

Population dynamics of dogs in the Mnisi area, Hluvukani, South Africa

Can we control the population?



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Abstract

The aim of this study is to determine how the dog population is formed in Hluvukani, South Africa. It is part of a baseline pilot study to see whether a temporary sterilization can be of use to stabilize or decrease the Hluvukani dog population. A lower turnover rate of the population will help to achieve a better vaccination grade against rabies. The ultimate goal is to eradicate rabies, to prevent human victims. During several rounds of house to house visits, research information about the dog population has been collected. People were questioned about their dogs and their willingness for female dog contraception. In 2011 the dog population consisted of 799 dogs. In 2013 it has increased to 870 dogs. The population increased with 5,9% over 12 months. Despite the free castrating campaigns only 10 female castrated dogs were found in 2011 and 12 female castrated dogs in 2013. The density of dogs in 2011 was 77/km² and 83,9/km² in 2013. A male:female ratio of 1,32 (2011) and 1,47 (2013) was found. To determine the population turnover rate more research is needed. The best method will be a Leslie matrix of the population. With the Leslie matrix it will be possible to run an computer stimulation to see the effect of Gonacon™ injection in female dogs on the dog population in Hluvukani.

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1. Introduction

1.1. Problem statement

The rabies virus belongs to the order of Mononegavirales, the family of Rhabdoviridae and the genus Lyssaviruses. It is an enveloped RNA virus with a rod-shaped morphology. The virus affects the central nervous system in most mammals and is mostly fatal, although there is a variation in susceptibility between species (domestic species and humans are believed to be moderately susceptible, while foxes, wolves, coyotes and jackals are believed to be highly susceptible). The virus is excreted in saliva and is usually transmitted through bite incidents.

Human rabies has been described in South Africa since the nineteenth century, but was not confirmed until 1928¹. According to the World Health Organization, nowadays more than 55000 people die of rabies every year in Africa and Asia alone². Although rabies is a reportable disease, the diagnosis of rabies in the poor countries is often not made. Most patients belong to lower socio-economic groups, so the actual number of rabies deaths that occur each year is not known³.

The domestic dog forms the most important vector for transmission to the human population^{4 5}. More than 95% of the human rabies cases are caused by bites of rabid domestic dogs⁶. Most clinical infections in dogs and humans are caused by the rabies virus of genotype 1⁶.

In the research of Weyer et al. over 70% of the affected humans were children and young adults and according to the World Health Organisation 45–60% of dog bite injuries and human deaths occur in children under 15, who rarely understand how rabies is transmitted, and often do not know how to behave around animals, especially dogs living in the same household or community^{1,7}. This makes it all the more important to get the incidence of human rabies under control.

The control of rabies in humans depends on the control of rabies in dogs and other host species. Rabies is widespread in many canine species and is associated with domestic dogs in several provinces in South Africa, including Eastern Cape, KwaZulu Natal, Mpumalanga, Free State, and Limpopo¹. Important canine reservoirs are foxes, coyotes, jackals, mongooses and possibly raccoon dogs⁸. Rabies also occurs in wildlife, like the black-backed jackals (*Canis mesomelas*), bat eared foxes (*Otocyon megalotis*) and the kudu antelope (*Tragelaphus strepsiceros*). A unique rabies virus variant is adapted to various herpestid or mongoose species in South Africa. There seem to be other rabies related lyssaviruses in Africa, respectively the Lagos Bat virus, Mokola virus and the Duvenhage virus⁸. There is less understanding of their characteristics and epidemiology.

The control of the virus in dogs might be twofold: it depends on effective vaccination of the dog population and on control of the dog population itself. A study performed in the Serengeti area of Tanzania showed that rabies was persistent in a dog population density of >5 dogs/km²⁹.

Vaccination against rabies in general is carried out in annual campaigns that tend to cover 70% of the dog population. This 70% coverage should keep the herd immunity above the critical levels of 25-40% to interrupt rabies transmission in dogs^{10 11 12}.

This has proven to be an effective method to control rabies in dog populations¹². In the dog population in the eastern cape province of South-Africa a team of animal health technicians try to vaccinate as many dogs as possible each year against rabies. In a field study carried out in the Transkei area of South Africa, 203 dogs were randomly selected after vaccination of 56% of the dog population, only 32% of dogs had a rabies titre of ≥ 0.5 IU/l which is likely to be protected against rabies virus infections¹³.

Successful vaccination can be achieved with parenteral vaccines often containing the inactivated virus or sometimes live-attenuated viruses. There are also several oral bait vaccinations available, which have proven to be successful¹⁴. Oral bait vaccination can be done with a modified live rabies vaccine⁸. This will be safe for parenteral vaccinated dogs and humans. This will be much more effective in communities with free roaming animals since dogs are not always approachable for parenteral vaccination. Although the stability under the conditions in the field are unknown and the cost aspect may make this approach not feasible⁸.

Population control can be carried out in two methods: culling and sterilization. However, culling proved to be ineffective in controlling rabies in several host species (including foxes in New York State and Denmark and dogs in Indonesia, Korea and Israel), while simultaneous vaccination of other host species during the outbreaks was effective to control the disease¹².

It is unclear what explains the ineffectiveness of culling. Perhaps one is not able to cull enough animals for it to be effective, or there have been human errors like translocation of unvaccinated dogs from other areas or culling of vaccinated dogs. Besides the ineffectiveness, culling is also socially and ethically unacceptable¹².

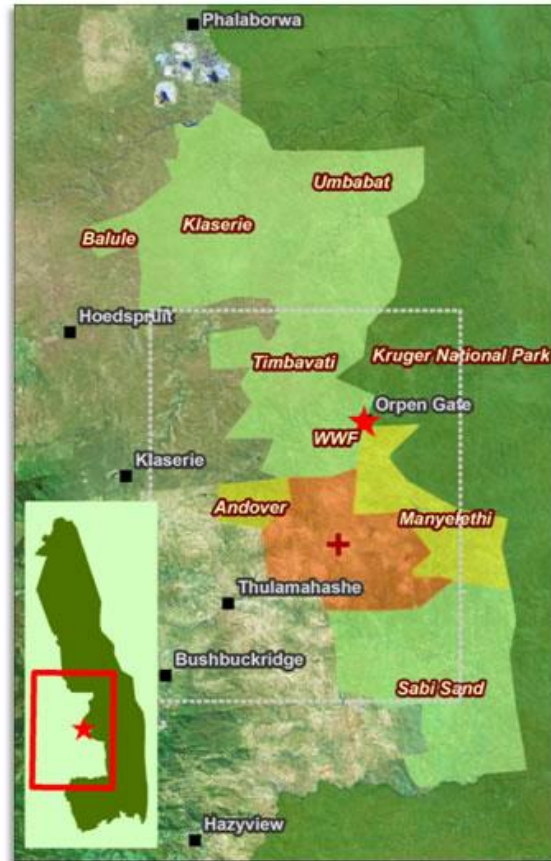
Sterilisation however may be effective, but there is not yet enough evidence to decide and to base policy on. Only surgical sterilisation has been carried out in the field and has been combined with simultaneous vaccination. This combination of sterilisation and vaccination may have the potential to reduce the incidence of rabies and decrease the population of dogs in certain areas¹². Surgical sterilisation however is an expensive and time consuming method¹⁵.

Population control through GnRH-vaccination has not yet been carried out in the field, but could be the method of the future. The GnRH vaccine is responsible for an antibody response targeted against Gonadotropin-Releasing Hormone (GnRH), secreted by GnRH neurons in the hypothalamus. If there is no GnRH available, the pituitary gland is not stimulated to produce Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) and so the testes and ovary will not produce gametes, rendering the male and female animal sterile¹⁶. It seems possible to combine rabies and GnRH in one vaccine (vaccinated mice were 100% protected from rabies and produced antibodies against GnRH, although it was not tested if the mice were sterile or not), making it cost- and time-effective¹⁷. GnRH vaccines have been tested and found effective in male dogs under laboratory conditions¹⁸. The duration of infertility caused by Gonacon™ in dogs is not yet clear. Gonacon™ can be used in combination with rabies vaccines without any adverse effects for immunity against rabies¹⁹¹⁷. However, this method is still in development.

1.2. Mnisi area

The Mnisi area is situated in the north-eastern region of the Bushbuckridge Municipal area. At the western region it is connected by Manyeleti, at the southern region it is connected to Sabi Sand and at the northern region it is connected to Timbavati. Manyeleti, Sabi Sand and Timbavati are private game reserves. They are all connected to the Kruger National Park. The Mnisi area is a developmental area, formed by a few communities.

The University of Pretoria runs a program in this area; the Mnisi community program. The focus is on cattle disease and management of rabies in dogs.



FFig. 1
Mnisi area with the communities and game reserves. Red cross points Hluvukani out.

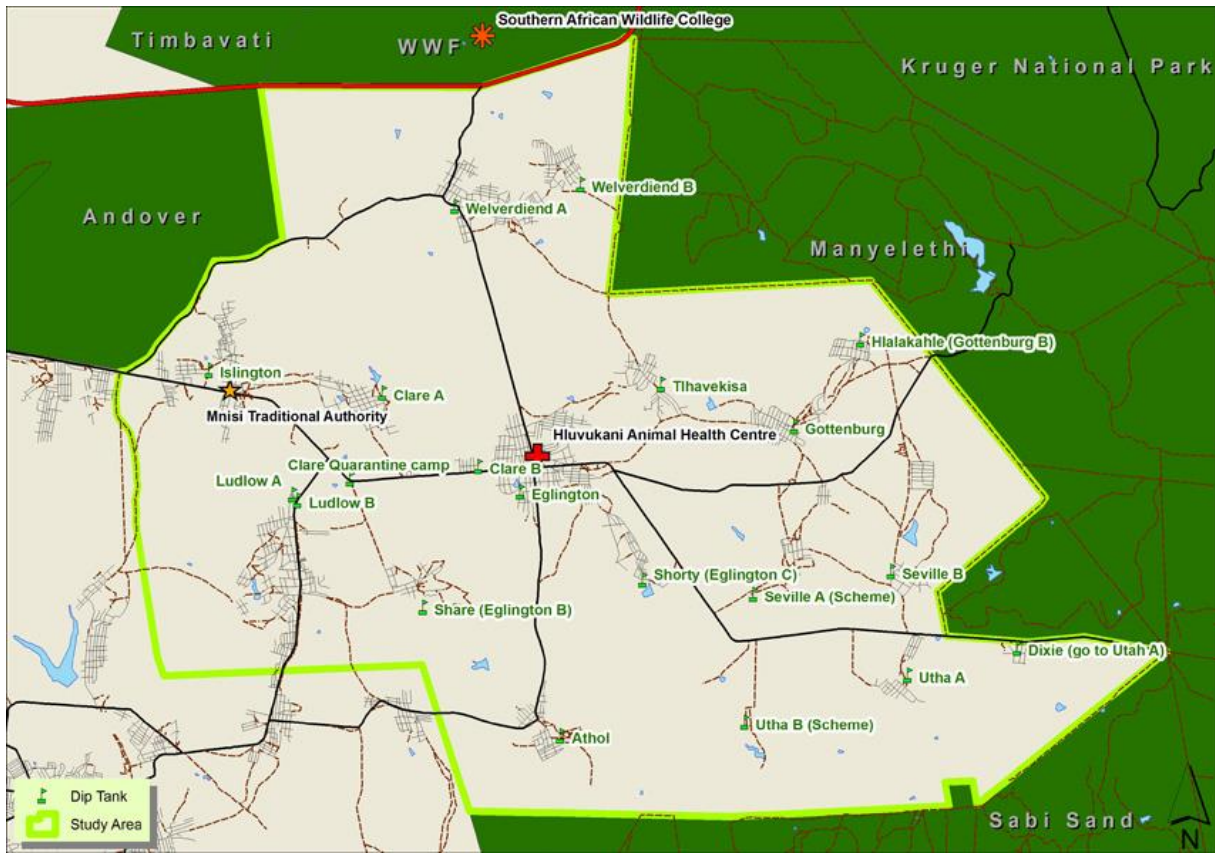


Fig 2
Mnisi area

1.3. Aim of this study

The aim of this study is to determine how the dog population is formed in Hlulukani, South Africa (S 24° 39' 00", E 31° 20' 00"). On the basis of the population details, we can determine the population turnover rate and see if the population is stable over time or whether it declines or rises. It is part of a baseline pilot study to see whether a temporary sterilization can be of use. If so, future studies could test a GnRH vaccine, Gonacon™ in this population. When the current population is stable the vaccination campaigns with Gonacon™ will be effective, because it will decline the population and prevent fast turnover rates.

In this pilot study there is a focus on the village it is one of the communities centred in the Mnisi area. To reach a bigger area, other studies started in 2013 to focus on the dog population in the communities close by.

1.4. Dogs and dog owners

In communities like Hluvukani dogs are mostly free roaming. This complicates identifying and locating/tracking the dogs in the area during the first visit but also during follow-up visits. This implicates some difficulties in identifying and follow up of dogs in the area. Besides the fact that the dogs are free roaming, they are also needed for herding the cattle. This makes that the dogs interact with others on long distances. To prevent diseases in cattle, farmers go to the dip tank once a week to have their cattle checked by a state veterinarian. The dogs interfere with each other around the dip tank.

People tend not to spend money on their dogs, if not necessary. Most dogs are not castrated, unless they are enrolled in a free castration campaign. Most of the dogs are not socialized like dogs kept indoors, this makes the owner really important to be able handle the dogs enrolled in the project.

Dog owners are really important to be able to collect the information needed about their dogs. This implies that the owners should be able to give the information. Illiteracy is also a problem making it more difficult to obtain all the information we need for the pilot study.

1.5. GnRH vaccine

In order to prevent a fast turnover rate of the population, future studies in the Hluvukani dog population will use a GnRH vaccine. A lower turnover rate of the population will help to achieve a better vaccination grade against rabies. The GnRH vaccine is responsible for an antibody response targeted against Gonadotropin-Releasing Hormone (GnRH), secreted by GnRH neurons in the hypothalamus. If there is no GnRH available, the pituitary gland is not stimulated to produce Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) and so the testes and ovary will not produce gametes, rendering the male and female animal sterile¹⁶.

This is a very effective method of immunocontraception (in theory), because the target is situated at the top of the hormone cascade. However, GnRH is released into the bloodstream in a pulsatile manner and will only travel a short distance in the blood, so the antibodies must reach a very high concentration in the bloodstream to be effective²⁰.

GonaconTM is such a GnRH-vaccine developed by the USDA APHIS Veterinary Services. It has been tested in a many different species of animals both male and female and has shown to cause contraception. This infertility is 2-3 times longer in females than males. It is believed that this will also be true for dogs, which is why this method is believed to be more promising in female dogs rather than male dogs¹⁹. GnRH-vaccines have been tested and found effective in male dogs under laboratory conditions²¹.

GonaconTM can be used in combination with rabies vaccines without any adverse effects for immunity against rabies¹⁹.

2. Materials and methods

2.1. Study design

The pilot study started in 2011, the first round was done. Because there was no infrastructure and map of the area at the beginning of the study, it was difficult to determine whether all households were visited. To cover this problem four more rounds have been done since 2011. The second round took place at the beginning of 2012, the third round mid-2012, the fourth round end-2012 and the fifth round at the beginning of 2013. During the consecutive rounds the survey area has been extended, because Hluvukani is a growing community.

All households were visited in an effort to include all dogs of Hluvukani. A survey by questionnaire was done by field workers in Shangaan, the local language. The main goal of the questionnaire (reproduced in Appendix A) was to obtain information about the dogs kept at the properties of Hluvukani. Property owners were asked the number of dogs they have, their sex and the puppies they had, sex, pregnancies and origin about all their dogs.

2.2. Microchips

All dogs (older than 2 months) received a microchip during the survey. The microchip was placed between the shoulder blades. The microchip has a unique number to identify the dog, during follow-up this will be more secure. The dog was registered in our database combined with the information of the property and a picture of the dog.

2.3. Data collection

At each round all households were visited to include all dogs of Hluvukani. Dog owners were asked about the number of dogs they have, their sex and the puppies they had.

During the first four rounds the program 'survey to go' with a PDA was used to enter the data collected. The fifth round open data kit (ODK) was used to enter the data collected. For the fifth round a questionnaire was introduced to trace missing dogs.

2.4. Sample size

All households of Hluvukani were visited during the rounds described above. 1939 households were visited in total.

2.5. Cooperation main pilot study

A part of the main pilot study was a pilot study about the incidence of pregnancy and estrus in bitches. To determine incidence rates of pregnancy and estrus, blood and vaginal smears were collected, estrus behaviour and physical signs (vulvar reflex, vulva swelling, tail reflex, lordosis, discharge etc.) were reported and abdominal palpation to diagnose pregnancy was carried out. Also the owner was asked about recent signs of heat and previous pregnancies. A follow-up of pregnant bitches in two rounds was done, five weeks apart, among a hundred randomly selected female dogs older than one year (range: 1-13 years) in Hluvukani.

2.6. Questionnaire contraception

To assess whether owners are willing to use a new kind of contraception, a survey by questionnaire was done among 78 households in Hluvukani. The female dogs in the households were randomly selected for the pilot study to determine the baseline pregnancy rates. A questionnaire (reproduced in Appendix B) was used to find out whether people would want their female dog(s) to be sterile, and if so, why. It was also to find out if people prefer temporary or permanent sterility for their female dog(s). The questionnaire as described above was designed by the study group. The interview was held by one of the animal technicians or translated to Shangaan.

3. Results

3.1. Population

During the round in 2011, an unknown number of households was visited. Overall 386 households owned one or more dogs. In total 799 dogs were registered during the survey. In the 5th round, 1939 households were interviewed before the 1st of May 2013. Overall 407 households, 21%, owned one or more dogs. In total 870 dogs were registered during the survey. 19,9% of the households owned multiple dogs, with an average of 2,07-2,14 dogs per household.

Despite the free castrating campaigns there were only 10 female castrated dogs registered in 2011 and 12 female castrated dogs in 2013.

The Hluvukani area is 10,37 km². The density of dogs in 2011 was 77/km². The density of dogs in 2013 was 83,9/km².

An age class distribution of the Hluvukani dog population has been constructed based on the results of the questionnaire. The age classes are not equally divided. The age distribution is shown in table 1.

Age (years)	Number of dogs November 2011	Percentage (%) November 2011	Number of dogs May 2013	Percentage (%) May 2013
0-1	294	36,8	210	24,1
1-3	288	36	324	37,2
3-5	146	18,3	198	22,8
5+	39	4,9	103	11,8
unknown	32	4	35	4,1

Table 1

Based on these results the age of dogs in days could be estimated. The results are in table 2.

	Age (day) November 2011	Age (day) November 2013
Minimum	4,1	1,1
Mean	758,4	920,4
Median	625,9	769,2
Maximum	3913	4460

Table 2

3.2. Growth rate

The growth rate found is 71 dogs in 18 months. If we assume there will be no seasonal influences on the reproduction of the dogs, the population growth will be 47 dogs over 12 months. This means an increase of the population of 5,9% over one year.

3.3. Sex ratio

The male: female ratio found in the survey was 1.32 during the round in 2011. The male: female ratio was 1,47 in 2013. There are obviously more male dogs than female dogs in the population.

3.4. Outcome questionnaire contraception

Out of the 78 households questioned, 52 people answered that they want their dog to be sterile. 6 people don't know which choice they will make because they are a family member of the owner instead of the owner and 20 people do not want their dogs to be spayed.

The reasons why people do not want their female dog(s) to be sterile are the following:

- They want puppies (n=18)
- Do not care whether the dog gives birth (n=1)
- The dog does not get pregnant (n=1).

The reasons why people want to sterilize their female dog(s) are the following:

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- To prevent unwanted litters (n=32)
- To prevent unwanted estrus behaviour (n=12)
- All puppies die after being born (n=5)
- There are too many dogs who misbehave and bite people (n=2)
- To prevent sickness (like transmissible venereal tumors) (n=2),
- The bitch eats the puppies after they are born (n=1).

People tend to prefer permanent sterilization (n=47) compared to temporary infertility (n=2).

To find out the dedication of dog owners to the main study people were asked for their willingness to pay for infertility of their female dog(s).

- Willingness to pay for the injection (n=19)
- Only willing to pay for a permanent solution (n=6)
- The amount of people who want to pay for a temporary solution:
 - o R5 (n=1)
 - o R20 (n=2)
 - o R50 (n=2)
 - o R100 (n=6)
 - o There are people who like to pay, but who cannot afford it because of financial reasons (n=8)
- The amount of people who want to pay for a permanent solution:
 - o R20 (n=2)
 - o R100 (n=4)

3.5. Cooperative pilot study; incidence rates of pregnancy and estrus

In one of the pilot studies the incidence of pregnancy and estrus in bitches was monitored. A incidence of dogs in (pro)estrus of 6% and 4,8% was found in round 1 and 2. 12 dogs gave birth during the sampling period. 57 puppies were born with a male: female ratio of 1,7.

4. Discussion

4.1. Turnover rate

The basic reproduction number (R_0) characterizes the maximum reproductive potential of a pathogen. The R_0 is defined as the average number of infections produced by one infected subject in a susceptible population. For an epidemic to spread R_0 must be > 1 . The population density is closely related to the R_0 . The threat of rabies virus transmission to humans from dogs increases when the density of dog exceeds the threshold density at which canine rabies is maintained¹⁹. The threshold density for canine rabies has been estimated to be 4,5 dogs/km²¹⁹. We found a population density of 77/km² during 2011 and 83,9/km² dogs in 2013. This means this currently rabies-free population is at risk for outbreaks when rabies is introduced from outside. To prevent the population from being susceptible for outbreaks of the rabies virus, vaccination campaigns are carried out. Dog vaccination campaigns should aim to achieve 70%

coverage, to maintain population immunity above critical levels of 25-40% and should cover for susceptible dogs that are brought into or are born in the area and immune dogs that leave it or die^{10 11 12}. The dogs in Hluvukani are free roaming and taken to particular places like the diptanks, this might implicate more contact between dogs. However the vaccination level and susceptibility of this dog population is unknown due to the probability of a fast population turnover.

4.2. Male: female ratio

Over the whole population the male: female ratio found in the survey was 1,32 in 2011 and 1,47 in 2013. In the population of the Eastern Cape Province of South Africa a male: female ratio of 1,7:1 is found. Similar numbers have been reported in several other African studies¹³. The distorted male: female ratio in an adult population can be since the onset of the birth or it can be a result of selection by the owners themselves, they might believe that male dogs are better in hunting and/or herding or because people are willing to control their number of dogs¹³. In the cooperative pilot study of Kim Koman, the results showed that more male pups are born initially. The male: female ratio in pups was 1,7:1.

4.3. Limitations

In the beginning of the study in 2011, there was no map of Hluvukani and infrastructure was poor. Houses were identified by a property ID. Now the government tries to implement a new system with stand numbers. There are still households without a stand number or a property ID. To determine these households GPS coordinates were used. There might have been some households which are not visited. For this reason we repeated the rounds five times.

There is a lack in registration of the new-born puppies and follow up by their owners and by governmental institutions. A lot of puppies are born in Hluvukani each year, not all of them survive because of several reasons. Owners do not always know how many puppies were born, whether they were male or female and if they died when and why. This makes it difficult to estimate population growth.

Hluvukani is a growing community, it extends every day. At the moment Hluvukani has two compartments, Eglington and Clare B. It looks like it is extending and connecting to other communities around soon. To be able to do rounds we can't reach too far from the center of Hluvukani. For this reason we imagined virtual boundaries around Hluvukani for our survey. This may influence the results, because dogs will roam further than our boundaries.

Animal technicians try to vaccinate all dogs of the Hluvukani population against rabies. Despite their willingness to complete the campaign successfully, sometimes other emergencies interfere with the campaign, for example the outbreak of foot and mouth disease in August 2013. In those situations, there is less manpower and the campaign is put on hold.

Dog owners are really important as a source of information about their dogs. This implies that the owners should be able to give the information. Illiteracy is also a problem making it more difficult to obtain the information and to ensure sufficient quality.

4.4. Wildlife vaccination

Wildlife might influence the relationship between disease incidence, host density and transmission in dogs under field conditions ¹².

There is a possibility that by vaccination of at least 70% of the dogs in the community a reciprocal increase (real or relative) of the disease in wildlife species may occur ¹³. An important canine reservoir is foxes, coyotes, jackals, mongooses and possibly raccoon dogs ¹³.

Oral bait vaccination can be done with a modified live rabies vaccine ⁸. Oral bait vaccination can be used both in wildlife and domestic dogs. This will be safe method even if the dog is parenteral vaccinated. For humans this is also a safe method. This will be much more effective in communities with free roaming animals since dogs are not always approachable for parenteral vaccination. Although the stability under the conditions in the field are unknown and the cost aspect may make this approach not feasible ⁸.

4.5. Importance for future work

To estimate the survival time of a dog, a life table can be of use. The information gathered in this pilot study is not sufficient to construct such a table. Further research is needed to be able to create a life table for this specific population. The life table and other details about the population dynamics can be used to construct an age structured Leslie matrix for the population²².

An age structured Leslie matrix will enable prediction of the effect of control scenario's on the size, structure and growth of the dog population. For example, one can do computer simulation of the effect on the population of the Gonacon™ injection in a given percentage of female dogs in Hluvukani because it enables to stimulate outcome in the presence ^{23 22}. This model gives a good indication when the animals will stay in the same environment and conditions as the animals used to create the matrix.

Fast population turnover indicates death at young age. The cause of death at young age is still unknown. Further research need to be done.

Future studies should aim to give an estimation on how many dogs must be injected with Gonacon™ to decrease the population rate and incidence of rabies. Subsequent studies are necessary to determine the effectiveness of the Gonacon™ vaccine in the field to prevent bitches to become pregnant.

4.6. Population available for Gonacon™ vaccination

At 52 households people are willing to let their female dog(s) be infertile. Most of these people will make the choice for a permanent solution instead of a temporary solution. A permanent solution can be achieved by repeating the GnRH vaccine after 2-3 years, to extend the temporary infertility. Because it is not yet known how long the effects of the GnRH vaccine will be in field conditions, there might be some difficulties in managing to prevent the bitches from becoming fertile again before the second vaccination round starts.

Overall people are willing to help to eradicate rabies. This could benefit the acceptance and implementation of the GnRH vaccine in the Hluvukani community.

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6. References

1. Weyer J, Szmyd-Potapczuk AV, Blumberg LH, et al. Epidemiology of human rabies in South Africa, 1983-2007. *Virus Res.* 2011;155:283-290.
2. World Health Organisation (WHO), rabies factsheet #99. . 2012:<http://www.who.int/mediacentre/factsheets/fs099/en/index.html>
3. Lembo, Tiziana Attlan, Michaël Bourhy, Hervé Cleaveland, Sarah Costa, Peter de Balogh, Katinka Dodet, Betty Fooks, Anthony Hiby, Elly Leanes, Fernando Meslin, François-Xavier Miranda, Mary Müller, Thomas Nel, Louis Rupprecht, Charles Tordo, Noël Tumpey, Abigail Wandeler, Alexander Briggs, Deborah. Renewed global partnerships and redesigned roadmaps for rabies prevention and control. *Veterinary medicine international.* 2011;2011:923149-923149.

4. Knobel, Darryn Cleaveland, Sarah Coleman, Paul Fèvre, Eric Meltzer, Martin Miranda, M Elizabeth G Shaw, Alexandra Zinsstag, Jakob Meslin, François-Xavier. Re-evaluating the burden of rabies in Africa and Asia. *Bull World Health Organ.* 2005;83:360-368.
5. Rupprecht C. A tale of two worlds: public health management decisions in human rabies prevention. *Clinical infectious diseases.* 2004;39:281-283.
6. Quinn. Veterinary microbiology and microbial disease. *Veterinary Microbiology and Microbial Disease.* Hoboken: John Wiley Sons; 2011.
7. World Health Organisation (WHO).
8. Nel LM, Wanda. Lyssaviruses. *Crit Rev Microbiol.* 2007;33:301-324.
9. Cleaveland SD, C. Maintenance of a microparasite infecting several host species: rabies in the Serengeti. *Parasitology.* 1995;111 Suppl:S33-S47.
10. 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;21:1965-1973.
11. Hampson. Transmission dynamics and prospects for the elimination of canine rabies. *PLoS Biology.* 2009;7:1000053.
12. Morters. Evidence-based control of canine rabies: a critical review of population density reduction. *J Anim Ecol.* 2013;82:6-14.
13. Van Sittert SJ, Raath J, Akol GW, Miyen JM, Mlahlwa B, Sabeta CT. Rabies in the Eastern Cape Province of South Africa - Where are we going wrong? *J S Afr Vet Assoc.* 2010;81:207-215.
14. Zhang, Shoufeng Liu, Ye Fooks, Anthony Zhang, Fei Hu, Rongliang. Oral vaccination of dogs (*Canis familiaris*) with baits containing the recombinant rabies-canine adenovirus type-2 vaccine confers long-lasting immunity against rabies. *Vaccine.* 2008;26:345-350.
15. Root Kustritz MV. Managing the Reproductive Cycle in the Bitch. *Vet Clin N Am : Small Anim Pract.* 2012;42:423-437.
16. Naz R. Contraceptive vaccines: success, status, and future perspective. *American journal of reproductive immunology (1989).* 2011;66:2-4.
17. Wu. Development of combined vaccines for rabies and immunocontraception. *Vaccine.* 2009;27:7202-7209.
18. Ladd, A Tsong, Y Y Walfield, A M Thau, R. Development of an antifertility vaccine for pets based on active immunization against luteinizing hormone-releasing hormone. *Biol Reprod.* 1994;51:1076-1083.

19. Bender. No adverse effects of simultaneous vaccination with the immunocontraceptive GonaCon™ and a commercial rabies vaccine on rabies virus neutralizing antibody production in dogs. *Vaccine*. 2009;27:7210-7213.
20. Munks. Progress in development of immunocontraceptive vaccines for permanent non-surgical sterilization of cats and dogs. *Reproduction in domestic animals*. 2012;47:223-227.
21. Ladd, A Tsong, Y Y Walfield, A M Thau, R. Development of an antifertility vaccine for pets based on active immunization against luteinizing hormone-releasing hormone. *Biol Reprod*. 1994;51:1076-1083.
22. LESLIE PH. On the use of matrices in certain population mathematics. *Biometrika*. 1945;33:183-212.
23. Cáceres MC, Iris. Random Leslie matrices in population dynamics. *J Math Biol*. 2011;63:519-556.

Attachment A

Questionnaire

General information

1. Date
2. Number of entry
3. Name of the observer
4. Property ID
5. Village of property
6. Standnumber

Property information

7. How many people normally live on the property?
8. How long have you/ has your family been resident at this property?
9. Are any dogs (resident) kept on the property?

Dog information

10. How many dogs are on the property?
→ 0, go to section D.
11. How many dogs entered the property since last visit (last entry).
→ 1 or more, go to section A.
12. How many dogs left the property since last visit (last entry).
→ 1 or more, go to section B.
13. Dogs we also saw last visit (last entry)
→ go to section C.

A. Individual dog information, new dog

14. Does the dog come from Hluvukani?
15. What was the amount the new owner paid for the dog?
16. What was the time since entering this property?
17. Is the dog still resident at the property?
18. Name of the dog
19. Microchip number
20. Breed
21. Sex
22. How many litters has the dog had previously?
23. Is the dog sterilized?

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24. Has the dog ever been vaccinated against rabies?
25. When was the dog last vaccinated against rabies?
26. Age

B. Individual dog information, leaving the property

27. Microchip number
28. Sex
29. Because of what event the dog was leaving the property?
30. What were the circumstances of death?
31. Did it go to another household in Hluvukani?
32. If the dog was sold, what was the amount of money did the owner received?
33. If the dog was exchanged, what did the owner receive in exchange?
34. What was the time since leaving the property?
35. Was the dog pregnant since the last visit (last entry)?
36. How did you know the dog was pregnant?
37. How many puppies were born alive?
38. How many puppies were born dead?
39. When were the puppies born?

C. Individual dog information

40. Microchip number
41. Sex
42. Is the dog sterilised?
43. Is/was the dog pregnant?
44. How do you know the dog is/was pregnant?
45. Estimated delivery date?
46. How many puppies were born alive?
47. How many puppies were born dead?
48. When were the puppies born?
49. Has the dog ever been vaccinated against rabies?
50. When was the dog last vaccinated against rabies?

D. Cats

51. Number of cats at the property

*Picture of the dog, to collect it in a database.

Attachment B

Contraception questionnaire

General information

1. Date
2. Number of entry
3. Name of the observer
4. Property ID
5. Village of property
6. Standnumber

Oestrus prevention

7. Do you want oestrus prevention for your female dog?
8. If so, why?
 - a. To prevent from unwanted litters
 - b. To prevent from unwanted estrus behaviour
 - c. Other reasons
9. Why not?
 - a. They want puppies
 - b. Do not care
10. What amount are you willing to pay for the temporary infertility injection?
 - a. R 200
 - b. R 100
 - c. R 50
 - d. R 20
 - e. R other
11. Comments