the 54 puppy deaths classified as ‘natural’, one was a case of sudden death without observed clinical signs. The remaining 53 were considered to be due to infectious and/or parasitic causes, with reported signs indicating one or more organ systems affected (circulatory n = 18, nervous n =16, respiratory n = 13, and digestive n = 6). Of the 23 deaths classified as ‘deliberate’, six were considered to have been caused by poisoning and 17 by trauma. The 47 accidental, non-anthropogenic deaths were considered to be due to the following causes: eaten by mother (n = 18), starvation (n = 8), laid on by mother (n = 7), animal bite (n = 5), drowning (n = 4), suffocation (n = 3) and crushed by a brick (n = 2). The single accidental, anthropogenic death was caused by a motor vehicle accident. Five were puppies were euthanized because they were unwanted.

4.3.5.2 Necropsy results

Of the dead puppies, 22(16.3%) were submitted for necropsy. Most owners did not call the study team, or called too late. Of the 22 submitted for post-mortem, 17 carcasses were in an advanced stage of autolysis and did not undergo necropsy. Necropsy results for the remaining six puppies are shown in Table 4.15, together with the cause of death identified through verbal autopsy.

Table 4.15: Comparison of cause of death in six puppies submitted for post-mortem examination and verbal autopsy.

Dogs	Morphological/ Histopathologicaldiagnosis	Verbal autopsy diagnosis	Verbal autopsy classification of cause of death
1	Severe splenic EMH	Infectious (circulatory)	Natural
2	Severeanaemia/mild nephrosis	Infectious (circulatory)	Natural
3	Acute heart failure (congenital atelectasis), nephrosis	Sudden death	Natural
4	Regenerative anaemia	Infectious (circulatory)	Natural
5	Internal/external parasites, anaemia, hepatocellular atrophy	Infectious (circulatory)	Natural
6	Hepatic abscessation, anaemia, peritonitis, helminthosis	Accident (starvation)	Non-natural/Accident/non-anthropogenic

CHAPTER 5 : DISCUSSION

Data from this study has provided more detailed information on mortality rates, survival and causes of death in owned, mostly free-roaming dogs in a resource-constrained community in a rabies-endemic area in South Africa. Mortality rates may affect the resultant herd immunity to rabies in a vaccinated population. We observed a mortality rate of 77.6 per 1,000 dog-years and a risk of death of 7.3% in the adult cohort during a one-year period. This study observed a low mortality rate in the adult cohort. This is higher than the rate of 39 per 1,000 dog-years seen over a five-year period in a study of insured Swedish dogs in their first year of life (Bonnett et al., 2005), but much lower than the risk of death seen in another study in Tanzania, which reported 450 deaths per 1,000 dog population (Hampson et al., 2009). Neither of these studies looked specifically at mortality in adult dogs only (one year of age or older).

Mortality was highest in the cohort of 5 years and above (133.9 per 1,000 dog-years), especially among the female cohort at305.8 deaths per 1,000 dog-years. This is consistent with an earlier study in insured Swedish dogs, where in all the breeds sampled, the risk of death increased with age (Egenvall et al., 2000).This may explain why the majority of dogs in sub-Saharan Africa are young dogs, as older dogs die of old age (Proschowsky et al., 2003). Another study in Iringa, Tanzania, showed that the majority of the dog population were young with an average age of 2.23 years (Gsell et al., 2012). In our study, the survival time of the older dogs of ages 5 years and above was 338.5 follow-up days and this is lower when compared with the other age categories. Very few data is available in published literature on the survival rates of dogs in longitudinal studies. In a vaccinated population, the death of these older, vaccinated dogs would have made an impact on the herd immunity of the population for important diseases like rabies if the turnover rate in the population remains high (Hampson et al., 2009).

In this study, the adult female cohort produced 62 litters with an average litter size of 5.6, which was similar to the average litter size of 5.5 observed in research carried out in Iringa, Tanzania (Gsell et al., 2012), and higher than what was observed in Zimbabwe (4.6) (Butler and Bingham, 2000) and in rural Tanzania (4.7); (Hampson et al., 2009). In this study, it was observed that 57(34.8%) females whelped during the study period. There appeared to be seasonality in whelping during the study, as more litters were born around the month of June. Recent research findings in the same study area has confirmed that more births were recorded between April to June (Conan et al., 2015). Seasonality in puppy births in free-roaming populations has not been consistently reported in the literature. Other workers have recorded seasonal pregnancies and oestrus in populations of free-roaming dogs in India (Reece et al., 2008, Totton et al., 2010a). Another study in Zimbabwe, an area also located in southern Africa, observed a peak in the birth of puppies from around June to August (Butler and Bingham, 2000). In a survey carried out in Johannesburg, South Africa no seasonality was observed in births in the dog population studied (Morters et al., 2014). Similarly, no definite whelping season was observed in Iringa, Tanzania (Gsell et al., 2012). In Sweden, no significant difference has been observed between seasons of distribution of whelping in the Swedish kennel club and the private kennels (Gavrilovic et al., 2008). This seasonality in birth and mortality rates suggests that there will be an effect on the dog population and targeted vaccination campaigns should be distributed among various age groups accordingly.

Mortality in puppies was high with 2389.3 deaths per 1000 dog-years recorded, with a mean survival time of 60 days. Conan et al (2015) have reported the same trend in the HDSS-dog study in the same study area. In that study, puppies 0-3 months of age that were vaccinated against rabies had a significantly lower mortality rate than their unvaccinated counterparts. A possible explanation for this may be a specific or non-specific protective immunological effects against rabies or other infectious diseases, or a confounding effect of owners who have their puppies vaccinated against rabies, and against other infectious diseases(Conan et al., 2015). We did not test for this effect, asno vaccination was administered to the puppies during the study period. Further controlled studies are needed to shed more light on these perceived factors. The risk of death in puppies (41.0%) recorded in our study was slightly lower but similar to the deaths of puppies in Iringa, Tanzania where out of 204 puppies, 107 (52.5%) puppies died before they were three months old (Gsell et al., 2012). In contrast to other studies, this study did not record any stillbirths in the study area.

No significant difference exists in the survival rates between the two sexes of puppies (p= 0.3). By contrast, survival analysis between the two sexes in the adult cohort showed a significant difference, with females having significantly lower survival rates than males. A study in Kenya had earlier revealed lower survival rates for females (Kitala et al., 2001), but other studies did not observe any significant difference in the survival rates between the two sexes (Hampson et al., 2009, Gsell et al., 2012). Sex-specific mortality rates may vary overtime, as seen in the study by Conan et al (2015). Higher mortality in adult female may explain why the sex-ratio was skewed towards males as observed in this population (Conan et al., 2015). Detailed longitudinal studies on the contributory factors to male dominance in population sex ratios are needed. Importantly, this study did not put external in-migration of puppies in perspective, so it will be ideal for future studies to take in-migration into consideration.

Several reasons still remain as to why more male dogs are kept than female dogs. This could be attributed to the fact that people prefer to keep male dogs for the purpose of using them for security and to guard their livestock, while they tend to avoid female dogs because of some factors like the unwanted whelping and the periodic oestrus cycles that affect the behavior exhibited by female dogs. Therefore similar to what is seen in studies elsewhere; the ratio of male population is higher than that of the females (Hampson et al., 2009, Morters et al., 2014, Gsell et al., 2012, Kitala et al., 2001, Butler and Bingham, 2000, Ratsitorahina et al., 2009, Brooks, 1990, Rautenbach et al., 1991). Our study showed that the demand for dogs was mainly for security. This is consistent with another study which found that African dogs are kept for the purpose of guarding, herding, hunting, and infrequently for cultural and religious beliefs (Gsell et al., 2012). On the other hand, in countries like India, the practice is different, because dogs have a special purpose in the community: they are seen as reincarnated souls, and the dogs are well provided for, even though they are not owned (Bollée and der Wissenschaften, 2006). This high male population may also be attributed to the higher mortality rates observed in the female population, or out-migration of females because they are unwanted (Conan et al., 2015).

The demand for dogs is reflected in the level of resource provisioning by households. In this study, the feeding patterns of the dogs, the type of food fed and the number of times the dogs were fed in their households were documented. The household-level management practices showed that nearly all households fed their dogs at least once a day and provided drinking water. Data from this study shows that free-roaming dogs in Hluvukani are provided for and not dependent on environmental resources. This study is also in agreement with management practices seen elsewhere in sub-Saharan Africa, where free-roaming dogs were owned and fed regularly by their owners (Brooks, 1990, De Balogh et al., 1993, Butler and Bingham, 2000). Free-roaming dogs in our study were not dogs cared for by the neighborhood (so-called 'community owned'), but were traced to particular households and owners. The respondents all agreed that all the dogs seen around the study area were domesticated, owned and allowed to roam freely, but cared for by their owners in terms of day-to-day provision of resources.

A similar pattern was seen in a study in Zimbabwe where it was observed that all dogs in the study area were owned and there was no evidence of feral population (Butler and Bingham, 2000).The majority of adult dogs in this study (77.1%) were not confined to the household. This showed that these dogs were vulnerable to being killed, and to rabies transmission. The study in Tanzania (Gsell et al., 2012), showed some contrast to the confinement status in our study in that majority of the dogs in that study were confined. In general, free-roaming dogs are the source of major public health and animal welfare concerns in many parts of the world (Slater, 2001), and responsible dog ownership practices should include confinement of dogs in a humane manner.

This study observed that 333 (90.7%) of the adult dogs remained alive after one year of follow-up, and that these adults remained in the households of enrollment. This is consistent with the study in rural Tanzania (Gsell et al., 2012), where surviving dogs remained in the household,1.9% were lost and none was sold or given out. We also observed that 68 (20.6%) puppies born to the female cohort remained alive after 120 days of follow-up. These puppies remained in the household where their litter was born; this is also consistent with the study in rural Tanzania where 80% of the surviving puppies remained in the household of the dam, 11% of the puppies were sold or given out within the homes of the owners, while 6% of the puppies were sold or given away outside the home-ward of the owners (Gsell et al., 2012). In this study, 126 puppies were lost to follow-up; these puppies were either sold, given out, lost or stolen. It has been suggested that killing of unwanted dogs by owners may be under-reported (Morters et al., 2014), hence more longitudinal studies will help to give clarity on the phenomenon of puppies reported as lost or stolen.

Data from this study has shown that 86.1% of dogs were vaccinated against rabies in the previous year. This is well above the WHO-recommended target coverage of 70% during annual campaigns, and the critical threshold of 20-40% for herd immunity. This will disrupt rabies transmission and help prevent an outbreak (Hampson et al., 2009). Only 51 (13.9%) of the dogs in this area were unvaccinated or their vaccination status was unknown; it is expected that this low unvaccinated population reported in this study would not affect the outbreaks of rabies in the area. The low mortality rate in the adult cohort in this study might have been associated with the vaccination campaigns that conferred adequate herd immunity on the population. It is possible that, when dogs are vaccinated against rabies, they are sometimes vaccinated against other infectious diseases like canine distemper, parvoviral enteritis, para-influenza and canine hepatitis, with resultant fewer infections and lower mortality rates. This rabies vaccination coverage also suggests that achieving the WHO-recommended target of 70% is feasible, and that eliminating canine rabies both in the dog and the human populations in resource-constrained communities such as Hluvukani is possible. Similar outcomes has been reported in previous studies (Gsell et al., 2012). It can be hypothesized from this study, that with or without population management (such as sterilization), vaccination alone is capable of achieving the targets of rabies control and elimination.

Whereas verbal autopsy has been widely used in human populations and has been a good tool for determination of causes of death (Fortney et al., 1986, Gray et al., 1990, Bang and Bang, 1992, Ross, 1992, Snow et al., 1992, Snow et al., 1993, Chandramohan et al., 1994), this was the first report of a verbal autopsy method being used to ascertain the probable causes of death in animals. As previously suggested, ongoing or current causes of health problems in dog population can be identified by looking at mortality rates and estimates of the proportion of deaths due to a particular cause (Bonnett et al., 2005). The information presented here can be used to inform and evaluate health promotion strategies. All the dogs that died during the course of our study had verbal autopsies done with their caregivers. This proved to be a cost-effective and potentially reliable method of ascertaining cause of death, because it was observed that the respondents actually have a good understanding of the clinical signs exhibited by their pet. This also showed that the caregivers or the owners had a good understanding of some of the illnesses that may have caused deaths in their dogs, as suggested in other human studies (Snow et al., 1992). The verbal autopsy interview indicated that most adult dogs died of natural causes (infectious or parasitic diseases, and envenomation). However, others died of non-natural causes, including three that died due to accidents. Seven non-natural deaths were deliberate and two dogs which were unwanted by the owner died by euthanasia. In the puppies, verbal autopsy was used to confirm that more deaths occurred by non-natural causes, and a higher proportion of these deaths occurred by accident compared to through deliberate causes and undetermined causes. Accidental deaths from non-anthropogenic causes were higher that anthropogenic cause, of which a single death occurred due to an automobile accident. The second most common cause of death was natural causes, which may be infectious or parasitic. Unwanted puppies were euthanized, as seen in other studies (Fleming et al., 2011). The euthanized dogs were classified separately because they could not be classified as either infectious or non-infectious cause of death.

Although the verbal autopsy method proved useful in this study, more studies should be carried out to validate the use of this tool in animal populations. A previous study in people has shown that a single verbal autopsy instrument that covered deaths in all age-groups, can be satisfactorily validated for children under 5 years and adults 15 years and older, and can be used effectively in areas without vital registration, to prioritize public health problems, inform resource allocation, and target and evaluate community-based interventions (Kahn et al., 2000). Understanding rates and causes of mortality in dog populations will in turn give understanding of population turnover, and can inform the design of interventions to create stable, healthy population of dogs. Verbal autopsy could prove to be a useful tool for rural communities that are resource-constrained and especially where rabies is endemic.

Post-mortem examination and histopathology were conducted in our study to determine the causes of mortality in the adult cohort and the puppies, with the intention to use the findings to partially validate the verbal autopsy method and also to use post-mortem examination on its own to diagnose causes of death, similar to studies in North America (Fleming et al., 2011) and England (O'Neill et al., 2013),where organ system and pathophysiology classification of causes of death were used. However, we encountered difficulty in obtaining viable carcasses from the respondents and with advanced autolysis of other carcasses. Results from the few dogs that were posted and examined by histopathology showed five adult dogs had infectious causes of death, and three of these post-mortem diagnoses were correlated with the organ system classification of diagnosis using verbal autopsy, which served as some form of validation for the verbal autopsy. In the puppies, six were diagnosed using histopathology and five of these post-mortem diagnoses correlated with the organ system diagnosis from the verbal autopsy. This is an indication that both verbal autopsy and post-mortem examination when used concurrently can achieve good results to determine the cause of mortality in dogs in resource-constrained areas, and to monitor the spread of infectious diseases like rabies. Both the post-mortem and verbal autopsy findings could not diagnose any case of rabies during the study period.

CHAPTER 6: CONCLUSION

In conclusion, free-roaming dogs in this study were owned, and were provided for by their owners, without reliance on environmental resources. Vaccination coverage in the adult cohort was sufficient to prevent rabies outbreaks. Mortality rates were low in the adult dogs and relatively high in older female dogs. Infectious causes of death were the highest in the adult dog cohort.

Birth rate in the female cohort was relatively high and the mortality rate was equally high in the puppies, before they reached 120 days of life, with accidental and deliberate causes as the principal causes of death in puppies.

This study recommends carefully-designed validation studies on the use of verbal autopsy in determining causes of death in underserved, resource-constrained communities like the Hluvukani village in the Mnisi community area of Mpumalanga Province, South Africa.

REFERENCES

ADAMS, V., EVANS, K., SAMPSON, J. & WOOD, J. 2010. Methods and mortality results of a health survey of purebred dogs in the UK. *Journal of Small Animal Practice,* 51**,** 512-524.

ANDERSON, R. & MAY, R. 1991. Infectious Diseases of Humans Oxford University Press. Oxford.

ANKER, M., BLACK, R. E., COLDHAM, C., KALTER, H., QUIGLEY, M. A., ROSS, D. & SNOW, R. W. 1999. *A standard verbal autopsy method for investigating causes of death in infants and children,* World Health Organization Geneva.

BANG, A. T. & BANG, R. A. 1992. Diagnosis of causes of childhood deaths in developing countries by verbal autopsy: suggested criteria. The SEARCH Team. *Bulletin of the World Health Organization,* 70**,** 499.

BARRAT, J., BLASCO, E., LAMBOT, M., CLIQUET, F., BROCHIER, B., RENDERS, C., PASTORET, P. & AUBERT, M. Is it possible to vaccinate young canids against rabies and to protect them. Proceedings of the Sixth Southern and Eastern African Rabies Group/World Health Organization meeting, Lilongwe, Malawi, 2001. 151-163.

BELOTTO, A., LEANES, L., SCHNEIDER, M., TAMAYO, H. & CORREA, E. 2005. Overview of rabies in the Americas. *Virus Research,* 111**,** 5-12.

BENTUBO, H. D. L., TOMAZ, M. A., BONDAN, E. F. & LALLO, M. A. 2007. Expectativa de vida e causas de morte em cães na área metropolitana de São Paulo (Brasil). *Ciência Rural,* 37**,** 1021-1026.

BERAN, G. W. & FRITH, M. 1988. Domestic animal rabies control: an overview. *Review of Infectious Diseases,* 10**,** S672-S677.

BOLLÉE, W. B. & DER WISSENSCHAFTEN, B. A. 2006. *Gone to the dogs in ancient India*, Verlag der Bayerischen Akademie der Wissenschaften.

BONNETT, B., EGENVALL, A., HEDHAMMAR, Å. & OLSON, P. 2005. Mortality in over 350,000 insured Swedish dogs from 1995–2000: I. Breed-, gender-, age-and cause-specific rates. *Acta Veterinaria Scandinavica,* 46**,** 105.

BOTHA, J. & BRADSHAW, D. 1985. African vital statistics--a black hole? *South African Medical Journal= Suid-Afrikaanse Tydskrif vir Geneeskunde,* 67**,** 977-981.

BROOKS, R. 1990. Survey of the dog population of Zimbabwe and its level of rabies vaccination. *The Veterinary Record,* 127**,** 592-596.

BUTLER, J. & BINGHAM, J. 2000. Demography and dog-human relationships of the dog population in Zimbabwean communal lands. *The Veterinary Record,* 147**,** 442-446.

CHANDRAMOHAN, D., MAUDE, G. H., RODRIGUES, L. C. & HAYES, R. J. 1994. Verbal autopsies for adult deaths: issues in their development and validation. *International Journal of Epidemiology,* 23**,** 213-222.

CHANDRAMOHAN, D., RODRIGUES, L. C., MAUDE, G. H. & HAYES, R. J. 1998. The validity of verbal autopsies for assessing the causes of institutional maternal death. *Studies in Family Planning*, 414-422.

CHAPPUIS, G. 1998. Neonatal immunity and immunisation in early age: lessons from veterinary medicine. *Vaccine,* 16**,** 1468-1472.

CLEAVELAND, S. 1998. Epidemiology and control of rabies: The growing problem of rabies in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 92**,** 131-134.

COETZEE, P. & NEL, L. H. 2007. Emerging epidemic dog rabies in coastal South Africa: a molecular epidemiological analysis. *Virus Research,* 126**,** 186-195.

COHEN, C., SARTORIUS, B., SABETA, C., ZULU, G., PAWESKA, J., MOGOSWANE, M., SUTTON, C., NEL, L. H., SWANEPOEL, R. & LEMAN, P. A. 2007. Epidemiology and molecular virus characterization of reemerging rabies, South Africa. *Emerging Infectious Diseases,* 13**,** 1879.

COLDHAM, C., ROSS, D., QUIGLEY, M., SEGURA, Z. & CHANDRAMOHAN, D. 2000. Prospective validation of a standardized questionnaire for estimating childhood mortality and morbidity due to pneumonia and diarrhoea. *Tropical Medicine and International Health,* 5**,** 134-144.

CONAN, A., AKERELE, O., SIMPSON, G., REININGHAUS, B., VAN ROOYEN, J. & KNOBEL, D. 2015. Population Dynamics of Owned, Free-Roaming Dogs: Implications for Rabies Control. *PLoS Neglected Tropical Diseases,* 9**,** e0004177.

DE BALOGH, K., WANDELER, A. I. & MESLIN, F.-X. 1993. A dog ecology study in an urban and a semi-rural area of Zambia. *The Onderstepoort Journal of Veterinary Research,* 60**,** 437-443.

DODET, B. 2009. The fight against rabies in Africa: From recognition to action. *Vaccine,* 27**,** 5027-5032.

DU TOIT, P. 1930. Rabies in South Africa. *Papers Veterinary Section, no. 33*.

EGENVALL, A., BONNETT, B. N., SHOUKRI, M., OLSON, P., HEDHAMMAR, Å. & DOHOO, I. 2000. Age pattern of mortality in eight breeds of insured dogs in Sweden. *Preventive Veterinary Medicine,* 46**,** 1-14.

FLEMING, J., CREEVY, K. & PROMISLOW, D. 2011. Mortality in North American Dogs from 1984 to 2004: An Investigation into Age-, Size-, and Breed-Related Causes of Death. *Journal of Veterinary Internal Medicine,* 25**,** 187-198.

FORTNEY, J. A., SUSANTI, I., GADALLA, S., SALEH, S., ROGERS, S. M. & POTTS, M. 1986. Reproductive mortality in two developing countries. *American Journal of Public Health,* 76**,** 134-138.

FOX, J. P., ELVEBACK, L., SCOTT, W., GATEWOOD, L. & ACKERMAN, E. 1971. Herd immunity: basic concept and relevance to public health immunization practices. *American Journal of Epidemiology,* 94**,** 179-189.

GAVRILOVIC, B. B., ANDERSSON, K. & FORSBERG, C. L. 2008. Reproductive patterns in the domestic dog—A retrospective study of the Drever breed. *Theriogenology,* 70**,** 783-794.

GRAY, R. H., SMITH, G. & BARSS, P. 1990. The use of verbal autopsy methods to determine selected causes of death in children. *Liege, Belgium, International Union for the Scientific Study of Population [IUSSP]***,** 46 p.

GSELL, A. S., KNOBEL, D. L., CLEAVELAND, S., KAZWALA, R. R., VOUNATSOU, P. & ZINSSTAG, J. 2012. Domestic dog demographic structure and dynamics relevant to rabies control planning in urban areas in Africa: the case of Iringa, Tanzania. *BMC Veterinary Research,* 8**,** 236.

HAMPSON, K., DUSHOFF, J., BINGHAM, J., BRÜCKNER, G., ALI, Y. & DOBSON, A. 2007. Synchronous cycles of domestic dog rabies in sub-Saharan Africa and the impact of control efforts. *Proceedings of the National Academy of Sciences,* 104**,** 7717-7722.

HAMPSON, K., DUSHOFF, J., CLEAVELAND, S., HAYDON, D. T., KAARE, M., PACKER, C. & DOBSON, A. 2009. Transmission dynamics and prospects for the elimination of canine rabies.

HERZENBERG, L. 1928. Two cases of hydrophobia. *Journal of the Medical Association of South Africa,* 2**,** 659-661.

JOHN, T. J. & SAMUEL, R. 2000. Herd immunity and herd effect: new insights and definitions. *European Journal of Epidemiology,* 16**,** 601-606.

KAARE, M., LEMBO, T., HAMPSON, K., ERNEST, E., ESTES, A., MENTZEL, C. & CLEAVELAND, S. 2009. Rabies control in rural Africa: evaluating strategies for effective domestic dog vaccination. *Vaccine,* 27**,** 152-160.

KAHN, K., TOLLMAN, S. M., GARENNE, M. & GEAR, J. S. 1999. Who dies from what? Determining cause of death in South Africa's rural north-east. *Tropical Medicine and International Health,* 4**,** 433-441.

KAHN, K., TOLLMAN, S. M., GARENNE, M. & GEAR, J. S. 2000. Validation and application of verbal autopsies in a rural area of South Africa. *Tropical Medicine and International Health,* 5**,** 824-831.

KING, A. A., FOOKS, A. R., AUBERT, M. & WANDELER, A. 2004. Historical perspectives of rabies in Europe and the Mediterranean Basin. *Paris, World Organisation for Animal Health*.

KITALA, P., MCDERMOTT, J., KYULE, M., GATHUMA, J., PERRY, B. & WANDELER, A. 2001. Dog ecology and demography information to support the planning of rabies control in Machakos District, Kenya. *Acta Tropica,* 78**,** 217-230.

KNOBEL, D. L., CLEAVELAND, S., COLEMAN, P. G., FÈVRE, E. M., MELTZER, M. I., MIRANDA, M. E. G., SHAW, A., ZINSSTAG, J. & MESLIN, F.-X. 2005. Re-evaluating the burden of rabies in Africa and Asia. *Bulletin of the World Health Organization,* 83**,** 360-368.

LEMBO, T., HAMPSON, K., KAARE, M. T., ERNEST, E., KNOBEL, D., KAZWALA, R. R., HAYDON, D. T. & CLEAVELAND, S. 2010. The feasibility of canine rabies elimination in Africa: dispelling doubts with data. *PLoS Neglected Tropical Diseases*, 4**,** e626.

MESLIN, F., FISHBEIN, D. & MATTER, H. 1994. Rationale and prospects for rabies elimination in developing countries. *Current Topics in Microbiology and Immunology*, 187**,** 1.

MORTERS, M. K., MCKINLEY, T. J., RESTIF, O., CONLAN, A. J., CLEAVELAND, S., HAMPSON, K., WHAY, H. R., DAMRIYASA, I. & WOOD, J. L. 2014. The demography of free-roaming dog populations and applications to disease and population control. *Journal of Applied Ecology*, 51**,** 1096-1106.

MURRAY, C. J. 1994. Quantifying the burden of disease: the technical basis for disability-adjusted life years. *Bulletin of the World Health Organization*, 72**,** 429.

MURRAY, C. J., LOPEZ, A. D., FEEHAN, D. M., PETER, S. T. & YANG, G. 2007. Validation of the symptom pattern method for analyzing verbal autopsy data. *PLoS Medicine*, 4**,** e327.

NEITZ, W. & MARAIS, I. 1932. Rabies as it occurs in the Union of South Africa. *Eighteenth Report of the Director of Veterinary Services and Animal Industry*.

NEVILLE, J., KING, A., FOOKS, A., AUBERT, M. & WANDELER, A. 2004. Rabies in the ancient world. *Historical Perspective of Rabies in Europe and the Mediterranean Basin: A Testament to Rabies by Dr Arthur A. King***,** 1-13.

O'NEILL, D., CHURCH, D., MCGREEVY, P., THOMSON, P. & BRODBELT, D. 2013. Longevity and mortality of owned dogs in England. *The Veterinary Journal*, 198**,** 638-643.

PRECAUSTA, P., SOULEBOT, J., CHAPPUIS, G., BRUN, A., BUGAND, M. & PETERMANN, H. 1985. NIL2 Cell Inactivated Tissue Culture Vaccine Against Rabies—Immunization of Carnivores. *Rabies in the Tropics.* Springer.

PROSCHOWSKY, H. F., RUGBJERG, H. & ERSBØLL, A. K. 2003. Mortality of purebred and mixed-breed dogs in Denmark. *Preventive Veterinary Medicine*, 58**,** 63-74.

QUIGLEY, M., SCHELLENBERG, J. A. & SNOW, R. W. 1996. Algorithms for verbal autopsies: a validation study in Kenyan children. *Bulletin of the World Health Organization*, 74**,** 147.

RATSITORAHINA, M., RASAMBAINARIVO, J. H., RAHARIMANANA, S., RAKOTONANDRASANA, H., ANDRIAMIARISOA, M.-P., RAKALOMANANA, F. A. & RICHARD, V. 2009. Dog ecology and demography in Antananarivo, 2007. *BMC Veterinary Research*, 5**,** 21.

RAUTENBACH, G., BOOMKER, J. & DE VILLIERS, I. 1991. A descriptive study of the canine population in a rural town in southern Africa. *Journal of the South African Veterinary Association*, 62**,** 158-162.

RCORETEAM 2013. R Foundation for Statistical Computing. *Vienna, Austria*, 3.

REECE, J. & CHAWLA, S. 2006. Papers & Articles. *The Veterinary Record,* 159**,** 379-383.

REECE, J. F., CHAWLA, S. K., HIBY, E. F. & HIBY, L. R. 2008. Fecundity and longevity of roaming dogs in Jaipur, India. *BMC Veterinary Research,* 4**,** 6.

ROSS, D. A. 1992. *Monitoring Cause-specific Infant and Child Mortality Rates in Areas where Death Certification Systems are Weak,* WHO.

RUPPRECHT, C. E., HANLON, C. A. & HEMACHUDHA, T. 2002. Rabies re-examined. *The Lancet Infectious Diseases,* 2**,** 327-343.

SABETA, C. T., WEYER, J., GEERTSMA, P., MOHALE, D., MIYEN, J., BLUMBERG, L. H., LEMAN, P. A., PHAHLADIRA, B., SHUMBA, W. & WALTERS, J. 2013. Emergence of rabies in the Gauteng Province, South Africa: 2010-2011. *Journal of the South African Veterinary Association,* 84**,** 00-00.

SCHNEIDER, M., BELOTTO, A., ADE, M., HENDRICKX, S., LEANES, L., RODRIGUES, M., MEDINA, G. & CORREA, E. 2007. Status of dog-transmitted human rabies in Latin America. *Cad Saude Publica*.

SHIMADA, K. 1971. The last rabies outbreak in japan. *Rabies. Baltimore: University Park***,** 11-28.

SHONE, D. 1962. Rabies in Southern Rhodesia 1900–1961. *Journal of the South African Veterinary Medical Association,* 33**,** 567-71.

SLATER, M. R. 2001. The role of veterinary epidemiology in the study of free-roaming dogs and cats. *Preventive Veterinary Medicine,* 48**,** 273-286.

SNOW, B., MARSH & , K. 1992. How useful are verbal autopsies to estimate childhood causes of death? *Health Policy and Planning,* 7**,** 22-29.

SNOW, R. W., DE AZEVEDO, I. B., FORSTER, D., MWANKUYSE, S., BOMU, G., KASSIGA, G., NYAMAWI, C., TEUSCHER, T. & MARSH, K. 1993. Maternal recall of symptoms associated with childhood deaths in rural East Africa. *International Journal of Epidemiology,* 22**,** 677-683.

SNYMAN, P. 1940. The Study and Control of the Vectors of Rabies in South Africa. *Onderstepoort Journal of Veterinary Science,* 15**,** 9-140.

SWANEPOEL, R., BARNARD, B., MEREDITH, C., BISHOP, G., BRÜCKNER, G., FOGGIN, C. & HÜBSCHLE, O. 1993. Rabies in southern Africa. *The Onderstepoort Journal of Veterinary Research,* 60**,** 325-346.

TALBI, C., HOLMES, E. C., DE BENEDICTIS, P., FAYE, O., NAKOUNÉ, E., GAMATIÉ, D., DIARRA, A., ELMAMY, B. O., SOW, A. & ADJOGOUA, E. V. 2009. Evolutionary history and dynamics of dog rabies virus in western and central Africa. *Journal of General Virology,* 90**,** 783-791.

THERNEAU, T. 2014. A package for survival analysis in S. 2014, R package version 2.37-7.

TODD, K. H., LEE, T. & HOFFMAN, J. R. 1994. The effect of ethnicity on physician estimates of pain severity in patients with isolated extremity trauma. *Jama,* 271**,** 925-928.

TOPLEY, W. & WILSON, G. 1923. The Spread of Bacterial Infection. The problem of herd-immunity. *Journal of Hygiene,* 21**,** 243-249.

TOTTON, S. C. 2009. *Stray dog population health and demographics in Jodhpur, India following a spay/neuter/rabies vaccination program*, University of Guelph.

TOTTON, S. C., WANDELER, A. I., GARTLEY, C. J., KACHHAWAHA, S., SUMAN, M., RIBBLE, C. S., ROSATTE, R. C. & MCEWEN, S. A. 2010a. Assessing reproductive patterns and disorders in free-ranging dogs in Jodhpur, India to optimize a population control program. *Theriogenology,* 74**,** 1115-1120.

TOTTON, S. C., WANDELER, A. I., ZINSSTAG, J., BAUCH, C. T., RIBBLE, C. S., ROSATTE, R. C. & MCEWEN, S. A. 2010b. Stray dog population demographics in Jodhpur, India following a population control/rabies vaccination program. *Preventive Veterinary Medicine,* 97**,** 51-57.

UMENO, S. 1921. A Study on the Antirabic Inoculation of Dogs, and the Results of its Practical Application. *Kitasato Archives of Experimental Medicine,* 4**,** 89-108.

VELASCO-VILLA, A., REEDER, S. A., ORCIARI, L. A., YAGER, P. A., FRANKA, R., BLANTON, J. D., ZUCKERO, L., HUNT, P., OERTLI, E. H. & ROBINSON, L. E. 2008. Enzootic rabies elimination from dogs and reemergence in wild terrestrial carnivores, United States. *Emerging Infectious Diseases,* 14**,** 1849.

WANDELER, A., BUDDE, A., CAPT, S., KAPPELER, A. & MATTER, H. 1988. Dog ecology and dog rabies control. *Review of Infectious Diseases,* 10**,** S684-S688.

WEYER, J., SZMYD-POTAPCZUK, A. V., BLUMBERG, L. H., LEMAN, P. A., MARKOTTER, W., SWANEPOEL, R., PAWESKA, J. T. & NEL, L. H. 2011. Epidemiology of human rabies in South Africa, 1983–2007. *Virus Research,* 155**,** 283-290.

WHO 1999. World survey of rabies: no. 34: for the year 1998: . *RABNET: The Electronic WWW Rabies Data Bank: 1988 to 1998*.

WHO 2013. WHO Expert Consultation on Rabies. Second report. *World Health Organization Technical Report Series***,** 1.

WUNNER, W. H. & CONZELMANN, K.-K. 2013. Rabies virus. *AC Jackson. Rabies, 3rd Edition. Oxford, UK: Acad Press/Elsevier***,** 17-60.

ZAHR, C. A. 2007. *Verbal autopsy standards: ascertaining and attributing cause of death*, World Health Organization.

APPENDIX

APPENDIX 1

Consent to participate in a research study

Rates and causes of death in dogs

Study leader:	Prof. Darryn Knobel	Dept. Veterinary Tropical Diseases
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	Rianne Vergeer	Utrecht University

We are from the University of Pretoria and Utrecht University. We would like to do a research study in your village. The purpose of this study is to find out more about what dogs in Hluvukani are dying from, and how many dogs die each month. We will do this by doing an interview with the owners of dogs that die, and recording information of those dogs. We will also examine some of the dogs that die each month to try to find out what they died from, and take samples from the dead dogs to test for different diseases. We will do the study in adult dogs, and in puppies up to 4months old. The information is important to help us to understand what diseases, accidents or injuries kill dogs in Hluvukani, so that we can help the Hluvukani Animal Clinic and the Mpumalanga Veterinary Services to improve the lives of dogs in this area.

We now ask if you would like to be a part of the study. You are being asked to take part because your dog is enrolled in the Health and Demographic Surveillance System study, and has been randomly selected to take part in the new study. This new study will continue for one year. With your permission, we will visit you every two weeks to find out if your dog is still alive. If your dog dies, we would like you to inform us as soon as possible by telephone or SMS, e.g. by sending a “please call me” message. In these cases, we would like to visit you at your home to conduct an interview with you or a member of your household to try to find out a bit more about what the dog died from. The interview will take about 30 minutes. We would also like to examine the dog within 12 hours after death (a ‘post-mortem’ examination) and take some tissue samples to test for some conditions that might have killed the dog. To do this, we will take the body of the dog away and perform the examination at the research station, not at your home. After we have examined the dog and taken the tissues, we will burn the body of the dog. We will do many of the tests soon after we take the tissues, and we will provide feedback to you. We would also like your permission to store the tissues and to make them available to other studies that may want to test for other diseases.

If you have a female dog, we would also like you to inform us by telephone or SMS if she gives birth. We will then visit your home as soon as possible to count the puppies. We would then like to visit every week, until the puppies are 4months old, to see if all the puppies are still alive. If any of the puppies dies, we would like you to inform us by telephone or SMS. In these cases, we will also do an interview and may do a post-mortem examination.

To inform us of a death or a birth, you can send a ‘Please call me’ to the following number, or you can phone/SMS the number:

076 044 3803

If you use any airtime in trying to contact us, we will provide you with an airtime voucher from your service provider for the amount that you spent.

We would also like to ask your permission to contact you via SMS. This will be to send out reminders about the study, or to inform you of anything related to the project. We promise not to send you messages more than once per week. We also promise not to share your contact details with anyone not related to the study. If you give permission for us to contact you, please provide your cell phone number at the end of this form.

There are no direct benefits to you, your family or your dog if you participate in this study. Your participation will help us to understand what dogs are dying from, and what things can be done to improve the health of dogs in this and similar communities.

You are free to decide if you want to participate in the study or not. If you do decide to participate today, but change your mind later, you are free to leave the study at any time without any consequences to you, your family or your dog.

If you have any questions about this study, you should feel free to ask them now or anytime throughout the study by contacting Julia Sithole (081 854 2442) at the Hluvukani Animal Health Clinic.

By signing this consent form, you are indicating your consent to participate in this study.

Name:__________________________ Village: _________________ Stand no: _________

Signature ____ Date _____________

Do we have your permission to contact you via SMS? Yes ☐ No ☐

If 'Yes', what is your cellphone number? ____

APPENDIX 2

Questionnaire: births of puppies in Hluvukani

Date:____________________________ Village:_________________________________
Stand number:__________________________

1. Identification of the bitch
Microchip number:______________________________

If microchip number is not available, note dog ID (Village-Stand-Sex-Name):
____

2. The litter
When was the litter born?
Lower Estimate________________________________
Upper estimate________________________________
Unit : Hours/ Days/Weeks

Do you have access to the litter?
- o Yes
- o No

2.1 Puppies born alive
How many puppies are born alive? _____________________

Of the puppies born alive, how many are male? ________________

Of the puppies born alive, how many are female? ______________

2.2 Stillborn puppies
How many puppies are stillborn? _______________________

Of the stillborn puppies, how many are male? ________________________

Of the stillborn puppies, how many are female? ________________

2.3 Puppies that died after birth
Did any puppies that were born alive die within 48 hours after birth?
- o Yes
- o No
- o Don't know

If yes, how many puppies died within 48 hours after birth? _____________

How many of those puppies were male? ______________________

How many of those puppies were female? ____________________

How many hours/days/ after birth did those puppies die?
Time after birth:

Lower estimation______________________
Upper estimation______________________
Unit: hours/days

APPENDIX 3

VERBAL AUTOPSY QUESTIONNAIRE

This verbal autopsy questionnaire has nine (9) sections as shown below:

6. Background information on dog and household
    - Name
    - ID no of dog/home address
7. Background information about the interview
    - Language
    - Interviewer name
    - Date of interview
    - Date of entry into the computer
8. Background information about owner/care giver/respondent
9. Information about the dog
    - Age of the dog
    - Date of death
    - Where the dog died
10. Open history questions
    - Description of illness in caregivers own words
    - No specific prompting, and it's a detailed narrative
    - Length of illness
    - Type of care given---hospital----home----elsewhere

6a. Accidents (puppy)

- Injuries
- Accidents
- Poisoning
- Bites
- Burns
- Drowning

6b. Puppy death (Puppies<120days)

7a. Accidents (Adult)

- Injuries
- Accidents
- Poisoning
- Bites
- Burns
- Drowning

7b. Adult death (Adults≥12 months)

8. Treatment and Record

Questionnaire Id

Instruction to interviewer: Introduce yourself and explain the purpose of your visit. Ask to speak to an adult caretaker who was present during the illness that led to death. If this not possible, arrange a time to revisit the household when the caretaker will be home.

Section 1: Background information on dog and household

(to be filled in before interview)

1.1 Address of household;

Village: ☐Eglington Stand no: _________

☐Clare

1.2 Name of dog: ___________________________________

Microchip number_____________________________________

If no microchip, ID number ("Name"-"Sex"-"Village"-"Stand Number"):

____

1.3 Sex of dog

☐ Male

☐ Female

☐ Unknown

Section 2: Background information about the interview

2.1 Interview language ☐Xitsonga

☐English

☐Other: Specify________________________________

2.2 Interviewer name: ____

2.3 Dates:

	Day/month/year;Time
2.3.1 Date of first interview attempt	
2.3.2 Date and time arranged for second interview attempt	
2.3.3 Date and time arranged for third interview attempt	
2.3.4 Date of interview	
2.3.5 Date form checked by supervisor	
2.3.6 Date entered in computer	

Section 3 **Information about respondent**

3.1 Which person takes care of the dog?

- ☐ Father of the family
- ☐ Mother
- ☐ Child
- ☐ Other: Specify____

3.2 Do you feed the dog regularly? (*refers to respondent, not household*)

- ☐ Yes
- ☐ No

3.3 Do you handle the dog regularly?(*refers to respondent, not household*)

- ☐ Yes **(go to section 4)**
- ☐ No (**go to 3.3.1**)

3.3.1 Can you handle the dog if needed?(*refers to respondent, not household*)

- ☐ Yes
- ☐ No

Section 4 Information about the dog

4.1 Age of the dog at the time of death

Lower estimate: _ _

Upper estimate: _ _

- ☐ Unit: Days
- ☐ Months
- ☐ Years

4.2 Do you know the day the dog died?

- ☐ Yes **(go to 4.2.1)**
- ☐ No **(go to 4.3)**

4.2.1 what day did the dog die ? (d/m/y) _ _/_ _/_ _ _ _

4.2.2 When did the dog die?

Lower estimate: _ _

Upper estimate: _ _

- ☐ Unit: Days
- ☐ Months
- ☐ Years

4.3 What was the length of time the dog was ill before it died?

- ☐ 0-6 days
- ☐ ≥7 days but < 14days
- ☐ ≥14 days but < 21days
- ☐ 21 days and above

4.4 Where didthe dog die?

- ☐ Home/Stand
- ☐ Neighbourhood
- ☐ HAC (Hluvukani Animal Clinic)
- ☐ On the way to HAC
- ☐ Other: Specify: ________________________________

***Note** to interviewer….Is this interview for a puppy or adult*

☐ puppy ☐ Adult

Section 5: **Open history question**

5.1 Could you tell me about the illness / circumstancesthat led to death?

Prompt: **Was there anything else?**

***Instruction to interviewer**- Allow the respondent to tell you about the illness in his or her own words. Do not prompt except to ask whether there was anything else after the respondent finishes. Keep prompting until the respondent says there was nothing else. While recording, underline any unfamiliar termsand write down principalclinicalsigns(use the categories given in Annex)*

(Take time to tick items mentioned spontaneously in the open history questionnaire)

If dog was less than 120 days old at the time of death, go to Section 6Puppyaccident/deaths

If the dog was 1 year old or more at the time of death, go to Section 7Adult accident/deaths

Section 6a: Accident (Puppy)

Write the number of puppy deaths you are investigating______________

6. Litter size:

6.0.1 How many puppies were born alive: __________________________

6.0.2 How many puppies were stillborn: ___________________________

6.1 Did your puppy die from an injury, accident, poisoning, bite, burn or drowning?

- ☐ Yes **(go to 6.1.1)**
- ☐ No **(go to section 6b)**
- ☐ Don't know **(go to section 6b)**

6.1.1 What kind of injury or accident?

Allow respondent to answer spontaneously. If respondent has difficulty identifying the injury or accident, read the list slowly.

- ☐ Violence
- ☐ Motor vehicle accident
- ☐ Fall
- ☐ Drowning
- ☐ Poisoning
- ☐ Bite or sting by a venomous animal
- ☐ Burn
- ☐ Bite or injury from another animal
- ☐ Other injury: Specify: ____

6.2 How long did the puppy survive after the **accident / incident** ?

☐ Died suddenly

☐ Died within 24 hours

☐ Died 1 day later or more

6.3 Was the puppy ill before the accident / incident?

Yes ☐ **(For puppies: go to section 6b.)**

No ☐ **(go to section 8)**

Don't know ☐ **(go to section 8)**

Section6b **Puppy death**

6.4 Was the late part of the pregnancy, labour or delivery complicated?

- ☐ Yes **(go to 6.4.1)**
- ☐ No **(go to 6.5)**
- ☐ Don't know **(go to 6.5)**

6.4.1 What complications occurred during the pregnancy, labour or delivery? ***(record all responses)***

- ☐ Bitch had convulsions
- ☐ Puppies stuck
- ☐ Excessive bleeding
- ☐ Emergency caesarean section
- ☐ Others:

Specify:____

(After respondent finishes prompt) **Was there anything else***?*

(Keep using this prompt until the respondent replies that there were no other complications.)

6.5 Were there any **bruises** or marks of **injury** on the puppy's body at birth?

- ☐ Yes
- ☐ No
- ☐ Don't know

6.6 Did the puppy have any **malformations** at birth?

- ☐ Yes **(go to 6.6.1)**
- ☐ No **(go to 6.7)**
- ☐ Don't know **(go to 6.7)**

6.6.1 Where were the **malformations**? (***Tick one or several boxes***)

- ☐ Head
- ☐ Body
- ☐ Fore limbs
- ☐ Hind limbs
- ☐ Other: Specify:______________________________

(*After respondent finishes prompt*): Were there malformations anywhere else?

(*Keep using this prompt until the respondent replies that there were no malformations anywhere else*).

6.7 At the time of birth was the puppy…

- ☐ Very small
- ☐ Smaller than usual
- ☐ About average
- ☐ Larger than usual
- ☐ Don't know

6.8 Was the puppy able to **breathe** after birth

***(Note: this does not include gasps or very brief efforts to breathe*)**

☐ Yes

☐ No

☐ Don't know

6.9 Was the puppy able to **suckle** (or bottle feed) in a normal way after birth?

☐ Yes

☐ No

☐ Don't know

6.10 During the illness that led to death did the puppy have **spasms** or **convulsions**?

☐ Yes

☐ No

☐ Don't know

6.11 During the illness that led to death, did the puppy have **tetanus**?

☐ Yes

☐ No

☐ Don't know

6.12 During the illness that led to death, did the puppy become **unresponsive/unconscious**?

☐ Yes

☐ No

☐ Don't know

6.13 During the illness that led to death, did the puppy have **difficulty in walking**?

☐ Yes

☐ No

☐ Don’t know

6.14 During the illness that led to death, did the puppy have a **fever**?

☐ Yes

☐ No

☐ Don’t know

6.15 During the illness that led to death, did the puppy have **yellow eyes** or ***gums***?

☐ Yes

☐ No

☐ Don’t know

6.16 During the illness that led to death, did the puppy have **pale eyes** or **gums**?

☐ Yes

☐ No

☐ Don’t know

6.17 *If the puppy was more than 10 days old, ask*: During the illness that led to death was the puppy **blind**?

☐ Yes

☐ No

☐ Don’t know

6.18 During the illness that led to death, did the puppy **vomit**?

☐ Yes **(go to 6.18.1)**

☐ No **(go to 6.19)**

☐ Don't know **(go to 6.19)**

6.18.1 For how long did the puppy **vomit** before death?

☐ 0-6 days

☐ ≥7 days but < 14days

☐ ≥14 days but < 21days

☐ 21 days and above

6.19 During the illness that led to death, did the puppy have frequent **loose** or **liquid stools**?

☐ Yes **(go to 6.19.1)**

☐ No **(go to 6.20)**

☐ Don't know **(go to 6.20)**

6.19.1 For how long did the puppy have loose stool or liquid stool?

☐ 0-6 days

☐ ≥7 days but < 14days

☐ ≥14 days but < 21days

☐ 21 days and above

6.19.2 Was there **blood** in the loose or liquid stools?

☐ Yes **(go to 6.19.2.1.)**

☐ No **(go to 6.19.3)**

☐ Don't know **(go to 6.19.3)**

6.19.2.1. If there was blood in the loose stool, was the stool **foul smelling**?

☐ Yes

☐ No

☐ Don't know

6.19.3 During the time with loose or liquid stools, did the puppy **drink lots of fluid**? (Dehydration)

☐ Yes

☐ No

☐ Don't know

6.20 During the illness that led to death, did you see **worms** in the stool?

☐ Yes **(go to 6.20.1)**

☐ No **(go to 6.21)**

☐ Don't know **(go to 6.21)**

6.20.1 Whatcolour was the worm?

☐ Red

☐ White (Boiled rice- like)

☐ Don't know

6.21 During the illness that led to death, did the puppy have a **swollen belly**?

☐ Yes

☐ No

☐ Don't know

6.22 During the illness that led to death did the puppy have a **cough**?

- ☐ Yes **(go to 6.22.1)**
- ☐ No **(go to 6.23)**
- ☐ Don't know **(go to 6.23)**

6.22.1 For how long did the **cough** last?

- ☐ 0-6 days
- ☐ ≥7 days but < 14days
- ☐ ≥14 days but < 21days
- ☐ 21 days and above

6.23 During the illness that led to death, did the puppy have a **secretion** from the **nose**?

- ☐ Yes **(go to 6.23.1)**
- ☐ No **(go to 6.24)**
- ☐ Don't know **(go to 6.24)**

6.23.1 What kind of **secretion**? *Select more than one if necessary**(prompt to describe)***

- ☐ Watery
- ☐ Thick
- ☐ Bloody
- ☐ Foamy
- ☐ Greenish
- ☐ Yellowish

6.24 During the illness that led to death, did the puppy have **pneumonia**?

- ☐ Yes
- ☐ No
- ☐ Don't know

6.25 During the illness that led to death, was the puppy **salivating** a lot?

☐ Yes

☐ No

☐ Don't know

6.26 Before the illness that led to death, did any dog **bite the puppy**?

☐ Yes **(go to 6.26.1)**

☐ No **(go to 6.27)**

☐ Don't know **(go to 6.27)**

6.26.1 How long ago was the puppy bitten?

☐ 2 weeks or less;

☐ 3 weeks to 3 months;

6.27 If the puppy was biting people/animals, did the puppy die on its own or was the dog killed by people?

☐ Died on its own

☐ Kill by people/owner

6.28 During the illness that led to death, do you think the puppy had **rabies**?

☐ Yes

☐ No

☐ Don't know

6.29 During the illness that led to death, did the puppy have a **skin condition**?

☐ Yes

☐ No

☐ Don't know

6.30 During the illness that led to death, did the puppy have ***ectoparasites***on its body?

☐ Yes **(go to *6.30.1*)**

☐ No **(go to 6.31)**

☐ Don't know **(go to6.31)**

6.30.1What kind of ectoparasite?*Select one or more options*

☐ Ticks

☐ Fleas

☐ Lice

☐ Others: Specify:___________________________

6.31 During the illness that led to death, did the puppy **lose a lot of hair**?

☐ Yes

☐ No

☐ Don't know

SECTION 7a **ACCIDENT** (Adult)

7.1 Did your dog die from an injury, accident, poisoning, bite, burn or drowning?

- ☐ Yes **(go to 7.1.1)**
- ☐ No **(go to section 7b)**
- ☐ Don't know **(go to section 7b)**

7.1.1 What kind of injury or accident?

Allow respondent to answer spontaneously. If respondent has difficulty identifying the injury or accident, read the list slowly.

- ☐ Violence
- ☐ Motor vehicle accident
- ☐ Fall
- ☐ Drowning
- ☐ Poisoning
- ☐ Bite or sting by a venomous animal
- ☐ Burn
- ☐ Bite or injury from another animal
- ☐ Other injury: Specify: ___________________________________

7.2 How long did the dog survive after the **accident / incident** ?

- ☐ Died suddenly
- ☐ Died within 24 hours
- ☐ Died 1 day later or more

7.3 Was the dog ill before the accident / incident?

- ☐ Yes **(go to section 7b)**
- ☐ No **(go to section 8)**
- ☐ Don't know **(go to section 8)**

Section 7b: **Adult dog death**

7.4 During the illness that led to death, did the dog stop **eating**?

- ☐ Yes
- ☐ No
- ☐ Don't know

7.5 During the illness that led to death, was the dog **thin/emaciated**?

- ☐ Yes
- ☐ No
- ☐ Don't know

7.6 During the illness that led to death did the dog have **spasms** or **convulsions**?

- ☐ Yes
- ☐ No
- ☐ Don't know

7.7 During the illness that led to death, did the dog have **tetanus**?

- ☐ Yes
- ☐ No
- ☐ Don't know

7.8 During the illness that led to death, did the dog become **unresponsive /unconscious**?

- ☐ Yes
- ☐ No

☐ Don’t know

7.9 During the illness that led to death, did the dog have **difficulty in walking**?

☐ Yes
☐ No
☐ Don’t know

7.10 During the illness that led to death, did the dog **cry /whine** all through the night?

☐ Yes
☐ No
☐ Don’t know

7.11 During the illness that led to death, did the dog have a **fever**?

☐ Yes
☐ No
☐ Don’t know

7.12 During the illness that led to death, did the dog **vomit**?

☐ Yes **(go to 7.12.1)**
☐ No **(go to 7.13)**
☐ Don’t know **(go to 7.13)**

7.12.1 For how long did the dog vomit before death?

☐ 0-6 days
☐ ≥7 days but < 14days
☐ ≥14 days but < 21days
☐ 21 days and above

7.13 During the illness that led to death, did the dog have frequent **loose or liquid stools**?

- ☐ Yes **(go to 7.13.1)**
- ☐ No **(go to 7.14)**
- ☐ Don't know **(go to 7.14)**

7.13.1 For how many days did the dog have loose stool or liquid stool?

- ☐ 0-6 days
- ☐ ≥7 days but < 14days
- ☐ ≥14 days but < 21days
- ☐ 21 days and above

7.13.2 Was there **blood** in the loose or liquid stools?

- ☐ Yes
- ☐ No
- ☐ Don't know

7.13.3 If there was blood in the loose stool, was the stool **foul smelling**?

- ☐ Yes
- ☐ No
- ☐ Don't know

7.13.4 During the time with loose or liquid stools, did the dog **drink lots of fluid**?

- ☐ Yes
- ☐ No
- ☐ Don't know

7.14 During the illness that led to death, did you see **worms** in the stool?

- ☐ Yes **(go to 7.14.1)**
- ☐ No **(go to 7.15)**
- ☐ Don't know **(go to 7.15)**

7.14.1 Whatcolour was the worm?

- ☐ Red
- ☐ White (Boiled rice- like)
- ☐ Don't know

7.15 During the illness that led to death, did the dog have a **swollen belly**?

- ☐ Yes
- ☐ No
- ☐ Don't know

7.16 During the illness that led to death, did the dog have a **cough**?

- ☐ Yes **(go to 7.16.1)**
- ☐ No **(go to 7.17)**
- ☐ Don't know **(go to 7.17)**

7.16.1 For how long did the dog cough before death?

0-6 days ☐

☐ ≥7 days but < 14days

☐ ≥14 days but < 21days

☐ 21 days and above

7.17 During the illness that led to death, did the dog have **difficulty breathing**?

☐ Yes

☐ No

☐ Don’t know

7.18 During the illness that led to death, did the dog have a **secretion** from the **nose**?

☐ Yes **(go to 7.18.1)**

☐ No **(go to 7.19)**

☐ Don’t know **(go to 7.19)**

7.18.1 What kind of **secretion**? *Select more than one if necessary* ***(prompt to describe)***

☐ Watery

☐ Thick

☐ Bloody

☐ Foamy

☐ Greenish

☐ Yellowish

7.19 During the illness that led to death, did the dog have **pneumonia**?

☐ Yes

☐ No

☐ Don’t know

7.20 During the illness that led to death, did the dog **salivate** a lot?

- ☐ Yes
- ☐ No
- ☐ Don't know

7.21 Before the illness that led to death, did any other dog **bite the dog**?

- ☐ Yes **(go to 7.21.1)**
- ☐ No **(go to 7.22)**
- ☐ Don't know **(go to 7.22)**

7.21.1 How long ago was the dog bitten?

- ☐ 2 weeks or less;
- ☐ 3 weeks to 3 months;
- ☐ 4 to 11 months ,
- ☐ 12 months or more

7.22 If the dog was biting people/animals, did the dog die on its own or was the dog killed by people?

- ☐ Died on its own
- ☐ Kill by people/owner
- ☐

7.23 During the illness that led to death, do you think the dog had **rabies**?

- ☐ Yes
- ☐ No
- ☐ Don't know

7.24 During the illness that led to death, did the dog have **skin condition**?

☐ Yes

☐ No

☐ Don't know

7.25 During the illness that led to death, did the dog have **ectoparasites**on the body?

☐ Yes **(go to 7.25.1)**

☐ No **(go to 7.26)**

☐ Don't know **(go to 7.26**)

7.25.1 What type of **ectoparasite**?

☐ Ticks

☐ Fleas

☐ Lice

☐ Others: Specify:____________________________

7.26 During the illness that led to death, did the dog **lose a lot of hair**?

☐ Yes

☐ No

☐ Don't know

7.27 During the illness that led to death, did the dog have **pale eyes** or **gums**?

☐ Yes

☐ No

☐ Don't know

7.28 During the illness that led to death, did the dog have **yellow eyes** or **gums**?

☐ Yes

☐ No

☐ Don’t know

7.29 During the illness that led to death, did the dog have any **swelling/growth** on any part of the body?

☐ Yes

☐ No

☐ Don’t know

Section 8: TREATMENT AND RECORD

I would like to ask a few questions about any medicationyour dog may have received during the illness that led to death

8.1 Was care sought outside the home while the dog was ill?

- ☐ Yes **(go to 8.1.1)**
- ☐ No **(go to 8.2)**
- ☐ Don't know **(go to 8.2)**

8.1.1 Select all the places that help was sought outside the house? *(select one or more options)*

- ☐ AHT (including dip tank)
- ☐ HAC (including dip tank/UP)
- ☐ Pharmacy
- ☐ Traditional healer
- ☐ Other: Specify:____________________________________

8.2 Did the dog receive any medication during the illness?

- ☐ Yes **(go to 8.2.1)**
- ☐ No **(go to 8.3)**
- ☐ Don't know **(go to 8.3)**

8.2.1 What kind of medication did the dog receive?

____

____

8.3 Was the dog ever vaccinated?

☐ Yes **(go to 8.3.1)**
☐ No **(go to end)**
☐ Don't know **(go to end)**

8.3.1 Was it vaccinated against rabies?

☐ Yes **(go to 8.3.1.1)**
☐ No **(go to 8.3.2)**
☐ Don't know **(go to 8.3.2)**

8.3.1.1 When was rabies vaccine done?

☐ Less than 1 year ago
☐ 1 year ago
☐ 2 years ago
☐ 3 years ago
☐ Don't know

8.3.2 Was it vaccinated with "5 in 1" vaccine?

☐ Yes **(go to 8.3.2.1)**
☐ No **(go to end)**
☐ Don't know **(go to end)**

8.3.2.1 When was "5 in 1" vaccine done?

Less than 1 year ago ☐
1 year ago ☐
2 years ago ☐
3 years ago ☐

END OF QUESTIONNAIRE

ANNEX

USE THIS TO GUIDE THROUGH THE REST OF THE QUESTIONNAIRE

5.1.1	DIARRHOEA
5.1.2	COUGH
5.1.3	FEVER/THIRST/WATER
5.1.4	SKIN LESIONS (when/where)
5.1.5	INJURY
5.1.6	UNCONCIOUS
5.1.7	SEIZURES
5.1.9	TETANUS
5.1.10	RABIES/SALIVATION
5.1.11	ASCITES/SWOLLEN STOMACH
5.1.12	STRANGE BEHAVIOUR
5.1.13	DIFFICULT BREATHING (fast or slow)
5.1.15	COMPLICATED BIRTH
5.1.16	MALFORMATION
5.1.18	VERY SMALL AT BIRTH
5.1.19	VERY THIN/emaciated
5.1.21	PNEUMONIA
5.1.22	ACCIDENT
5.1.23	DISTEMPER/PARVO
5.1.24	YELLOW MUCOUS MEMBRANE
5.1.24	PALE MUCOUS MEMBRANE
5.1.25	CONGESTED MUCOUS MEMB (reddish)

APPENDIX 4

Additionalquestionnairefor house-level and dog-level management

Standnumber: c40189
Dogname: Noname, Noname (left missing)
Dog ID:
Date:
NOT OWNER WHO ANSWERED

1. Why do you keep dogs?
- As a pet ☐
- For security ☐
- Cultural believes ☐
- Other ☐
- Don't know

2. How often do you feed the dog?
- Twice a day or more ☐
- Once a day ☐
- Not every day, but regularly ☐
- Sometimes ☐
- Never, the dog finds its own food ☐
- Don't know

3. What do you feed the dog?
- Pap ☐
- Pap + soup ☐
- Pap + meat/bones ☐
- Meat ☐
- Vegetables ☐
- Dog food ☐
- Other ☐
-

4. What is the source of water for the dog?
- Tap water from the household ☐
- Tap water from community ☐
- Rainwater on property ☐
- Finds his own water source ☐
- Other ☐

5. How do you keep the dog?
- Inside or in a cage the whole day ☐
- On the property all day ☐
- Partially on the property, partially free roaming ☐
- Free roaming all day ☐
☐

- Other

6. Is the dog vaccinated against Rabies?
- Yes ☐
- No ☐

7. When was the dog vaccinated against rabies?
- This year ☐
- One year ago ☐
- Two years ago ☐
- Three or more years ago ☐
- Never ☐

8. Is the dog sterilized
- Yes ☐
- No ☐

9. How many dogs did you have in June 2014
- One ☐
- Two ☐
- Three ☐
- Four ☐
- Five ☐
- Six ☐
- Seven or more ☐

10. How many dogs do you have now?
- One ☐
- Two ☐
- Three ☐
- Four ☐
- Five ☐
- Six ☐
- Seven or more ☐

11. What are the sexes of the dogs?

female
…………………………………………………………………………………………………
…………………………………………

APPENDIX 5

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Mortality rates and survival analysis of a cohort of owned adult dogs and puppies in Hluvukani, Buschbuckridge, South Africa**
PROJECT NUMBER	**V028-14**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. F Kolo**

STUDENT NUMBER (where applicable)	**UP_11233509**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Canine**	
NUMBER OF ANIMALS	**400**	
Approval period to use animals for research/testing purposes		**April 2014-April 2015**
SUPERVISOR	**Prof. D Knobel**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	5 December 2015
CHAIRMAN: UP Animal Ethics Committee	Signature	

S4285-15