Fall 12-9-2016

Rabies Control and Prevention within Wildlife Populations of Skunks, Raccoons, and Foxes

Cassidy M. Smith
University of Wyoming, csmit106@uwyo.edu

Rabies Control and Prevention within Wildlife Populations of Skunks, Raccoons, and Foxes

Cassidy Smith

December 20, 2016

Abstract

Rabies is a zoonotic virus that is extremely lethal to humans and many wildlife species such as raccoons, skunks, foxes, as well as domestic animals such as cats, dogs, and livestock. Reservoirs of the rabies virus throughout species specific populations pose an enormous risk of exposure and infection of domestic animals that have direct contact with humans. The most effective way to prevent wildlife spillover of rabies is through the use of vaccine programs. The wildlife targeted with vaccines are mainly skunks, raccoons, and foxes since these species hold the most significant reservoirs of the virus within the United States. Modified live or attenuated vaccines and whole inactivated or killed vaccines will be discussed as well as their effectiveness and drawbacks. Different forms of administration of vaccines will be compared and contrasted to decide the effectiveness of the types. The social and habitual behaviors of skunks, raccoons, and foxes need to be understand in order to administrate the vaccines at the most effective time to ensure a larger number of wildlife are vaccinated. The cost effectiveness of type of vaccine, administrative methods, and the cost of distribution must be analyzed to reduce costs of the vaccine programs. Further research must be done to create different strains of vaccines that are cost effective, have very few drawbacks, and are effective at preventing the virus from infecting wildlife populations. It may not be possible to eradicate the rabies virus within the near future due to its ability to infect many species but the control and prevention of the virus in the wildlife populations is essential in order to reduce the risk of domestic animals being infected and ultimately prevent humans from becoming infected.

Wildlife spillover of rabies into domestic animals is a major concern in rabies prevention and control within the United States. Rabies is a virus (RABV) that infects many different species within wildlife such as skunks, raccoons, foxes, and many domestic species including cats, dogs, and livestock. It is lethal to all species including humans, being the most lethal infectious disease in the world (WHO, 2005). Reservoirs of the rabies virus throughout wildlife populations pose an enormous risk of exposure and infection of domestic animals that have direct contact with humans. In order to reduce the number of domestic animal rabies cases, RABV must be controlled and prevented within wildlife populations. The most effective way to do this is through the use of vaccination programs in specific wildlife populations. The vaccines used in wildlife vaccination programs are mainly modified live or attenuated vaccines and killed or whole inactivated vaccines. These vaccines can be administrated via injection subcutaneously or intramuscularly and orally. Each vaccine and method of administration has its strengths and weaknesses and costs will vary with each type of vaccine as well as method of administration. The wildlife targeted for vaccinations are mainly skunks, raccoons, and foxes since these species hold the most significant reservoirs of the virus within the United States. Furthermore, the social and habitual behaviors of skunks, raccoons, and foxes are analyzed in order to administrate the vaccines at the most effective time to ensure a larger number of the species population are vaccinated. Each species breeding season, habitat location, activity level, and territory size throughout each different season will be discussed. These behaviors will determine where to vaccinate and when to vaccinate based on when there will be larger populations of a species in an area. This will ensure the vaccination of the most animals as possible. Each species presents their own unique challenges when it comes to vaccination for the rabies virus. The optimal wildlife vaccination program for these species will have minimal costs and will be more effective

in vaccinating more animals as compared to previous wildlife vaccination trials. Through a better understanding of the virus and the wildlife species it infects, greater improvements may be made in the efforts to eradicate RABV.

Rabies is a negative-sense or negative polarity RNA virus, meaning that the virus's genetic information is in the orientation 3' to 5' (Hicks, 2012). The genome of the virus is non-segmented, about 12 kilo-bases long and encodes for 5 viral proteins. These proteins include the nucleoprotein (N), phosphoprotein (P) or otherwise known as M1 protein, matrix protein (M) also known as M2 protein, glycoprotein (G), and polymerase (L) (Hicks, 2012). RABV has a characteristic bullet-shaped appearance. The viruses are 100-300 nm in length and have a diameter of 75 nm (WHO, 2005). The rabies virus is enveloped, which it obtains when budding (exiting) the hosts cells after replication. Trimers of the glycoproteins compose the spike like projections on the envelope which allow for the entry of the virus into the host cell. The trimer of glycoproteins bind to the virus specific receptors on the membrane of the host cell resulting in receptor mediated endocytosis of the virus. The helical ribonucleocapsid of the rabies virus is composed of the RNA genome associated with nucleoproteins (N), polymerase (L), and phosphoprotein (P). This complex allows for the transcription and replication of the virus which occurs in the cytoplasm of the host cell. Matrix protein (M) is responsible for the budding of the virus and is located between the ribonucleocapsid and the envelope. M2 protein also gives the virus its bullet-shaped appearance (Hicks, 2012). The locations of the proteins and the morphological structure can be shown in Figure 1 (Hunt, 2016). Rabies is a virus specific to the

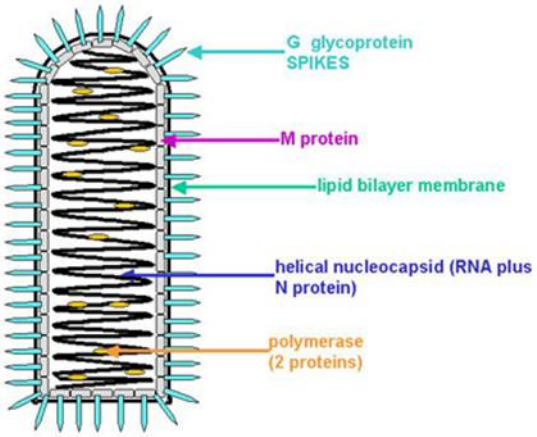

Figure 1

Mononegavirales order, *Rhabdoviridae* family and *Lyssavirus* genus. The genus *Lyssavirus* stems from the name of the Greek goddess of furry and rage (Müller, 2014).

RABV acute encephalitis is an ancient a zoonotic disease, being recognized as far back as ancient Egyptian times (Cliquet, 2015). The RABV causes the acute encephalitis in humans and other mammalian species. Rabies virus is transmitted via direct contact with mucosal surfaces of an infected host such as a bite. From there, the virus can directly enter the peripheral nervous system or replicate within the local area of muscle cells. The incubation period of the virus is on average of 2 to 3 months but varies greatly in species, as well as amount of virus that infects host, and the site of infection. If it enters the peripheral nervous system directly, the virus will travel to the central nervous system (CNS) via retrograde axoplasmic flow (Jackson, 2013). The virus must reach the central nervous system in order to be pathogenic. Once in the CNS, signs and symptoms begin to occur such as spasms, agitation, confusion, and paralysis, and eventually leading to death (Jackson, 2013). However, it has been documented that RABV can cause very mild neurological signs and symptoms and associated inflammation of neuro cells in infected humans and animals. This is most likely due to the virus's ability to evade the host's immune system in its effort to keep the nervous system intact so it can continue to survive in the host (Ito, 2016). Survival includes the ability to infect and spread within in the nervous system and continued replication until it reaches the salivary glands. The virus's objective is to reach the salivary glands in order to be given the opening to infect a new host since through saliva via transport by a bite/wound. The current host of the virus will unavoidably succumb to the virus's lethal effects and once that occurs the virus will die as well unless it infects a new host (Jackson, 2013). Despite the virus's very simplistic structure and morphology, it has proven to be a very

complex in the ways that it avoids the host's immune system and its ability to mutate to confuse the immune system (Ito, 2016).

There are several ways to be diagnose RABV acute encephalitis. Clinical diagnosis coupled with laboratory diagnosis is required for definite diagnosis of rabies. Clinical diagnosis includes the observation of signs of encephalitis within the patient. The presence of spontaneous inspiratory spasms until death often aids clinical diagnosis of a rabid patient (WHO, 2005). Paralytic rabies is harder to diagnosis due to the absence of these signs but if coupled with probable cause of rabies, it can be clinically diagnosed. Another diagnostic tool used for clinical diagnosis is magnetic resonance imaging (WHO, 2005). If images of the brainstems, hippocampus, hypothalamus, or white and gray matter within the brain are abnormal, then there is a strong indication that the patient has been infected with rabies (WHO, 2005). These images must be performed to rule out other viral encephalitis possibilities. Laboratory diagnosis of rabies is required to diagnosis the patient regardless of clinical diagnosis. Such laboratory tests should be performed in a biosafety level 2 (BSL-2) laboratories and personnel handling any rabies suspect tissue samples should have pre-exposure rabies vaccine and personal protection equipment (PPE) must be worn when handling samples (WHO, 2005). The fluorescent antibody (FA) technique is a rapid and sensitive method for diagnosing rabies infection in animals and humans. It is the most efficient and accurate diagnostic test for rabies in laboratories (WHO, 2005). The tests uses the antibody/antigen specificity nature to diagnose the virus. Tissues from the possible infected host are looked at under a microscope with fluorescent light after they have been treated with anti-rabies serum or globulin conjugated with fluorescein isothiocyanate (WHO, 2005). If the virus is present, the anti-rabies serum will bind to the virus and the conjugated fluorescence molecule will fluoresce when a fluorescent light is shown on the tissue.

Tissues from the brainstem, thalamus, cerebellum, and the hippocampus are the recommended sources for FA postpartum and from saliva in current, living patients. Polymerase chain reaction (PCR) can also be used to diagnose RABV but is not common practice in laboratories anymore and is only done if laboratories lack the equipment needed to perform FA (WHO, 2005). Treatment of rabies encephalitis is ineffective once clinical signs have occurred. Keeping the patient comfortable is the only form of treatment for advanced rabies encephalitis through the use of sedatives and mental health support (WHO, 2005).

Direct prevention strategies for human rabies are pre exposure vaccines and post exposure vaccines for the rabies virus. Pre-exposure vaccination entails a 1 ml vaccine via intramuscular injection on days 0, 7, 21 and 28. Boosting of pre-exposure vaccine is recommended at 3–5-years after each pre-exposure vaccine interval (Middleton, 2015). Post exposure vaccines for RABV are a series of rabies post-exposure prophylaxis (RPEP) injections that should be administrated on the day of exposure to avoid the progression of RABV in the body which will lead to acute encephalitis. These shots include a four-dose series of human diploid cell vaccines along with rabies immune globulin. These shots should be administrated as follows; two intramuscular regimens and two intradermal regimens on days 0, 3, 7, 14 (Middleton, 2015).

The incidence of rabies infection occurs worldwide, excluding a few RABV-free locations. RABV-free areas include countries such as United Kingdom, Ireland, Sweden, Norway, Iceland, Japan, Australia, New Zealand, Singapore, most of Malaysia, Papua New Guinea, the Pacific Islands and some islands in Indonesia (Cliquet, 2004). In order to be considered rabies free, there must be no indigenously acquired cases in humans or animals during a 2 year time period. The number of incidences per year is highly debated due to the fact

that many third world countries do not report every case and many cases go undiagnosed. Majority of cases being in Asian and African countries that are underdeveloped and do not have preventative measures for RABV nor educational programs that are informative about rabies. North America has eliminated street rabies in canines through vaccination efforts and Western Europe has been extremely successful in eliminating rabies from the red fox completely by implementing an oral vaccine program (Müller, 2014). Within the United States, 92% of reported cases of terrestrial rabies are in wildlife, majority being raccoons, skunks, and foxes (Dyer, 2013). This includes raccoon populations within major cities or otherwise known as village raccoons (Slavinski, 2012). Figure 2 shows the distribution of rabies within North America of raccoon, skunks, and foxes (Dyer, 2013). Skunk rabies strains dominate the middle of North America and raccoon rabies strains dominate the eastern coast of North America. Even though there is much error in the exact number of cases of RABV infection of the human population, rabies has the highest fatality rate of any infectious disease worldwide, having a fatality rate of nearly 100% in all species (Hicks, 2012). The approximate human deaths per year range from 25,000 to 160,000 and more than 10 million exposures are estimated a year but due to insufficient reporting of RABV and lack of availability of diagnostic testing, those numbers could be much lower than the actual number of deaths and exposures due to the virus (WHO, 2005). Global distribution of the rabies virus and the risk of being exposed to rabies in each location can be shown in figure 3 in which red regions are very high risk and light yellow are

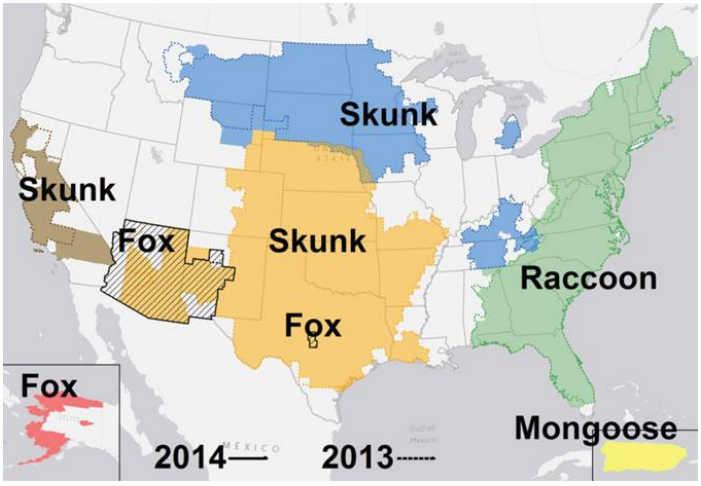

Figure 2

low risk areas (WHO, 2005). Control of RABV in wildlife can significantly reduce the cases and exposures of rabies to the human and domestic animal populations.

Several control methods are used throughout the world in order to lower the occurrence of rabies within species specific wildlife populations. These control methods are culling of the specific species populations that commonly harbor the virus also known as population control as well as vaccination programs in wildlife. Culling of species that are commonly infected with RABV is a common practice for many animal and veterinary medicine facilities and affiliated wildlife departments (Mähl, 2014). Culling of the animals involved the trapping of these species within the proximity of human populations, humanely euthanizing the animals, and collecting tissue samples to send to a BSL-2 laboratory for diagnostic testing. This method of control however did not show much impact on the occurrence of the virus in the specie populations of skunks, raccoons, and foxes (Mähl, 2014).

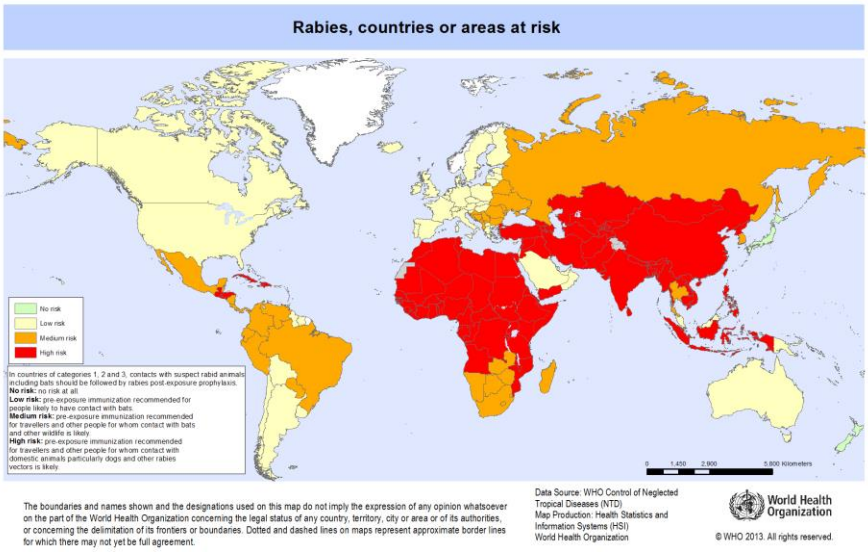

Figure 3

The second method in the efforts to control RABV is the implementation of wildlife vaccination programs. The first type of vaccine most commonly used in wildlife vaccination programs is the modified live (MLV) or attenuated vaccines. Live viral vaccines induce mild infections with live organisms resulting from non-target hosts or attenuated through passage in different cell line cultures or they are created by inducing mutations in the viral genome which

cause reduced virulence of the vaccine (Hicks, 2012). Since the virus is still alive, it can enter host cells, replicate, and bud off. Due to the virus retaining this ability, it activates both humoral and cell mediated immunity. Consequently, MLV vaccines induce the greatest immune response to RABV, so once exposed, the immune system of the host can target the virus easier through antibodies made from primed B cells and a greater CD8 and CD4 T cell response (Meeusen, 2007). The vaccine has primed the immune system to mount such a high immunity to the virus. Unlike other vaccines, the virus is alive and as a result there is no need to add adjuvants or other carrier molecules for the vaccine virus. This makes the vaccine very versatile in the form of administration. It can be given in drinking water of animals, injected subcutaneously (SubQ), intramuscularly (IM), or orally administrated. Furthermore, MLV vaccines are less expensive to produce do to the fact that there is less processing to produce them, they do not require an adjuvant, nor do they require an inactivating agent and they can be produced in several cost effective administrative forms (Meeusen, 2007).

However, there are drawbacks to using a modified live vaccine to vaccinate for such a highly fatal virus. Since the virus is still alive, there is potential for the vaccine to revert back to virulence (Meeusen, 2007). Reverting to virulence is the ability that a modified virus has the ability to do. Instead of boosting the immunity of the animal, the vaccine will cause the disease. Each different vaccine produced has variance in the occurrences of reversion to virulence but the small percentage that do revert back can cause the spread of the virus, thus counterproductive (Meeusen, 2007). Due to MLV having this ability, many strict guidelines or tests must be passed by the vaccine in order to be able to be a product that can be used as a vaccine. MLV vaccines have to fulfill international regulations in order to guarantee their use and reliability, as well as their efficacy and safety in target and non-target species. In consequence, it can be more costly at

first to produce a live vaccine until a satisfactory product is made to pass these guidelines (Rossatte, 2009). Another drawback to MLV vaccines is the skewing of research results pertaining to cases of rabies in wildlife populations. Due to the virus still being alive in the vaccine and thus when administrated to an animal, being alive in the host, when samples are taken from the animal, they may test positive for RABV even though they are not infected (Cliquet, 2015). This can be problematic because it indicates a higher population of wildlife infected with RABV and in consequence more unnecessary money may be spent in order to vaccinate more animals and larger areas than necessary as well a mass culling of wildlife unnecessarily (Cliquet, 2015). Therefore, tracking of animals that have been vaccinated may be necessary to avoid such results when population RABV data in wildlife is being obtained.

The second type of vaccine commonly administrated is the killed vaccines or also known as whole inactivated vaccines. In this type of vaccine, the virus is killed by the use of recommended inactivating agent used in rabies vaccine production, beta-propiolactone (βPL) (Meeusen, 2007). This vaccine also contains an adjuvant. The virus is no longer viable and cannot enter the host cell and replicate and move throughout the host. An adjuvant is needed to traffic the vaccine around the host to get a better priming of the immune system (Meeusen, 2007). The benefit to whole inactivated vaccines is there inability to revert to virulence. Therefore there is zero risk of infecting animals with RABV, there is no possibility of getting false positives in RABV population research data, and it is much easier to produce due to have less strict guidelines and tests to pass to be a product (Meeusen, 2007).

Like MLV vaccines, killed vaccines have their drawbacks. The inactivating agent, beta-propiolactone (βPL), is a very expensive chemical (Meeusen, 2007). Not only the cost of the chemical but βPL has been proved to be potential carcinogen which is less than ideal. The

addition of an adjuvant also increases the costs of vaccine production because it's an extra component that has to be purchased and added into the vaccine (Meeusen, 2007). In addition to the drawbacks of production of whole inactivated vaccines, the immunity induced by the vaccine is by far inferior to the immunity generated by the modified live vaccines. When it comes to priming the immune system, MLV vaccines are the gold standard. Due to killed vaccines inability to infect cells and activate cytotoxic (CD8) T cells, it does not prime the immune system as adequately as live vaccines (Meeusen, 2007). The cell mediated (T cell) response is not induced by the vaccine, only the humoral (antibody/B cell) response is primed. Therefore, whole inactivated vaccines are not used as frequently in wildlife as modified live or attenuated vaccines. Once produced initially, MLV vaccines show greater cost reduction to generate than do whole inactivated vaccines. MLV vaccines also provide better priming of the immune system, inducing both humoral and cell mediated immunity, as killed vaccines only prime the humoral immune response. Although live vaccines show their weaknesses, their strengths outweigh their drawbacks and the benefits of killed vaccines and are used as the primary vaccine in wildlife vaccination programs (Cliquet, 2004).

As discussed previously, MLV vaccines can be administrated in several different methods such as in drinking water, SubQ, IM, and orally. In wildlife, in order to administrate subcutaneous and intramuscular vaccines, the animals must be trapped, restrained, the vaccine physical administrated by a human, and then released (Hicks, 2012). Such trap and release vaccination programs are the most costly because more equipment is needed and physical labor. It also only allows you to vaccinate one animal at a time (Slavinski, 2012). Though these programs do exist, they are not the most practical in administrating vaccine to large populations

of wildlife and put humans at risk of injury and exposure to any animal that may already be infected with RABV (Hicks, 2012).

Placing the vaccines within the drinking water would lower costs of equipment and physical labor but there are many drawbacks to this method. One drawback being that most wildlife drinking water is rivers and the vaccines would wash away from the target location and there would be no control of the dosage that each animal would obtain (Meeusen, 2007). Ethically, putting a modified live vaccine into drinking water that may be collected later on for human consumption would not be allowed by the Environmental Protection Agency (EPA), going against many water protection regulations (Meeusen, 2007). Thus, the last and most widely used option for wildlife vaccine programs is the distribution of oral modified live vaccines.

Oral modified live vaccine programs have shown to be the most effect and cost effective way to do a mass vaccination of wildlife species specific populations in efforts to eradicate rabies (Cliquet, 2015). The positives to this method is that the vaccine can be aerially distributed with minimum physical labor and equipment which reduces the costs of the program further. Oral vaccines are administrated via baits that certain species such as the skunk, raccoon, and fox are particular too. The animal ingests the bait and thus the vaccine (Meeusen, 2007). Oral RABV vaccine baits contain a liquid vaccine in a packet and a food-based coating that encloses the packet to entice target animals and encourage consumption. Color, texture, flavor, and scent of the bait encourage target species to ingest the vaccine (Bachmann, 1990). If a non-target species ingests the bait as well, recent studies showed that there is no great adverse effects on them from the currently used MLV vaccine strains used in the oral vaccination programs (Slate, 2014). Regardless, the intention is to vaccinate the targeted species so non-target species ingesting the

vaccines can increase costs of the programs. Therefore, the behavioral tendencies of the target species must be understood in order to place the vaccine in a location that is most accessible to the target species and less so to non-target species. Such behavior includes habitat location, food scavenging behaviors, breeding season, or anything specific that the target species does that other non-target species do not. Each target species will have its own unique challenges when it comes to oral vaccination programs and the understanding of the differences between the wildlife species characteristics will better help with the implementation of an effective vaccine program for each target species.

Creating a species-specific bait matrix for each target species has been the key to success for oral vaccination programs including when and where to administrate the oral vaccine. Skunks are one of the main target species for RABV oral vaccination programs because populations of this specie have significant reservoirs of the virus. Skunks in particular have their own unique challenges for oral vaccination programs. Although skunks night activity and home ranges are much less than other target species, their acceptance of the bait matrix is the most difficult to discern. They are much more selective of food than the raccoon and the fox (Bachmann, 1990). Furthermore, Skunk rabies has the broadest geographical distribution of all terrestrial rabies virus strains in the USA (Brown, 2014). This means that there needs to be multiple MLV vaccine strains created for the oral vaccine programs because skunks are susceptible to many strains of the virus. In the case of this species, trap-vaccinate-release programs are more successful in vaccination efforts although they are more labor intensive and have higher associated costs (Jojola, 2007). However, efforts are still being made to find a successful oral vaccine and bait matrix combination for skunks. Studies in bait selection of skunks have shown that skunks select meat based baits, such as fish or poultry, over cat food based baits and they prefer polymer baits

or coated sachets rather than slim based ones. Texture played a huge role in which baits were selected (Jojola, 2007). Additionally, size difference in baits impacted vaccine consumption. Due to skunks much smaller jaw and mouth, larger baits were much harder to ingest and therefore the vaccine packet wasn't punctured or ingested (Jojola, 2007). Once an optimal bait is discovered, costs of skunk vaccination efforts could drop considerably, making it more likely that the vaccination programs become a general practice for many countries.

The optimal oral vaccination program for skunks additionally depends on habitat use during the different seasons and breeding season. Dropping vaccine baits at the optimal time in which a larger population of the species is in a certain area will ensure a larger number of vaccinated animals. The breeding season for skunks occurs in the months of spring. During this time, the male and female home ranges increases, but more so in the males, having an increase of 4.9 times larger than their standard home ranges (Lesmeister, 2009). During the spring, the habitat selection of skunks is overwhelmingly young, shortleaf pine habitats which are the highest preference in all seasons in wooded areas (Lesmeister, 2009). Since skunks are known to habitat very diverse terrains aside from heavily wooded areas, the second most preferred habitat and foraging locations are agricultural fields. If woodland areas are not available, fields are the second highest preferred habitats by skunks (Neiswenter, 2007). Consequently, the optimal time and location to air-drop oral rabies vaccines is during the spring breeding season when home ranges are the largest and in shortleaf pine habitats or near agricultural fields.

Another species that is targeted by RABV oral vaccination programs are raccoons. Raccoons, as with skunks, are a common species to be infected with the virus. The unique challenge that vaccine programs are facing with vaccinating raccoons is pin pointing the exact location of raccoons and how to vaccinate such large areas. Racoons inhabit the most diverse

habitat out of all the target species for rabies vaccination. In recent studies, only 30% of the raccoon species in the target areas are ingesting the vaccine. The amount of raccoons that need to ingest the vaccine in order to make an impact on enzootic regions is 50-70% of the species population (Boyer, 2011). This indicates consumption of the vaccine by other non-target species that may be attracted by the fishmeal bait matrix of the vaccines or aerial drop location inaccuracies. Further studies conducted on the raccoon vaccine baits showed that raccoons do have a strong liking for the fish baits which indicates that it is most likely that the baits are being dropped in ineffective areas or that other non-target species in the area also have a strong liking for the fish baits.

Raccoons inhabit wooded areas, crop fields, urban areas, marshlands, and countless other habitats. Understanding the likeliness of certain habitats in certain season including breeding season may give an indication as to where and when to drop the vaccine baits. Breeding season for most raccoons occurs between February and May (Byrne, 2011). During these months, a larger population of raccoons will be in the same area making it easier to vaccinate a larger number of animals. During breeding season, the home ranges of raccoons increases meaning that their activity level increases and they are more likely to travel to many areas, making it easier for them to be exposed to an area that vaccine baits were air-dropped on (Byrne, 2011). Due to this, the optimal time to do oral vaccine bait drops is during February and May. The most common habitat during breeding season is very open fields for their home ranges followed by upland forest (Byrne, 2011). Home ranges for raccoons is the lowest in summer due to the fact that food is abundant during the season so not much movement is needed to find food (Byrne, 2011). This makes the summer season the least optimal season to implement the oral vaccine program.

Therefore, the optimal time and location for aerial drops of oral rabies vaccine is in February through March in open fields and upland forests.

The last species that is mainly targeted by wildlife vaccination programs that harbor the virus is foxes. The red fox in particular is the main target for oral vaccination programs and they too have their own unique challenges. Red foxes location and movement within their territories is greatly misunderstood because they are mostly active at night, making it difficult to follow their movements. The red fox is known to inhabit only very covered, dense wood-like habitats such as forests and corn fields (Cagnacci, 2004). Certain bait air-drop studies have shown that if baits are dropped in locations that would be indicative of their habitat, then there is a decrease in vaccine ingestion. However, if baits were dropped in more locations that the species are likely to have activity at night in, the ingestion of the vaccine baits increased. Baits distributed about $20/km^2$ over a much greater region of 98,000 km^2 rather than just cornfields and wooded areas proved to increase the consumption of the vaccine baits to greater than 50% (Bachmann, 1990). This indicates the species eating habits. The red fox consumes most of its food in open areas away from the den at night. Therefore, baits should be dropped in areas that the foxes would hunt or have a high activity level in at night, and not necessarily within the vicinity of its den. Foxes have very large home ranges so vaccine bait drops must be done over a larger area than other target species. Furthermore, ground beef baits are used for oral vaccination of the species, however, ground beef vaccine baits are rapidly depleted by non-target species in the area of the bait drops (Bachmann, 1990). Vaccine baits that are attractive to foxes specifically need to be developed in order to prevent this. Foxes have shown a strong inclination to consuming poultry more so than other species (Bachmann, 1990). There is a possibility to reduce non-target species

ingestion of vaccines by changing the composition of the baits to a food source that nearly only foxes like to consume.

When to vaccinate foxes depends largely on mating seasons which would bring many foxes into the same area. Red foxes mating season occurs between the months of February and May (Farstad, 1998). Breeding season is the only time that home ranges increase but habitats remain the same. Dense forested habitats are almost always selected for, while almost all sparse forest and open habitats were avoided during all seasons including breeding season (Cagnacci, 2004). In consequence to the strict habitat selections and the breeding season of February to May, the optimal place and time to vaccinate red foxes orally for rabies is during the breeding season in densely covered habitats.

In order to improve effectiveness of wildlife vaccine programs, future progress must be made in vaccine production, administrative methods, and in knowledge of target species changes in behavior, socialism, and habitat throughout the seasons. Although oral vaccination programs have prevented new areas from becoming enzootic, it has not decreased the current enzootic areas. Further innovation in zoonotic disease control must proceed in order to make an impact on these current enzootic locations. Recent studies have shown that within the United States, approximately only 30% of some target species are ingesting the oral vaccines despite more than 8 million bait drops a year. There needs to be at least 50-70% consumption of the vaccine in order to make in impact on the enzootic locations (Boyer, 2011). This indicates that the target species are not attracted to the current bait matrix or the vaccines are being dropped in inaccurate locations to where the target species are actually located are have a high activity in. Further research needs to be conducted in order to produce a bait that is highly appeasing to each target species to increase effectiveness of vaccination programs. In addition to understanding bait

preference, understanding of scavenging behaviors and activity levels and locations need to be better understood. Studies that track night activity, observes areas commonly trafficked, and focus on hunting and food collection behaviors by each target species needs to be conducted to supplement the current oral vaccination programs. If such knowledge is obtained and more strains of oral vaccinations are produced, 50% or greater immunization to RABV should be achievable for the target species of skunks, raccoons, and foxes (Bachmann, 1990).

Oral vaccination programs are the future for control, prevention, and possible elimination of enzootic rabies in skunks, raccoons, and foxes throughout the world. Switching to such vaccination programs from culling and population control will show greater progress in the efforts to reduce enzootic areas as well as reduce the number of cases of exposures of rabies from wildlife in humans' worldwide. Due to the expensive process of executing a vaccination program in which the success rate is currently undetermined deters a lot of countries from implementing the programs. With further research continuing in wildlife vaccination programs and if effectiveness can be shown in cost effective manner, then more third world countries will be more able to conduct such programs in wildlife to decrease the cases of rabies. As long as there is rabies prevalent worldwide, there is a need for vaccination programs. Where there is a need for a vaccination program, there is need for improvement and advances in tools and methods. It is imperative that veterinary medicine professionals and facilities continue to push forward with these advances until a permanent solution is identified.

Work Cited

Bachmann, P., Bramwell, R. N., Fraser, S. J., Gilmore, D. A., Johnston, D. H., Lawson, K.F., Macinnes, C. D., Matejka, F. O., Miles, H. E., & Pedde, M. A. (1990) Wild carnivore acceptance of baits for delivery of liquid rabies vaccine. *Journal of Wildlife Diseases,* 26, 486–501.

Boyer, J. P., Canac-Marquis, P., Guérin, D., Mainguy, J., & Pelletier, F. (2011). Oral vaccination against raccoon rabies: landscape heterogeneity and timing of distribution influence wildlife contact rates with the ONRAB vaccine bait. *Journal Of Wildlife Diseases*, *47*(3), 593-602.

Brown, L. J., Rosatte, R. C., Fehlner-Gardiner, C., Ellison, J. A., Jackson, F. R., Bachmann, P., & Donovan, D. (2014) Oral vaccination and protection of striped skunks (Mephitis mephitis) against rabies using ONRAB®. *Vaccine*, *32*(29), 3675-3679.

Byrne, M. E., & Chamberlain, M. I. (2011). Seasonal space use and habitat selection of adult raccoons (Procyon lotor) in a Louisiana bottomland hardwood forest. *American Midland Naturalist*, *166*(2), 426-434.

Cagnacci, F., Meriggi, A., & Lovari, S. (2004). Habitat selection by the red fox Vulpes vulpes (L. 1758) in an Alpine area. *Ethology, Ecology & Evolution*, 16(2), 103-116.

Cliquet, F. & Aubert, M. (2004) Elimination of terrestrial rabies in Western European countries. In: Schudel A, Lombard M., editors. Control of infectious animal diseases by vaccination. *Basel: Karger,* 185–204.

Cliquet, F., Picard-Meyer, E., Mojzis, M., Dirbakova, Z., Muizniece, Z., Jacevicience, I., Mutinelli, F., Matulova, M., Frolichova, J. Rychlik, I., & Celer, V. (2015) In-Depth Characterization of Live Vaccines Used in Europe for Oral Rabies Vaccination of Wildlife. *Public Library of Science*, *10*(10), e0141537.

Dyer, J. L., Wallace, R., Orciari, L., Hightower, D., Yager, P. & Blanton, J.D. (2013) Rabies surveillance in the United States during 2012. *Journal of the American Veterinary Medical Association*, 243, 805–815.

Farstad,W. (1998) Reproduction in foxes: current research and future challenges. *Animal Reproduction Science*, 53(1/4), 35-42.

Hicks, D. J., Fooks, A. R., & Johnson, N. (2012) Developments in rabies vaccines. *Clinical And Experimental Immunology*, *169*(3), 199-204.

Hunt, M. (n.d.). RNA Virus Replication. Retrieved December 11, 2016, from http://www.microbiologybook.org/mhunt/rna-ho.htm

Ito, N., Moseley, G. W., & Sugiyama, M. (2016). The importance of immune evasion in the pathogenesis of rabies virus. *Journal Of Veterinary Medical Science*, *78*(7), 1089-1098.

Jackson, A. C. & Fu, Z. F. (2013) Pathogenesis. pp. 299–349. In: Rabies, 3rd ed. (A. C. Jackson ed.), Academic Press, Oxford.

Jojola, S. M., Robinson, S. J., & Vercauteren, K. C. (2007) Oral rabies vaccine (ORV) bait uptake by captive striped skunks. *Journal of Wildlife Diseases*, 43, 97–106.

Lesmeister, D. B., Gompper, M. E., & Millspaugh, J. J. (2009). Habitat Selection and Home Range Dynamics of Eastern Spotted Skunks in the Ouachita Mountains, Arkansas, USA. *Journal of Wildlife Management*, 73(1), 18-25.

Mähl, P., Cliquet, F., Guiot, A., Niin, E., Fournials, E., Saint-Jean, N., Aubert, M., Rupprecht, C.E., & Gueguen, S. (2014) Twenty Year experience of the oral rabies vaccine SAG2 in wildlife: a global review. *Veterinary Research*, 45.

Meeusen, E. N., Walker, J., Peters, A., Pastoret, P. P., & Jungersen, G. (2007) Current status of veterinary vaccines. *Clinical Microbiology Review*, 20, 489-510.

Middleton, D., Johnson, K. O., Rosatte, R. C., Hobbs, J. L., Moore, S. R., Rosella, L., & Crowcroft, N. S. (2015) Human Rabies Post-Exposure Prophylaxis and Animal Rabies in Ontario, Canada, 2001-2012. *Zoonoses & Public Health*, *62*(5), 356-364.

Müller, T., Demetriou, P., Moynagh, J., Cliquet, F., Fooks, A.R., Conraths, F.J., Mettenleiter T.C., & Freuling, C. (2014) Rabies elimination in Europe- A success story. *Compendium of the OIE Global Conference on Rabies Control*, 31-43.

Neiswenter, S. A., & Dowler, R. C. (2007). Habitat use of western spotted skunks and striped skunks in Texas. *Journal Of Wildlife Management*, *71*(2), 583-586.

Rosatte, R.C., Donovan, D., Davies, J.C., Allan, M., Bachmann, P., Stevenson, B., Sobey, K., Brown, L., Silver, A., Bennett, K., Buchanan, T., Bruce, L., Gibson, M., Beresford, A., Beath, A., Fehlner-Gardiner, C., & Lawson, K. (2009) Aerial distribution of Onrab(r) baits as a tactic to control rabies in raccoons and striped skunks in Ontario, Canada. *Journal of Wildlife Diseases*, 45, 363–374.

Slate, D., Chipman, R. B., Algeo, T. P., Mills, S. A., Nelson, K. M., Croson, C. K., & ... Rupprecht, C. E. (2014). Safety and immunogenicity of Ontario rabies vaccine bait

(ONRAB) in the first us field trial in raccoons (Procyon lotor). *Journal Of Wildlife Diseases, 50*(3), 582-595.

Slavinski, S., Humberg, L., Lowney, M., Simon, R., Calvanese, N., Bregman, B., & Oleszko, W. (2012) Trap-vaccinate-release program to control raccoon rabies, New York, USA. *Emerging Infectious Diseases, 18*(7), 1170-1172.

WHO (2005) WHO expert consultation on rabies, 1st report. In *WHO Techn Rep Series Geneva: World Health Organization*, 931, 121.