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Rates and causes of mortality in adult dogs in an owned, free-roaming population

A demographic pilot study in Hluvukani, South Africa

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Contents

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Abstract

Introduction: It was estimated that yearly around 23,000 people die of rabies, inflicted by dog bites, in Africa. More than 95% of human rabies cases are caused by dog bites. Rabies is still a public health and economic burden. Vaccination campaigns in canine populations are very important in controlling rabies. To start a successful vaccination campaign, demographic information of the canine population is necessary. Mortality rates are a very important part of this demographic information. To decrease mortality rates, knowledge of the cause of death is also necessary.
To obtain more information about the mortality rates and causes in Hluvukani, South Africa a retrospective study Hluvukani (RSH) and longitudinal study Hluvukani (LSH) was started.
Hypotheses: Based on other studies, the mortality rate per month is expected to be around 3%. No difference is expected between the mortality rate per month in the RSH and LSH. It is assumed there is a relationship between mortality rates and sexes. Females have a higher mortality rates due to pregnancies and post partum mortality. Higher mortality rates are expected to be found in dogs with an age of five years or more. The most common cause of death in Hluvukani is thought to be diseases (unspecified which diseases exactly) or trauma (accidents, hit by car).
Methods & Results: A retrospective study obtained information over a period of 5 months through a questionnaire (n=509). After the retrospective study a longitudinal study was started. Enrolled dogs were visited every two weeks for about two months (n=368). Deaths were reported to the researchers during a revisit or by a phone call. Mortality rate for the retrospective study was 17.9% (3.6% per month) and 1.8% for the longitudinal study (0.9% per month). These mortality rates differ significantly. There was no relationship found between sexes and mortality rates. The mortality rate per age group (1-2, 3-4 or ≥5) did not differ. Most common cause of death in the longitudinal study was trauma (3/6). Causes of death were obtained by a questionnaire, because post mortem examination was not possible. Furthermore, the database of the Hluvukani Animal Clinic database was searched for mortalities (January 2012-May 2014). This study showed rabies (7/10) as the most common cause of death. Both conclusions on causes of death were based on low amount of data, causing these to be less reliable. It is likely that data obtained in the future will increase the reliability of this parameter.
Conclusion: To make sure these results are reliable a long-term longitudinal study is needed. This is currently being realized in Hluvukani, South Africa. To determine the cause of death, with more precision, post mortem examination is necessary. Results of this study do contribute to obtain further knowledge of the demography of the canine population in Hluvukani. Hopefully eventually leading to an effective vaccination campaign against rabies in the future.

Introduction

Free roaming dog populations have been studied in many parts of the world. Especially in area's with endemic zoonotic disease, as rabies, it is important to obtain a clear view of free-roaming dog populations. Africa is one of these areas were rabies is an endemic disease and still kills many people every year. Knobel et al 2005 have evaluated the public health and economic burden of endemic rabies, in canine populations, in Asia and Africa. Eight million people a year receive post exposure prophylaxis, after a dog bite, in Africa and Asia (1). Still around 23,000 people die yearly of dog inflicted rabies in Africa (1)(2). Ninety-nine percent of all human deaths, caused by rabies, occur in developing countries were rabies is endemic in canine populations and more than 95% of all cases of rabies in humans are caused by dog bites (3). Total costs of treatments after dog bites (including vaccination and immunoglobulins) and control of canine populations counts up to 20,5 million US

dollars a year (1). These numbers show that rabies in canine populations still causes an important public health and economic burden in Africa.
Rabies in canine populations can be controlled by two major measures. Firstly, by mass vaccination campaigns and secondly, by decreasing turn-over rates in the population (4). Vaccination against rabies has been shown to be a successful method to control rabies in canine populations (3, 5). To be able to vaccinate a canine population information of the population dynamics is needed (6, 7). The number of dogs in the population and the turn-over rates are needed to determine the percentage of dogs that needs to be vaccinated. The turn-over rate depends on the amount of dogs that die, are born and migrate.
To determine percentage of a population that needs to be vaccinated against any disease, information of the infectious rate of the disease is needed (8, 9). Information about the infectious rate is given by the basic reproduction number (R0). The R0 is a parameter that indicates how many individuals will get infected by one infected individual in a susceptible population. If the R0 is below one, the disease will not spread and eventually die off (9). The R0 in rabies is estimated between 1 and 2 (9), this means the disease will spread if the population is not protected. The vaccination coverage needed to control rabies can be calculated by using a formula (1-1/R0) to calculate the herd immunity threshold (10). The herd immunity threshold for rabies is 25-40% (11). Population immunity levels above these critical levels ensure rabies spread is interrupted (4). In the period between vaccination campaigns population, turn-over will lead to changes in the composition of the free roaming dog population, because of death of vaccinated dogs, birth of new dogs, in-migration of unvaccinated dogs and out-migration of vaccinated dogs (9, 12). Therefore, it is calculated that 70% of the dog population should be vaccinated during annual vaccination campaigns to prevent the vaccination coverage dropping below the critical threshold during the period between vaccination campaigns (3, 5, 12, 13). This is also recommended by the WHO (2). Study by Cleaveland et al (2003) showed a decline (69.5% after the first vaccination campaign and 97.4% after second campaign) in the incidence of rabies in a dog population with an initial vaccination coverage of 60-70% (5). The amount of rabid suspected dog bites also showed a successive decline of 92% after the third vaccination campaign (5).
By controlling the rapid turn-over of dogs in an area the risk of a rabies outbreak can be reduced. The risk is reduced because a minimum turn-over ensures the vaccination percentage to stay above the immunity threshold. In order to control a population of dogs, data on population dynamics are needed (6, 7, 12). These data includes death, births and migration ratios in the population. In order to diminish the total turn-over in the population the numbers of new births or migrations into the population must be reduced. The number of births can be reduced by contraception such as vaccination with an anti-Gonadotropin Releasing Hormone (GNRH) vaccine (4). In the Mnisi area in South-Africa the use of this GNRH vaccine will be studied. *Gonacon,* developed by the USDA APHIS Veterinary Services, has shown to cause contraception in both sexes. The Gonacon injection does not interfere with the rabies vaccine (14). Of course surgical contraception is also an effective method.
Culling has proven to be ineffective and inappropriate of controlling the canine population, because of the relationship between humans and domestic dogs (4).

Mortality rates and causes of death in dogs in Africa

Many studies have taken place to learn more about causes of death in dogs in general. The areas in which these studies have been done differ a lot and so do the methods that are used. They show that the cause of death depends on which part of the world dogs live. In the western part of Europe and North America veterinary care for animals is of a very high standard, this leads to higher a life expectancy and different causes of death, like neoplasia and diseases that come with old age (16-18). Another reason for the difference is the amount of purebred dogs and mixed bred dogs. Purebred dogs have a lot of genetic problems and many breeds have their specific main cause of death (17).
In Africa veterinary care is not as common and dogs are free-roaming . Dogs who live on the streets are likely to have a higher risk to get hit by a car. Furthermore, the dogs have more contact with each other, possibly leading to spreading of infectious diseases. These differences could contribute to higher mortality rates in Africa (19-21, 23)
Research in a free-roaming dog population in Tanzania showed a high mortality rate (19). The study took place from July to November 2005 and contained 2498 owned dogs. The mortality rates (five months) were 72% in dogs less than one year old and 80% in dogs of four years old (19).

A large demographic study was described for four area's in South Africa and Indonesia. Researchers were interested in the effects of humans and other environmental factors on the free-roaming canine population. To facilitate a comparison between two different environments and cultures two area's at Bali (Indonesia) and two area's in Gauteng Province(South Africa, SA) were chosen. Rabies is endemic in these four areas. Households were regularly visited and dogs were monitored from March 2008 until April 2011. The total amount of dogs monitored, per research, area were 1022(Zenzele, SA), 882(Braamfischersville, SA), 707(Kelusa, Bali) and 629(Antiga, Bali).They found no overall increase or decrease in mortality per month (6). Kelusa showed the highest mortality rate of 72.0% over three years, second highest was Zenzele with a mortality rate of 70.2%. Braamfischerville had the lowest mortality rate of 59,5% and Antiga had a mortality rate of 68.4%(6). Other reasons for the dogs to have left the study site were theft, being given away, disappearance, relocation with owner, dumping, not being found by researchers or other. In three years Zenzele lost (including mortality) 731 (71.5%) dogs, Braamfischersville lost 597 (67.7%) dogs, Kelusa lost 447 (63.2%) dogs and Antiga lost 386 (61.4%) dogs(6).
Another study on a dog population in the communal lands in Zimbabwe in 1994 showed a very high mortality rate over two years in dogs younger than one year old (71.8%) (20). After the age of one the mortality rate was consistently lower for all ages. The mortality rate for the other ages was around 5% per year (20).

Vaccination coverage
With numbers of mortality, birth and migration the turn-over rate of a population can be stated. This can be used to calculate to decrease of vaccination coverage in a population. The decrease in vaccination coverage in the four populations in Johannesburg and Bali is showed in Figure 1.

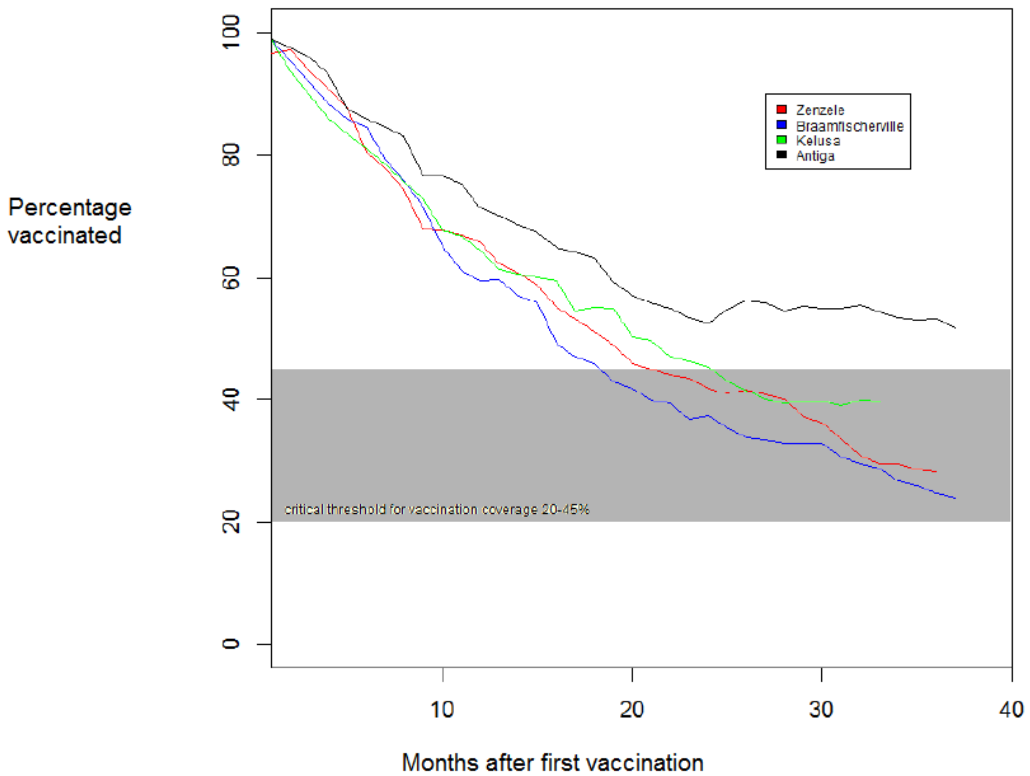

Figure 1. Decline in vaccination coverage after first vaccination campaign per research area (Morters et al 2014)

After approximately 20 months the vaccination coverage for Zenzele, Braamfischersvile and Kelusa dropped under the critical threshold (4). This implies that from this point the risk of a rabies outbreak in the population is higher. The two areas with the highest mortality rate also show the fastest decline in vaccination coverage. Thus high mortality rates cause a rapid decline in vaccination coverage. In populations with high mortality rates vaccination campaigns should have smaller intervals. For Zenzele, Braamfischersville and Kelusa a new vaccination programme should have started within 20 months to keep vaccination coverage from declining under the critical threshold.
The population in the Machakos district in Kenya also showed a rapid decline in vaccination coverage. The proportion of vaccinated dogs was 55% in the year after the last vaccination, 47% in the second year and 36% after two years (21). Meaning the vaccination coverage reaches the critical threshold around 2 years after vaccination. That seems to be around the same time as found in Johannesburg and Bali (6).

Life expectancy
Life expectancy also gives information about the turn-over rate in a population. If dogs die at early age Causing new susceptible dogs to come into the population and a decline in the vaccination coverage.
A demographic study in a free-roaming dog population in Kenya, showed a low life expectancy (21). The study included 305 dogs (182 male and 123 female). Only 39% of the female dogs survived to an age of 1 year. The overall mortality rate was estimated to be 36% during one year. The population had more male dogs in all age categories. Life expectancy for males (3.5 years) was longer than for female (2.3 years). The low life expectancy of the female dogs was caused by high neonatal and post partum mortality (21). The average life expectancy for the entire population was 2.9 years (21). This is higher than the 1,9 years determined by S.Gascoyne in 1994 for dogs in the Serengeti area in Tanzania. But lower than the life expectancy of 4.6 found in Manicaland, Zimbabwe (22). an even lower life expectancy of 1.1 (20). A total of 1085 dogs (614 males and 471 females) were surveyed, data was obtained by interview (20). These low life expectancies are mostly cause by high mortality rates in the first year of life (19-21, 23). An example of this is shown by the life expectancies found in Tanzania. The life expectancy at birth was 1.7 years but rose to 2.76 years after survival of the first year (19).

Cause of death
Not many demographic studies in Africa included the cause of death in dogs. This led to little knowledge of causes of death in canine populations in Africa. A study in Hluvukani in 2013 showed unspecified illness, trauma (accidents), euthanasia or killed by owner were common causes of death (24). In one case of euthanasia the reason was a tumour and distemper. In the other case the dog was unwanted (24).
In 434 dogs in Zimbabwe the most common causes of death were unspecified illness (35.5%) and unknown causes of disappearance (31.8%). The other causes were predation of leopards or lions. A few dogs died of diseases that come with old age (20).
Most deaths in a canine population in a urban area in Sierra Leone are due to lack of care and attention (23). Owners do not seek veterinary help in cases of illness or trauma. Sometimes even starvation can occur (23).
The most common cause of death found in the four area's in Johannesburg and Bali was that the dog was ill, this included injuries. The cause of the illness was not specified. The second most common cause of death was: "hit by a car" (6).
Another study, concerning causes of death, contains information about a very specific population. The authors followed a group of livestock-guarding dogs after retirement in Namibia. The most common cause of death was accidents (22/42). Accidents included drowning, snake bite, killed by baboon or hit by car. The second most common cause was culling by owner (12/42) (25). Other causes were killing by neighbour ranchers, disease or poisoning.

Study objective
Mortality rates are an important part of the population dynamics. High mortality rates cause a higher population turn-over and high turn-over rates cause a lower population immunity level (15). The population immunity level drops if dogs that are vaccinated die, causing the total amount of vaccinated dogs in the population to drop. The movement of dogs can increase with high mortality rates because

people get a new dog from another population. Movement of dogs from different populations increases the risk of infectious disease spreading and also makes changes in the immunity level of the population hence new probably susceptive animals enter the population. Canine populations with high turn-over rates need to be vaccinated after every 6-8 months (15).
This study was set up to obtain more knowledge about the mortality rates and causes in Hluvukani, South Africa. Hluvukani is located in the Mnisi community. This area was chosen because a large scale community at the human/wildlife interface is situated here. University of Pretoria implemented the Mnisi Community Program and developing research projects on One Health. One of them focuses on the population of free-roaming dogs. In 2011 a Health and Demographic Surveillance System (HDSS) was set up to collect information concerning the longitudinal measurement of demographic and health variables in dogs (9). All households within the research area were identified, as were all the dogs in the households. The longitudinal system of data collection continued with visits to all households every three months. At these visits, vital changes or events among dogs or the ownership status of the household were recorded (9). These changes included pregnancies, births (or other pregnancy outcomes), litter size, deaths and migrations. New dogs, born or migrated into the population, were enrolled. .
The aim of this study was to obtain more information about the mortality rates and causes in adult dogs in Hluvukani. Differences between sex and age specific mortality were of also of interest. With this information more insight in the demography of the population is obtained. This information can help lead to a plan to decrease the amount of mortality in the population and/or better vaccination campaigns. With the objective to achieve a vaccination coverage above the critical threshold, so the rabies transmission in the population is reduced. To answer these questions a retrospective and longitudinal study was started.

Materials and methods

1. Retrospective and longitudinal study Hluvukani (RSH and LSH)

Research area

The research areas of the retrospective study Hluvukani (RSH) and longitudinal study Hluvukani (LSH) fall within the Mnisi Community Programme (University of Pretoria, UP). This programme is located in the Bushbuckridge municipality in Mpumalanga Province in north-east South Africa. The Mnisi Community area is bordered by several game reserves as shown on the map (figure 2). The village of Hluvukani consists of two parts, Clare B and Eglington. These parts are divided by a tar road. In the areas residents own dogs, but these dogs live free on the streets and can have contact with all other dogs and humans of the village. Thus they are free-roaming.
The Hluvukani Animal Clinic was officially opened in 2009. The clinic is a project of the University of Pretoria and is used for teaching veterinary students as well as providing the area with sound veterinary care. The Mnisi Community Programme was started to study the complex interactions at the human/wildlife interface. Because of the geographic location of the area a lot of different relations between wildlife, livestock and humans can be studied. Public health is very important in this programme. Different researchers and students work together with environmental monitors (EM). The environmental monitors are specially trained to collect data of environment, wildlife and domestic animals. They work as a link between researchers and the people of the village.
All owned dogs in the Hluvukani are monitored and recorded by the HDSS-dog project (Health and Demographic Surveillance System for dogs). At the moment of this study, the Demographic Surveillance Area (DSA) consisted of 2218 households. The size of the DSA is 10.4 square kilometres, the part with households and shops has the size of 4.1 square kilometres (9). Dogs in the DSA are identified by microchip. If the dog did not have a microchip (aggressive dogs or puppies), an unique identification code was given. The code includes the name of the dog, sex, area of residence (Eglington or Clare) and stand number. A picture was taken of these dogs to verify the identity of the dog. The HDSS-database keeps track of all births, deaths and migrations of dogs in the area. The canine population consisted of 711 dogs in January 2014. The households are visited quarterly to update the population information.
The Hans Hoheisen Wildlife Research Station (HHWRS, UP) is located in Kruger National park nearby Orpen gate. The research station served as a home-base during this study.

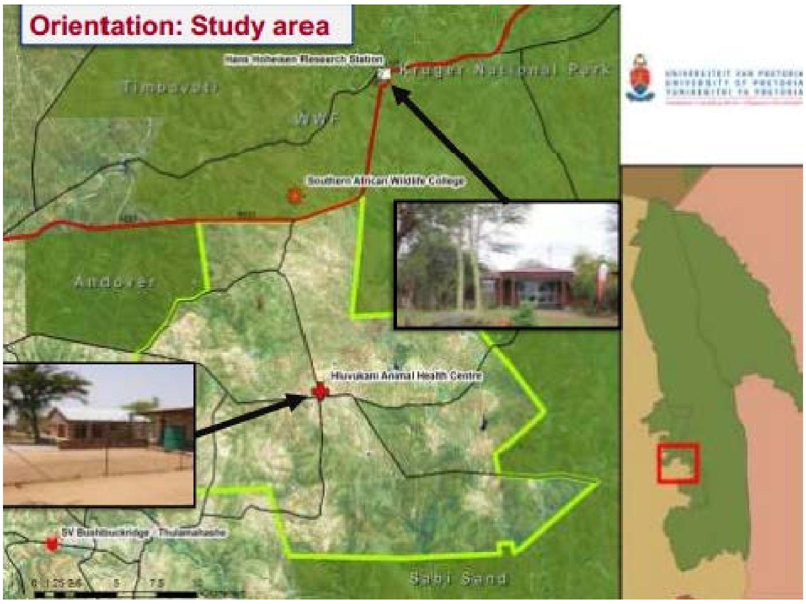

Figure 2. Map of the research area RSH and LSH. The Hluvukani Animal Clinic marked by the red cross in the middle of the Mnisi area.
The HHWRS is marked by the white dot. |The smaller map on the right shows the location of the area next to the wildlife reserves.

Selection of dogs
The aim was to enrol 200 adult female and 200 adult male dogs for the longitudinal study. The target population was all dogs aged one year or older. Dogs were randomly selected from the HDSS-database. All selected dogs were still alive in January 2014. During enrolment the retrospective study took place.
The selection for the male cohort of the longitudinal study was weighted by age. The categories consist of one to two year olds (1-2, group 1), three to four year olds (3-4, group 2) and five year and older (≥5, group 3). All male selected male dogs were visited till a number of 200 enrolled males was reached. Due to the low amount of female dogs in the population it was not possible to reach 200 enrolled female dogs. All selected females were visited.

Method of data collection
The owners of selected dogs were visited from the 12th of May 2014 till the 4th of June 2014.
The visits were carried out in two teams. Each team consisted of an Environmental Monitor (EM) and one or two fieldworkers. To be able to visit the households a map was made with all stand numbers on it. All households that had to be visited were marked on the map.
The EM communicated with the owner of the dog, while the fieldworker managed the interview and noted all the information obtained by the conversation. Interviews were in the local language (Shangaan). The following questions were asked for every dog: Is the dog still alive? If dead, what is the date of death? What was the cause of death? Is the dog still at the household? Is the microchip number the same as on list? What is the sex of the dog? What is the age of the dog?
The information obtained during the enrolment for the longitudinal study (LSH) formed the retrospective study (RSH). It provided information about the mortalities in the selected dogs from January 2014 (last update HDSS) until the date of visit (May 2014). If the dog was still alive and present at household owners were asked to participate in the LSH. The EM explained the procedure to the owner and let them sign a consent form. Owners had to sign a copy of the consent form, so they could keep one and one could be taken with the research team for administration. This consent form was drawn up in English (Appendix) and translated to Shangaan. After enrollment, enrolled dogs were visited every two weeks, from 01-06-2014 until 25-07-2014, to check if the dog was still alive. One household could own more than one enrolled dog. During a revisit the owner was asked if the dog was

still alive and if the fieldworkers could see it. If necessary the microchip number of the dog was checked. If the dog was still alive the owner was reminded of the study and asked to call or message the specific number if the dog should die.
If the dog had died previously, but the owners had not reported this, the carcass was checked to see if post mortem examination was still feasible. In all cases the owner was asked questions about the causes of death. The interviewer had to follow a verbal autopsy questionnaire. Parts of this verbal autopsy were recorded and translated into English by an EM.
In case of a deceased dog the owner was asked to send a “please call me” message or call the specific phone number for this project. If a report of a death of a dog was received, the household was visited as soon as possible (within 12 hours) to collect the carcass of the dog and to do a verbal autopsy. The carcass was brought to the Hans Hoheisen research station (HHWRS) to perform a post mortem examination.

Post mortem examination
The fieldworkers received additional post mortem training at Onderstepoort Faculty of Veterinary Science (OP) in Pretoria, for two weeks. A key feature of this training was taking correct samples like blood smears, brain smears, microbiology and histopathology samples. Another important feature was creating a morphological diagnosis. The morphological diagnosis always describes the severity, duration, location and distribution of lesions. It was important to train this, because all lesions found at post mortems needed to be well described with a morphological diagnosis. The pathologist, located at OP, used this morphological diagnosis, samples and pictures to give an interpretation and final diagnosis.
In the field the carcass of the dead dog was put in two plastic bags. Everyone who touched the dog during transportation had to wear gloves and disinfect his/her hands after handling the dog. The carcass was placed in a car and brought to HHWRS as soon as possible. In the special post mortem hall at the HHWRS the post mortem examination was performed. The carcasses were stored in the freezer at HHWRS.
Dogs were examined by following the standard protocol used at Onderstepoort Faculty of Veterinary Medicine. Samples were taken for bacteriology and histopathology and sent to the department of Pathology at Onderstepoort Faculty of Veterinary Science (OP). Bacteriology samples were only taken of suspected lesions and had to be taken sterile. This meant disinfecting forceps and scissors with formalin before collecting samples and in between taking samples. Blood and brain smears were always taken and were stained with a Diff-quick solution and stored for future research. Half of the brain was also kept in saline to test on rabies. Test used on the brain tissue was a fluorescent antibody test (FAT) and a histopathological stain. In stained samples Negri bodies are pathognomic for rabies. However, in only 50-80% of the infected samples Negri bodies are detected (26). The FAT test is seen as one of the most reliable tests for rabies (26, 27). Pictures were taken of all important lesions seen in the dog. The official cause of death was given by the pathologist of OP.

Data analysis
1.1 Retrospective Study Hluvukani (RSH)
Information of all visited dogs were put in an excel sheets. The percentages of all dogs alive, dead, migrated and of which the owner was not home were calculated for all dogs visited. The same was done separately for all female dogs and all male dogs.
Mortality rate for the total amount of studied dogs was calculated:

number of death/(total number selected dogs - dogs with owner not at home and/or migrated)

This formula only includes dogs of which was certain that they were alive or dead. Meaning migrated dogs and dogs of which the owner was not present at the household were excluded from calculations. Separate mortality rates for female and male dogs and by age group were also calculated using the same method.
To determine whether there was a difference in mortality rates by sex or by age, a chi-squared test was used. The chi-squared test was used with one degree of freedom (n-1). Rejecting the null hypothesis if $P<0{,}05$.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

To compare the different age categories in male dogs the same test was used. All three categories were tested at once, using two degrees of freedom. If the null hypothesis should be rejected, meaning there is a difference in the proportions of mortality between the age categories, all categories should be tested separately.
To make sure that censored proportions were equal in all calculations they were also tested with the Chi-squared test with respectively one or two degrees of freedom. This determined whether any categories contained more dogs that had migrated or of which the owner was not present at the household.

1.2 Longitudinal Study Hluvukani (LSH)
The amount of households, were the owner was not at home, were reported. The amount of dogs in these households were counted and percentages were calculated. These dogs were censored from the calculations of mortality rates.
Total amount of mortality in two months was noted. Mortality rate was calculated for these two months:

number of mortalities/total number of dogs at the beginning of the month

Separate mortality rates for the female and male cohort are calculated (mortality sex/total sample sex). The mortality rates were tested with the chi-squared test, using 1 degree of freedom. Rejecting the null-hypothesis when $p<0,05$.
Proportions of male dogs that died in every age category were calculated (mortality rate). These proportions were also compared with use of a chi-squared test using two degrees of freedom. Also rejecting the null-hypotheses when $p<0,05$. Since the exact date of death is known it can be determined whether there is a difference in the amount of deaths per month. Amount of deaths per month are counted and a mortality rate per month can be calculated.
The causes of death were also noted for each dog. Causes were categorized and the most common cause of death was determined.

Hypotheses
1. The mortality rates in the RSH and LSH, for one month, are expected to be around 3%, based on mortality rates found in other areas in Africa (6, 19, 21).
2. The mortality rates for females and males differ significantly ($p<0,05$). A higher mortality rate in females is expected in both studies. The life expectancy of females tends to be lower because of mortalities during and after pregnancy (21).
3. Very high mortality rates were seen in the first year of life. (19-21, 23) Causing low life expectancies in these populations. After the first year of life the mortality rates seem to decrease and stabilize (20). Though high mortality rates were found for dogs with increasing age (19, 23). Assuming this is the same for the canine population in Hluvukani we expect a higher mortality rate for dogs ≥5 years, in both studies, than for dogs in the other two age categories.
4. The mortality rate for June and July are expected to be the same in the longitudinal study. No seasonality has been found in previous studies (6)
5. Since not much is known about causes of death in dogs in Africa it is difficult to determine what to expect. Based on four studies found (6, 20, 24, 25) it can be said that a disease (without specific diagnoses), accidents (trauma) and culling by owners or neighbors are expected to be common causes of deaths. Because of the presence of rabies in the canine population, in and around Hluvukani, this is also an expected cause of death.

2. Database Hluvukani Animal Clinic

Research area

The database of the Hluvukani Animal Clinic contains information of an larger area, not only Hluvukani, is included in this database. The area in which the Animal Clinic is active is shown on the picture below. The clinic is based in Hluvukani and this is the centre of all activities. The area is located next to the Kruger National park, near Orpen Gate.

Dr. Greg Simpson, Environmental Monitors and undergrads veterinarian Students from OP work in this area daily to grant animal care to all animals in the area. They work at dip tanks for cattle shown on the map and work on call or appointments for all animal owners in the area.

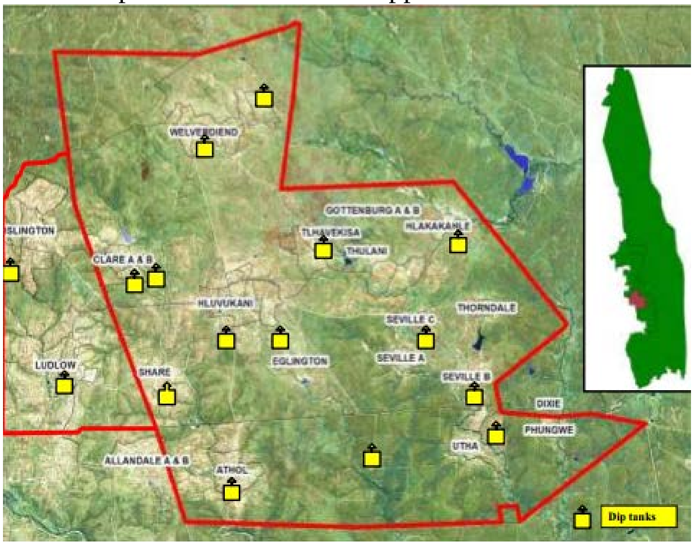

Figure 3. The Mnisi Community area, located in the bushbuck district of Mpumalanga, South Africa. Containing multiple villages around Hluvukani. Yellow marks are all diptanks in the area visited by the team of the Hluvukani Animal Clinic.

Data collection

The database of the Hluvukani Animal Clinic was consulted. The database consists of consultation reports recorded by the vet students and the vet on duty at the clinic. Only adult dogs (>1 year old) were used for this study. All treatments on dogs from 2012-2014 were searched. All dogs that had died were filtered. Sex, age, date of death and cause of death were reported. The information was collected in an excel sheet. The cause of death was placed in the following categories: Rabies, trauma, poisoning or unspecified disease. Trauma included car accidents, dog fights, wildlife attacks and violence inflicted by owner or neighbors. Unspecified disease included all dogs that showed signs of a disease as vomiting, diarrhea, anorexia, coughing, lethargy. In most cases the specific disease was not diagnosed. All cases of rabies noted were confirmed with positive test results. The dogs were placed in age categories of one-two year olds, three-four year olds and dogs older than five.

Data analysis

Because the amount of dogs in the area, at the time of the study, was unknown, no mortality rates could be calculated. Possible seasonality of mortality was determined by calculating the amount of deaths per month. These numbers per month were compared with a chi-squared test using one degree of freedom, rejecting the null-hypotheses if $p<0.05$.

The percentages females and males in all mortalities were determined. To see if there is a relation between sex and mortality a chi-squared test was used with one degree of freedom ($p<0.05$).

The amount of deaths in all age categories were calculated. These numbers were compared see if mortality was higher in one category.

The amount of causes of death per category were calculated. Percentages for all causes were also calculated. The most common cause of death was determined.

Hypotheses
1. Percentage of female mortalities is expected to be higher than percentage of male mortalities (21).
2. No seasonality in mortality is expected (6). Amount of death in each month should not differ.
3. Since not much is known about causes of death in dogs in Africa it is difficult to determine what to expect. Based on three studies found (6, 20, 24, 25) it can be said that a disease (without specific diagnosis), accidents (trauma) and culling by owners or neighbors are expected to be common causes of deaths. Because the presence of rabies in the canine population, in and around Hluvukani, this is also an expected cause of death.

3. Comparing mortality rates and causes of death.

Data analysis
To be able to compare the mortality rates of the LSH and RSH, a mortality rate per month was calculated. This was done by dividing the mortality rate by the amount of months. These mortality rates per month were compared by a chi-squared test with one degree of freedom. Null-hypothesis was rejected if $p<0.05$. The mortality rates for females and males were also compared using the same method.
The causes of death found in the LSH and database of the Animal Clinic were also compared, to see if the most common causes of death are similar.

Hypotheses
1. No significant difference is expected in the mortality rates for the LSH and RSH. Both mortality rates should be around 3% per month, based on studies in other areas in Africa (6, 19, 21).
2. The most common causes of death in the LSH and the database are expected to be similar. Both trauma and diseases are expected to be common causes of death. These diseases could still be unspecified in the database, but are expected to be diagnosed by post mortem examination in the longitudinal study.

Results

1.1. Retrospective Study Hluvukani (RSH)

Because of owners not being at home, some households had to be visited multiple times during the RSH. This caused a delay in the study. Another delay was caused by the high amount of dogs that had died or were no longer present at the household because of migration. The goal of finding 200 living female dogs was not reached after visiting all households in the first selection. Another selection was carried out and the same procedure applied. Because of the new selection some households had to be revisited. Still it was not possible to find 200 living female dogs. A total of 229 female dogs and 280 male dogs were visited during the RSH (enrollment). This means a total of 509 dogs. Three hundred sixty-eight (72%) dogs were still alive and present, thus enrolled into the longitudinal study. One hundred sixty-eight female and 200 male dogs were enrolled. The intention was to divide the male cohort in three equal age groups. However it was not possible to enroll enough male dogs in group 3. Table 1 shows the total of female and male dogs found alive in the RSH and thus enrolled in the LSH. The 509 visited dogs are 71.5% (509/711) of the total dog population of Hluvukani on the 1^{st} of January 2014. One hundred and twenty-one (27.7%) dogs were not enrolled because of the following reasons: The owners were not at home multiple times, the dog had died or the dog had migrated. All results found in RSH are shown in Table 2.
In case of a deceased dog the owner was asked for the exact date and cause of death. In most cases these questions could not be answered accurately. For this reason these data are not included in the study.

	Total	Female	Male	1-2	3-4	≥5
Dead	80	41	39	14	14	11
Alive	368	168	200	74	73	53
Owner not home	42	5	14	7	4	3
Migrated	19	15	27	8	10	9
Total	509	229	280	103	101	76
Mortality rates	17.9% n=448	19.6% n=209	16.3% n=239	15.9% n=88	16.1% n=87	17.2% n=64

Table 2. Amount of dogs in each category after questionnaire in retrospective study Hluvukani (January-May 2014). Mortality rate over period of five months, calculated after censoring two categories (owner not home and migrated).

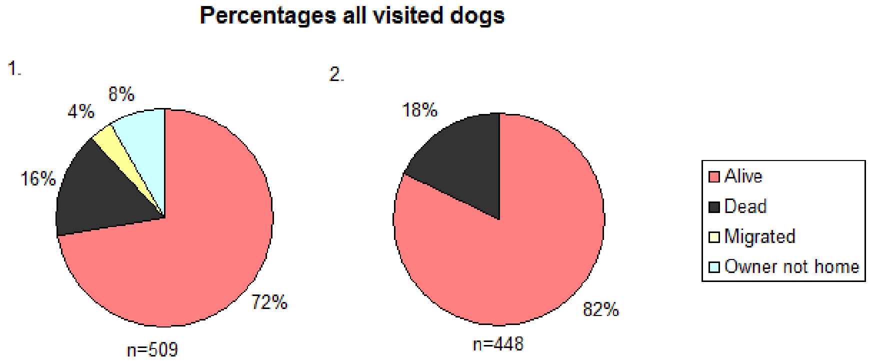

Figure 4. Percentages of all visited dogs found in the retrospective study in five months (January 2014-May 2014)1. All dogs.
2. Mortality percentages, after censorin). All dogs migrated and of which owner was not home excluded.

In the female cohort 229 dogs were visited of these 73.3% (168/229) was enrolled. The other 26.6% were also divided in categories. Forty-one (18%) female dogs had died, five (2%) had migrated and of 15 (7%) female dogs the owner was not at home. The male cohort showed similar kind of distribution in the different categories. Two-hundred (71%) of the male dogs were enrolled. Fourteen percent (39) of the male cohort had died, 5% (14) of the owners were not at home and 10% (27) of the dogs had migrated.
Since no actual information could be obtained for dogs in the households were the owner was not at home and where the dog had migrated these data were excluded for all mortality rate calculations. This leaves 209 female dogs and 239 male dogs, a total of 448 dogs.
After censoring the mortality rate for all dogs from January till May (five months) was 17.9% (80/448). The mortality rate in males (16.3%) seemed less than in females (19.6%), however, the difference was not significant (p=0.36). Meaning there is no relation found between sex and mortality rate.
In total 20 females and 41 males where excluded because the had migrated or the owner was not at home. The difference, between the amount excluded dogs per sex, is not significant (p=0.06).

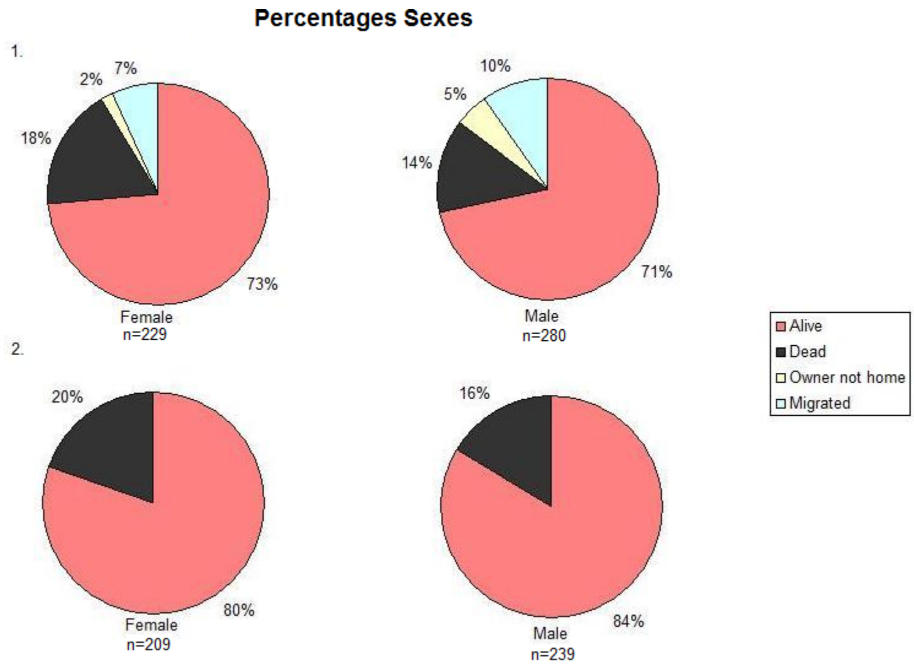

Figure 5. Results retrospective study in Hluvukani (January 2014-May 2014)
1. Percentages all visited females and males in retrospective study (five months).
2. Mortality percentages, after censoring, in females and male. All data of migrated dogs and dogs of which owner was not at home excluded.

Results male cohort

The male cohort was divided in age categories (group 1, 2 and 3). Group 1 contained 103 dogs. Fourteen (14 %) dogs in this category had died, seven had migrated and in eight cases the owner was not at home. Group 2, containing 101 dogs, showed an equal amount of dogs that had died. The amount of migrated dogs was four and for ten dogs no owner was present at the household. Less dogs were expected to be found alive in group 3. This seemed to be true, only 76 dogs were found in the HDSS-database. Of these only 53 were still alive. Eleven dogs in this age group had died. Three dogs were reported migrated and of nine dogs the owner was not at home.

Mortality rates slightly increased when the dogs age. The highest mortality rate found in dogs ≥5 and lowest mortality rate found in dogs from one to two. However, there is no significant difference found between the proportions of dead dogs between different age categories (p=0.98). Meaning there is no relationship found between mortality and age.

The number of dogs excluded was also did not differ significantly between the age categories (p=0.95).

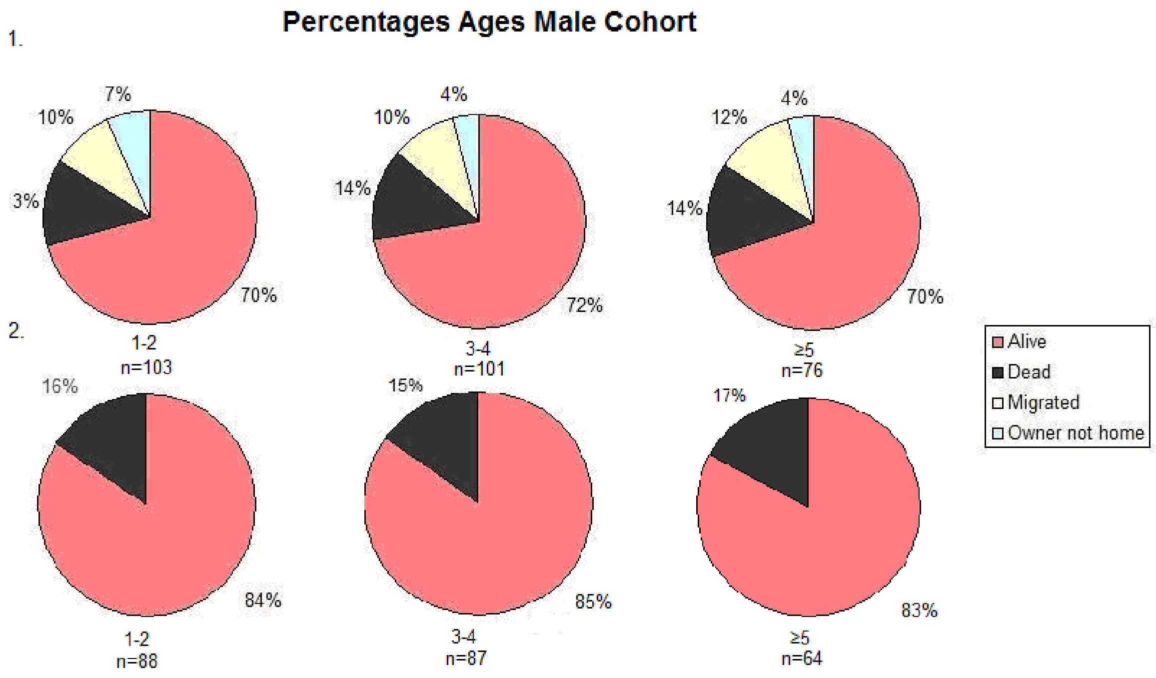

Figure 6. Results retrospective study in Hluvukani (January 2014-May 2014)
1. Percentages age categories male cohort..
2. Mortality percentages after censoring. All data of migrated dogs and owner not home excluded.

1.2 Longitudinal study Hluvukani

All, but one, selected dogs found alive during the RSH were enrolled in the LSH. Only one owner refused to sign the consent form. The remainder of visited dogs were categorized in three groups, dead, migrated and owner not home.

	Female	Male
Total	168	200
1-2	-	74
3-4	-	73
≥5	-	53

Table 1. Amount of dogs found alive in retrospective study Hluvukani, out of 509 dogs visited in May-June 2014. Showing total numbers of females and males enrolled in the longitudinal study Hluvukani.

After enrollment, at the first of June, the revisits started. Most households contained multiple enrolled dogs, leaving 219 households that had to be visited. It appeared that the first revisit took more time than two weeks, due to the start of a new research team. The first revisits took place from the nineteenth of June 2014 till the seventh of June 2014. The second revisit from tenth of June 2014 till the 23rd of June 2014. The next round of revisits was started in the week after the 23rd of July. In the first two rounds of revisits, the data of all owners who were not home was collected. In the first round this was the case in 22 households. These household were visited two times. These 22 (10%) households contained 37 (10%) dogs. During the second round of visits the owners were not present at 25 (11.4%) households. These households contained 38 (10.3%) dogs. In 13 households, were the owner was not at home during the first round, the owners were home during the second round. This way it was checked if the dog was alive. In the other 8 households the owner was not home during the second revisit as well. Leaving 38 (10.3%) dogs of which the status (alive or dead) was unknown after the second revisit. Of these dogs 21 were female and 17 were male. These dogs were censored from all mortality rate calculations, leaving 330 dogs (147 females and 183 males), of which status was known.

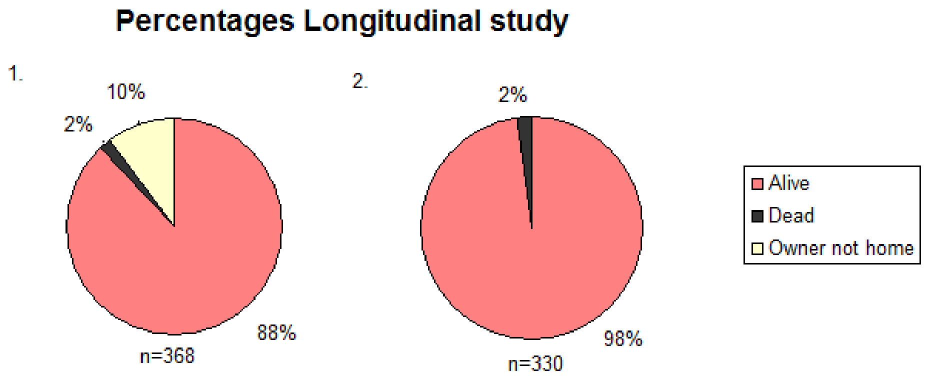

Figure 7. Proportions of all dogs in longitudinal study Hluvukani June and July 1. Percentages all enrolled dogs. 2. Mortality rate after censoring. All data of migrated dogs and of which owner was not home excluded.

It appeared that most owners had not called or reported it when their dog had died. Only one owner had come to the clinic to report the death of their dog. Other deaths were recorded during revisits. In two months a total of six dogs were reported or found death. The first death, Rax, was reported to the clinic. The carcass was collected and disposed of. The questionnaire outcome pointed to poisoning by rat poison. All other deaths were discovered by revisits. In these carcasses autolysis was already in advanced state, causing post mortem examination to be unfeasible. Therefore, cause of death was based on a questionnaire. Two (2/6) dogs seemed to be poisoned, probably with rat poison. Their carcasses showed blood loss and verbal autopsy indicated poisoning. In one (1/6) dog the exact cause of death is still unknown. Three (3/6) dogs died of trauma. Out of these dogs five were female, only one dog was a male. The male dog belonged to the age group of three to four year olds. For female dogs exact age was unknown.
The mortality rate for all dogs was 1.8% (6/330) in two months. The amount of mortalities in the female cohort seemed higher than in the male cohort. The mortality rate for females was 3.4% (5/147) and for males 0.5% (1/183). However, the difference was not significant (p=0.06). Because of only one death in the male cohort no comparison was made between age categories.
Five dogs died in June and only one died in July. Mortality rate for June is 1.5% (5/330), while the mortality rate for July is 0.3% (1/325). No significant difference found in mortality rates for June and July (P=0.11).

	Name	Sex	Age	Date of death	Cause of death
1.	Rax	Female	>1	10-06-2014	Poisoning
2.	Staywell	Female	>1	18-06-2014 t/m 25-06-2014	Trauma
3.	Bobby	Female	>1	13-06-2014	Poisoning
4.	No Name	Female	>1	14-06-2014	Trauma
5.	Snoppy	Male	3-4	24-06-2014 t/m 25/06-2014	Unknown
6.	Jj	Female	>1	08-07-2014	Trauma

Table 3. Results longitudinal study Hluvukani, all mortalities (6/368) June 2014-July 2014.

2. Database Hluvukani Animal Clinic

Information achieved from the database of the Hluvukani Animal Clinic showed a very low amount of deaths. Only 10 dogs were reported dead in the period of January 2012 to May 2014. Three (3/10) of them were female and six (6/10) were male. In one (1/10) of the dogs the sex was not recorded. The amount of male mortalities seemed to be higher than the amount of female mortalities. However, this was not a significant difference (p=0.18).

The total amount of dogs in the population was not known, as a result no mortality rates could be calculated.

Prominent is not only the low number of deaths, but also the distribution of deaths during this period. Two dogs died in January 2012, four dogs in October 2012, one in January 2013 and three in February 2013. This implicates the mortalities occur very clustered. Another striking variable was the cause of death. Seven out of ten dogs had died as a result of Rabies . The other three had died of unspecified disease (2/10) or trauma (1/10). The dog with trauma had an open fracture. No veterinary care was sought. The two dogs with an unspecified disease were both anorexic. No specific disease was diagnosed.

It was expected that cases of rabies would cause death in this population. However, it was not expected to be the most common cause of death.

Name dog	Sex dog	Age dog	Date of death	Cause of death
1. Nokia	Female	3-4	4-1-2012	Trauma
2 Dog 1	Male	1-2	5-1-2012	Rabies
3. Chuks	Male	1-2	4-10-2012	Rabies
4. Munyu	Female	3-4	4-10-2012	Rabies
5. Bobby	Male	3-4	14-10-2012	Unspecified disease
6. Sparky	Male	1-2	22-10-2012	Unspecified disease
7. No name	Female	1-2	8-1-2013	Rabies
8. Dog	Male	3-4	19-2-2013	Rabies
9. Unknown	Unknown	1-2	19-2-2013	Rabies
10. Dog	Male	1-2	19-2-2013	Rabies

Table 4. Mortalities found in database Hluvukani Animal Clinic (January 2012-June 2014).

Six (6/10) dogs had an age between one and two years. The other four (4/10) were three to four years old. Unfortunately no proportions of death in age categories could be calculated since these number are unknown.

3. Comparing mortality rates and causes of death

The mortality rate in the retrospective study in Hluvukani was 17.9% in five months. The mortality rate in the longitudinal study in Hluvukani was 1.8% in two months. To be able to compare these numbers they both have to be brought back to a mortality rate per month. For the RSH this was 3.6% and for the LSH this was 0.9%. This shows a much higher mortality rate per month from January to May than in June and July (p=0.0001), meaning there is a difference between the mortality rates from Jan-May and Jun-Jul.

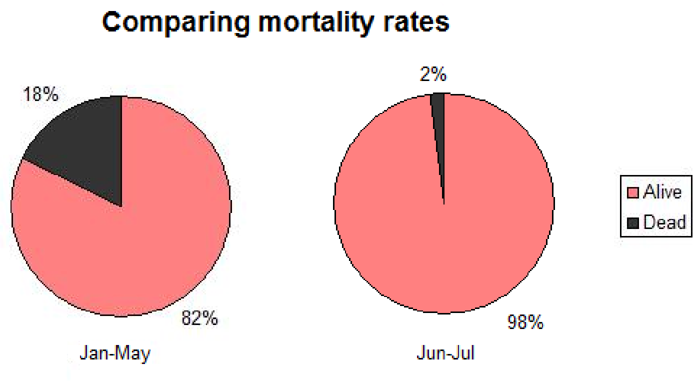

Figure 8. Mortality rates for retrospective (n=448, five months) and longitudinal study (n=330, two months).

To see if the difference is caused by one particular sex, these mortality rates were also compared. The mortality rate for females in the retrospective study was much higher (p=0.000001) than in the longitudinal study, respectively 19.6% and 3.0%. The difference between the males is also significant (p=0.0001). This implies that the mortality rates in both sexes differed from Jan-May and Jun-Jul differ significantly. It can not be said the difference in mortality rates is caused by one specific sex. The most common cause of mortality in the longitudinal study in Hluvukani was trauma (3/6), followed by poisoning (2/6). This differed from the most common cause of death in the study of the database Animal Clinic Hluvukani. The database showed the most common cause of death was rabies (7/10), followed by unspecified disease (2/10) and trauma (1/10).

Discussion

Mortality rates

The mortality rate obtained from the RSH is similar to mortality rates found in previous demographic studies in Africa (6, 21). To be able to compare these mortality rates all were brought back to mortality rates per month. The mortality rate found in the RSH was 3,6% per month. This is higher than the 3.0% expected at the beginning of this study and slightly higher than the mean mortality rate per month found in the four area's in Johannesburg and Bali (1,88%) and Kenya (3,0%) (6, 21). The mortality rates per month found in dogs in Tanzania were a lot higher (14,4% and 16%) (19). However these rates were age specific. The mortality rate of 16% was found in dogs older than four. Higher mortality rates in older dogs were also found in other studies (23). However, our result did not match with our hypotheses that, the mortality rate in dogs ≥5 would be higher than in other age categories. No significant difference was found. This outcome does match with mortality rates found in Zimbabwe (20). The study in Zimbabwe also showed a stable mortality rate per age after the first year of life. Although, the mortality rate of the older dogs did not differ significantly in our study, we could not find enough dogs ≥5. Implicating that not a lot of dogs survive up to five years. This does comply with studies in Africa showing the majority of the population to be very young (19, 21, 22). In Kenya and Tanzania half (50-52%) of the population had an age of one year or less (19, 21). Our study does not contain information about dogs with an age below one. To get a more reliable view on mortality rates per age category the female cohort should also have been divided into age categories in our study. This was not realised because not enough female dogs could be found to reach the aim of 200 female dogs. Still age classifications would have been useful. Due to some misunderstandings and lack of time, the age of most female dogs in our study is unknown.
The mortality rate found in the LSH differed significantly from the mortality rate found in the RSH. The mortality rate per month, in the LSH, was 0.9%. This is a lot lower than the hypotheses stated at the beginning of this study (3.0%). However, it is somewhat equal to the mortality rate found in Zimbabwe after reaching the age of one (1.0%) (20). The deviation from our hypotheses (mortality rates for both studies were the same) could be caused by the usage of different study methods. It could also be that some of the dead dogs in the retrospective, questionnaire based study were not really dead. Owners could assume the dogs is dead while is it actually missing. It could have been stolen or have

left the household by itself (6). Other reasons could be seasonality in mortality in this population or an outbreak of disease during the period of January 2014 to May 2014.
Even though, the mortality rates for females and males were expected to differ significantly, this was not the case. It could be that these results were not accurate, because of the reasons mentioned earlier. It could also be that the amount of pregnancies and post mortem mortalities are not as high in our population. This will also be studied in Hluvukani in the future.
Although, our results are similar to results found in other studies in Africa, we cannot really compare these results. The amount of data in this study was not sufficient enough to draw any reliable conclusions. Furthermore, the data was biased. This due to the RSH being based on owner information, two different research teams and other complications. Because of this all results found and assumptions made have to be doubted.

Vaccination coverage
The mortality rate per month found in the retrospective study was higher than the ones found in Braamfischersville, Kelusa and Zenzele (6) . Because of this we assume that the vaccination coverage, for rabies, in our population decreases faster (19). In the study of Morters et al (2014) the vaccination coverage dropped below the critical threshold around 20 months after vaccination. This would mean the vaccination coverage in our population would drop below the critical threshold before 20 months. Therefore, based on our findings, new vaccination campaigns should start within 20 months to protect this population.
However, not only mortality rates determine the vaccination interval. Births and migration also cause the vaccination coverage of the population to decrease (6). In Kenya half of the population was replaced each year (21). Assuming 70% of the population was vaccinated in the beginning of a year, the coverage will reach the critical threshold in one year in this area necessitating annual vaccination campaigns. To determine the vaccination interval in Hluvukani information about reproduction and migration is also needed. A pilot of the reproduction study has taken place in the same period as this study. Ultimately, data of these studies could be combined to get an idea of the turn-over rate in this population. Leading to a more accurate vaccination interval.
The WHO recommends to calculate a annual turn-over rate in canine populations to help determine the timing for vaccination campaigns (28). Not all demographic studies in Africa monitored the population for one year. Most demographic studies that have taken place in Africa monitored a population for one year or more (6, 20, 21). However, this pilot study does not contain information of one year. No real advise can be given about vaccination campaigns yet. This study does contribute to the long-term (one year) demographic study that takes place in Hluvukani at the moment (June 2014-June 2015).

Study methods
Different study methods were used in this study. Two retrospective and one longitudinal method were used. Longitudinal studies are also called prospective studies. Most other demographic studies in Africa used a longitudinal approach (4, 19, 21, 25). They followed a population of dogs during a period of time. The study in the communal lands in Zimbabwe did use a retrospective approach, they asked owners about mortalities in dogs in the last two years (20). Because of the use of different study methods results can differ. In the longitudinal study the dogs were monitored. It was made sure that the dog was still alive or had died. In the RSH it was more difficult to make sure the dog had died, as the information was questionnaire based and the actual carcass could not be seen. The response rate in the RSH was very high (>99%). Only one owner did not want to answer the questionnaire. In other demographic studies of canine populations the response rate was also high (68,5%-99,6%) (22, 28, 29). Although the owners were willing to give the information, most owners did not know the exact date of death in the RSH. Owners forgot the date or had never known the exact date. As a result mortalities per month could not be determined. The only information known about the time of death is that is was between January 2014 and May 2014. For the longitudinal visits the exact date of death was known in most cases. For two cases, where the owner had not reported the death, it was estimated. The same occurred for causes of death. In the RSH owners did not know the cause of death. They have forgotten or have never known the cause. In the LSH a questionnaire was done right after death or during revisit. This way the owners memory was still right. The other advantage was that the carcass

was still present.
However, the longitudinal study was performed by two different teams. This caused differences in the collection of data. This has lead to some ambiguities. Because of this some data of the last revisit round is missing. The author of this report did not receive the information about the presence of owners at the households. This information could have led to more or less dogs being censored from the mortality rate calculation, leading to a different mortality rate for the longitudinal study. The amount of owners not at home influenced the mortality rate. 10.3% (38/368) of the enrolled dogs had to be censored from the calculations. Thus the longitudinal study seemed to be more a more accurate study method in theory, however, there were also issues with the reliability.
Comparing results of these different study methods is less reliable. It would be better to compare two studies with the same study method. However, these studies are preliminary. The retrospective study acted as a pilot for the longitudinal study that will continue in Hluvukani for one year. The information contributes to the background knowledge and feasibility of the long-term study. The study will continue until June 2015. Monitoring the enrolled dogs every two weeks with revisits. Using a verbal autopsy and post mortem examination in case of a mortality. Leading to more insight in the mortality rates and mortality causes in this area.

Cause of death
The most common cause of death found in the database of the Hluvukani Animal Clinic is not the same as found in other area's in Africa. The database showed rabies as the most common cause of death (7/10). This does not comply with our hypotheses. Though not much is known about causes of death in canine populations, rabies has not been mentioned as a common cause of death in other studies (20, 24, 25). However, the amount of data in this study is not enough to draw a reliable conclusion. More data is needed to determine a more reliable common cause of death.
The amount of mortalities found in the Animal Clinic database was very low. According to the database only 10 dogs had died during two years. Since the exact number of dogs in the population of the Mnisi area is not known an expected mortality rate could not be determined. Assuming the Mnisi area contained more dogs than the Hluvukani area alone more than ten mortalities were expected in two years. This result could be caused by the fact that owners do not seek veterinary care for their dogs. A lot of deaths were probably missed because they were not reported to the clinic. Another cause could be that students or other employees of the clinic did not keep track of all mortalities in the database. The distribution of deaths is also very peculiar. It implies that mortality occurs very clustered. Caused by lack of consistency between students groups and possible rabies outbreaks. Looking at the causes of death (7/10 rabies) reported it seems that only rabies cases have been reported in the database. It could be that owners only sought veterinary care in cases of rabies. This way post mortem examination was only performed on rabies cases. These cases of rabies occur clustered, it seems like little outbreaks of rabies have taken place. The percentage of rabies cases also seems highly unlikely as no other studies mention rabies as common cause of death (20, 24, 25, 28). The outcome of this study does not seem not very reliable. The data do show that cases of rabies still occur in the canine population around Hluvukani.
The data of the LSH did not show rabies as the most common cause of death. In this study trauma (3/6) was the most common cause of death. This was compatible with our stated hypothesis. This is also more similar with results found in other studies (6, 24, 25). However, the common cause of death in the LSH is only based on six cases. This is not enough to give a reliable conclusion yet. Comparison between the two studies has not been made, because of the lack of data in both studies. Both studies do not seem to be reliable. Furthermore, the methods differed a lot, making comparison less feasible.
Due to an outbreak of Foot and Mouth disease in the research area, it was not allowed to transport the carcasses from the research area to HHWRS. It was prohibited to move samples from one area to another. This could cause spreading of the disease. A section 20 permit from the government was needed to be able to transport the carcasses from Hluvukani village to HHWRS. In the mean time no post mortem examinations could be done.
Another encountered problem for the post mortem examination was that owners mostly did not report the death of their dog. As a result in many cases carcasses already showed severe signs of autolysis when found. Post mortem examination was not feasible in these cases. This occurred because owners

did not report the death of their dog. Owners should be informed about the study frequently, in order to increase their reporting the death of their dog. This has been done by visits, messages by cell phone or community meetings. A community meeting was held during the study. Prof. D. Knobel gave a presentation about previous studies in the area and the necessity of this study. However, not many members of the community were present. Due to the lack of post mortem results the results about causes of death in this study are less reliable. They were only based on the verbal questionnaire, thus information of the owner. Post mortem examination would have given a more reliable cause of death.

Advise for long-term study

As mentioned the data found in our study is probably biased, thus not reliable. Most of all the data is not sufficient. To make sure the data found in the long-term study in Hluvukani is reliable and sufficient. Dr. Francis Kolo will continue this study for one year. He will follow the enrolled dogs and revisit them every two weeks. A big improvement is that post mortem examination is now allowed, hopefully leading to more reliable causes of death.

A reason of the bias on our study was that the RSH was based on stories of the owners. Sometimes two owners each told different stories or they had forgotten a lot of information about the history of the dog. In the long-term study the dogs will be monitored for a year. Every dog must be seen during the revisits so that it is made sure it is still alive. All information obtained during revisits must be noted in specially template excel sheets. Including the date of visit and whether the owner was at home or not. The information for all dogs must be noted the same way. It is best if only one person works in these sheets. In our study to many people worked on the data, causing differences and leading to biased data.

Furthermore, the owners must be kept informed. It is important to keep them up to date with the progress of the study so they will participate in the future and report dead dogs. Although making the people more aware of the importance of this study may also change their behaviour towards the dogs, their cooperation is very important. Still the influence of the study on the way owners treat their dogs must be taken in account.

To really be able to draw any conclusions on the relation between mortality and age the ages of the female dogs should also be included. This can be done by searching the HDSS-database for all enrolled females. This way more data about ages and mortality is available and the conclusion it not only based on one sex.

The main reason for this study was to set up a successful vaccination campaign against rabies in the canine population of Hluvukani. For this not only mortality rates are needed, but also birth and migration rated. An long-term study, of at least one year, should take place to obtain these rates.

Conclusion

The mortality rate for the canine population of Hluvukani, South Africa, found in the retrospective study, is 3.6% in per month. The mortality rate per month found in the longitudinal study in the same population is 0.9% . This difference is probably caused by the use of different study methods. Another cause could be a fluctuation in the amount of mortalities per month, with a possible seasonality.

No significant differences have been found in mortality rates in females and males. Furthermore no difference has been found in the mortality rates of the chosen age categories. The most common cause of death found in the longitudinal study in Hluvukani was trauma (3/6). Rabies was the most common cause of death (7/10) found in the database of the Hluvukani Animal Clinic. Data obtained from the database is probably biased, meaning no conclusion can be drawn from this.

The results of this study are a starting point for better knowledge of the mortality rates and causes in the Hluvukani canine population. The small amount of data obtained from the longitudinal study in Hluvukani and retrospective study of the database of the Hluvukani Animal Clinic make the results less reliable. Another obstacle was the fact that mortalities in the longitudinal study were not reported and post mortem examination could not be realized in all cases.

To be able to draw a more reliable conclusion about the mortality rates in the canine population of Hluvukani a long-term study is necessary. The population of dogs should be followed during at least one year, so the possible seasonality can be noticed. Dogs enrolled in the study should be monitored frequently (every one or two weeks). Post mortem examination is desirable to give a specific cause of

death. This kind of study is already being realized in the same canine population in Hluvukani at the moment (June 2014 – June 2015).
Although, most results are not reliable, this study served as a starting point hoping to contribute to more knowledge of mortality rates and causes in Africa. Eventually leading to a better vaccination campaigns against rabies in free-roaming canine populations.

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References

1. Knobel DL, Cleaveland S, Coleman PG, Fevre EM, Meltzer MI, Miranda ME, et al. Re-evaluating the burden of rabies in africa and asia. Bull World Health Organ. 2005 May;83(5):360-8.

2. Rabies fact sheet [Internet].; August 2014. Available from: http://www.who.int/mediacentre/factsheets/fs099/en/http://sfx.library.uu.nl/utrecht?sid=Refworks%3AUtrecht%20University&charset=utf-8&__char_set=utf8&genre=article&aulast=World%20Health%20Organization&date=August%202014&volume=2014&issue=08%2F26&atitle=Rabies%20fact%20sheet&au=World%20Health%20Organization%20&.

3. Cleaveland S,Kaare M, Knobel D, Laurenson MK. Canine vaccination--providing broader benefits for disease control. Vet Microbiol. 2006;117(1):43-50.

4. Morterd M, Restif O, Hampson K, Cleaveland S, Wood JLN, Conlan AJK. Evidence-based control of canine rabies: A critical review of population density reduction. J Anim Ecol. 2013;82(1):6-14.

5. Cleaveland S, Kaare M, Tiringa P, Mlengeya T, Barrat J. A dog rabies vaccination campaign in rural africa: Impact on the incidence of dog rabies and human dog-bite injuries. Vaccine. 2003 May 16;21(17-18):1965-73.

6. Morters MK, McKinley TJ, Restif O, Conlan AJK, Cleaveland,S. Hampson,K. Whayc,H.R. Damriyasad,I.M. Wood,J.L.N. The demography of free-roaming dog populations and applications to disease and population control. 2014.

7. Wandeler AI. Ecological and epidemiological data requirements for the planning of dog rabies control. 1985;Rabies in the tropics:657-61.

8. Zinsstag, JD, Penny MA, Mindekem R, Roth F, Menendez Gonzalez S, Naissengar S, Hattendorf J. Transmission dynamics and economics of rabies control in dogs and humans in an african city. Proc Natl Acad Sci U S A. 2009;106(35):14996-5001.

9. Knobe D, van Rooyen J, Simpson G, Akarele O. Research protocol: A health and demographic surveillance system for dogs in a rabies-infected area in south-africa . In press 2011.

10. Fine P, Eames K, Heymann D. "Herd immunity": A rough guide. Clinical infectious diseases. 2011;52(7):911-6.

11. Knobel D. Addendum to protocol V0033/11: A health and demographic surveillance system for dogs in a rabies-infected area in south-africa: Proposal for student research projects. In press 2014.

12. Hampson K, Dushoff J,Cleaveland S, Haydon, D, Kaare M, Packer C, Dobson A. Transmission dynamics and prospects for the elimination of canine rabies. PLoS Biology. 2009;7(3):e53-.

13. Coleman PG, Dye C. Immunization coverage required to prevent outbreaks of dog rabies. Vaccine. 1996 2;14(3):185-6.

14. Bender. No adverse effects of simultaneous vaccination with the immunocontraceptive GonaConTM and a commercial rabies vaccine on rabies virus neutralizing antibody production in dogs. Vaccine. 2009;27(51):7210-3.

15. Kaare M, Lembo T, Hampson K, Ernest E, Estes A, Mentzel C, et al. Rabies control in rural africa: Evaluating strategies for effective domestic dog vaccination. Vaccine. 2009 Jan 1;27(1):152-60.

16. Bonnett BN, Egenvall A, Hedhammar A, Olson P. Mortality in over 350,000 insured swedish dogs from 1995-2000: I. breed-, gender-, age- and cause-specific rates. Acta Vet Scand. 2005;46(3):105-20.

17. Fleming. 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. 2011;25(2):187-98.

18. Proschowsky HF, Rugjberg H, Ersboll AK. Mortality of purebred and mixed-breed dogs in denmark. Preventive Veterinary Medicine. 2003;58:63-74.

19. Gsell A, Knobel D, Kazwala R, Vounatsou P, Zinsstag J. Domestic dog demographic structure and dynamics relevant to rabies control planning in urban areas in africa: The case of iringa, tanzania. BMC veterinary research. 2012;8:236-.

20. Butler JR, Bingham J. Demography and dog-human relationships of the dog population in zimbabwean communal lands. Vet Rec. 2000 Oct 14;147(16):442-6.

21. Kitala P, McDermott J, Kyule M, Gathuma J, Perry B, Wandeler A. Dog ecology and demography information to support the planning of rabies control in machakos district, kenya. Acta Trop. 2001 Mar 30;78(3):217-30.

22. Brooks R. Survey of the dog population of zimbabwe and its level of rabies vaccination. Vet Rec. 1990 Dec 15;127(24):592-6.

23. Suluku R, Abu-Bakarr I, Jonathan J, Jonsyn-Ellis F. Post-war demographic and ecological survey of dog populations and their human relationships in sierra leone (A case study of urban freetown). Science Journal of Agricultural Research & Management. 2012.

24. Koman K. Baseline incidence rates for pregnancy and (pro)estrus in owned but free-roaming female dogs in the mnisi community area, mpumalanga province, south africa. University Utrecht and University of Pretoria. 2013.

25. Marker. Survivorship and causes of mortality for livestock-guarding dogs on namibian rangeland. Rangeland ecology & management. 2005;58(4):337-43.

26. Woldehiwet Z. Clinical laboratory advances in the detection of rabies virus. Clin Chim Acta. 2005 Jan;351(1-2):49-63.

27. Bourhy H, Rollin PE, Vincent J, Sureau P. Comparative field evaluation of the fluorescent-antibody test, virus isolation from tissue culture, and enzyme immunodiagnosis for rapid laboratory diagnosis of rabies. J Clin Microbiol. 1989 Mar;27(3):519-23.

28. Davlin SL, Vonville HM. Canine rabies vaccination and domestic dog population characteristics in the developing world: A systematic review. Vaccine. 2012 May 21;30(24):3492-502.

29. Knobel DL, Laurenson MK, Kazwala RR, Boden LA, Cleaveland S. A cross-sectional study of factors associated with dog ownership in tanzania. BMC Vet Res. 2008 Jan 29;4:5,6148-4-5.

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Appendix
1. Consent form

Consent to participate in a research study

Rates and causes of death in dogs

Study leader:	Prof. Darryn Knobel	Dept. Veterinary Tropical Diseases	072 754 3243
Researchers:	Dr Anne Conan	Dept. Veterinary Tropical Diseases	082 057 5603
	Dr. Francis Kolo	Dept. Veterinary Tropical Diseases	
	Kim Koman	Utrecht University	
	Anke Nas	Utrecht University	
	Bregje Leenders	Utrecht University	
	Rianne Vergeer	Rianne Vergeer	

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 die each month. We will do this by recording information of dogs that die, and doing an interview with their owner. We will also examine some of the dogs that die each month, 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 3 months 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 by telephone or SMS. 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 exam the dog 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.

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 3 months 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:

Fill in number here

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 ____________ (____________) at ____________, or ____________ (____________) at____________. If you believe that your rights have been violated in any way, please contact ____________ on ____________ .

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? ______________________________________