

DEVELOPMENT OF STRATEGIES TO ORALLY DELIVER VACCINE FOR
BOVINE TUBERCULOSIS TO WHITE-TAILED DEER OF
NORTHEASTERN LOWER MICHIGAN

By

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ABSTRACT

DEVELOPMENT OF STRATEGIES TO ORALLY DELIVER VACCINE FOR BOVINE TUBERCULOSIS TO WHITE-TAILED DEER OF NORTHEASTERN LOWER MICHIGAN

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A self-sustaining reservoir of bovine tuberculosis (bTB) occurs in free-ranging white-tailed deer (*Odocoileus virginianus*) in northeastern lower Michigan. An oral vaccine delivery system to inoculate deer against bTB has been proposed as a method to reduce prevalence. Our objectives were to (1) quantify deer abundance on agriculture fields during winter break-up, (2) evaluate deer preferences for oral vaccine delivery unit (VDU) candidates (3) quantify vegetation characteristics that may contribute to deer use of agriculture fields, and (4) quantify VDU consumption by deer with an effective biomarker. In 2015 and 2016, food preference trials of 4 types of VDUs were conducted and resulted in higher consumption of the alfalfa/molasses VDU compared to other VDUs. Biomarker trials with rhodamine B (Rb) were conducted in 2016 during late-winter using the alfalfa/molasses VDU. Rhodamine B causes a fluorescent band to appear on the vibrissae of deer after consumption. Vaccine delivery units were placed in 50-m x 20-m grids on agriculture fields with VDUs spaced 2.5-m apart. During biomarker trials, VDUs were distributed on 17 agriculture fields. From 16 March - 4 May 2016, USDA Wildlife Services euthanized 1-13 deer on each VDU grid. Six vibrissae were collected per deer and analyzed for the presence of Rb. A total of 107 deer were collected on alfalfa/molasses VDU grids with 69.2% of the deer having the biomarker present on vibrissae. Our research will allow us to make recommendations of how, when and what VDU to deploy a successful oral vaccine distribution system to reduce bTB prevalence in deer of DMU 452.

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This thesis is dedicated to the two most
supportive and hardworking parents a son
could have and to my best friend Amy.

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GENERAL INTRODUCTION

With an increase in urbanization, domestication of wildlife and a changing climate, zoonotic diseases present an increasing public health risk (Kruse et al. 2004; Mills et al. 2010). Researchers estimate that 61% of all infectious pathogenic diseases that pose a risk to humans are zoonotic (Taylor et al. 2001). Methods to control zoonotic diseases from reaching domesticated species such as cattle are extremely important due to the ability of these diseases to enter the human transmission cycle (Morand et al. 2014).

Breaking chains of zoonotic disease transmission must be managed on local and national levels. The World Health Organization has identified many neglected zoonotic diseases (NZD) that originate in animal hosts. These NZDs have a major effect on public health, public perception, local and global economies, and wildlife and livestock hosts. Some NZD and zoonotic diseases include rabies, brucellosis (*Brucella spp.*), bovine tuberculosis (*Mycobacterium bovis*) (bTB), *Echinococcosis*, avian influenza (H5N1) and dozens more caused by bacteria, viruses, and parasites (Veterinary Record 2015). It is estimated that 32% of free-ranging ungulates worldwide are hosts for zoonotic diseases (Han et al. 2016). Several of these zoonotic diseases have found wildlife reservoirs in a range of domestic and free-ranging hosts. The “One Health” approach attempts to incorporate a wildlife component into the human, livestock and wildlife interface, however, the complexities of wildlife disease reservoirs can be a challenge to understand and manage (Miller and Olea-Popelka 2013).

Controlling and eradicating zoonotic diseases can greatly improve the socio-economic condition of the public. Rapid response to a disease outbreak on a local level has the potential to effect global change in management and surveillance of zoonotic diseases (National

Research Council 2010). With emerging zoonotic occurrences, wildlife agencies can learn what conditions cause an outbreak and the importance of quick response. Disease management strategies must explore proper response to an outbreak and commit to a comprehensive prevention and management plan. Management plans for reducing the density of host species (Carstensen and DonCarlos et al. 2011; Ramsey et al. 2014), wildlife mitigation practices to protect domesticated cattle (Lavelle et al. 2015), and long-term vaccination programs of wildlife can effectively reduce disease prevalence (Fearneyhough 1998; Slate et al. 2009).

Public perception and unique characteristics of zoonotic diseases (e.g. multiple hosts, virulence, and pathogen diversity) pose several challenges that impede the prevention and control of many zoonotic diseases. The oversimplification of complex disease transmission chains can lead to a breakdown in communication between wildlife managers and the public (Peterson et al. 2006). For example, bTB and prion diseases such as chronic wasting disease spread through horizontal and vertical transmission and have the potential to spread through contaminated environmental components (Palmer and Whipple 2006; Mathiason et al. 2009). Environmental transmission can be effected by temperature, shade/sunlight, presence of water sources, or soil composition (Walter et al. 2012; Barasona et al. 2016). Social dimensions of acceptable forms of wildlife management of diseases can be negatively affected by any miscommunication. Financial and political motivation can also inhibit the ability for wildlife managers and the public to safely and effectively reduce the cases of zoonotic disease. The Food and Agriculture Organization of the United Nations (FAO) has prompted the need for strengthening the surveillance system of zoonotic diseases and translating science into policy.

With zoonotic diseases, such as bovine tuberculosis or brucellosis, the public may not perceive the indirect economic and social impacts of these diseases. Nonlethal management strategies have been supported by the public for managing well known diseases (e.g. *E. coli*, bovine tuberculosis) (Peterson et al. 2006). While culling of wildlife reservoirs of zoonotic diseases has been effective in some cases, decreased public acceptance is evident. Thus, additional management strategies beyond culling must be explored if control or eradication is truly the objective (Carstensen and DonCarlos 2011; Manjerovic et al. 2014; Abdou et al. 2016). In conjunction with culling, long-term vaccination programs have potential in control and eradication of some zoonotic diseases (Abdou et al. 2016).

A self-sustaining reservoir of bTB has been a management challenge in free-ranging white-tailed deer (*Odocoileus virginianus*) ('deer' hereafter) in a four county area of northeastern lower Michigan (NELM; Montmorency, Alpena, Alcona, Oscoda) (Schmitt et al 1997). Surveillance of this four county area began on 15 November 1995 after a 4.5-year-old harvested deer tested positive for bTB in 1994 (Schmitt et al. 1997). This was the second known case of bTB in Michigan deer since another bTB-positive deer was harvested in 1975 only 14.5 km from the 1994 case (Hickling 2002). In 1996, a core area of ~1,500 km² designated as 'Deer Management Unit (DMU) 452' was established by the Michigan Department of Natural Resources (MDNR) to encompass the core area of the bTB infection. In 2016, 29 bTB-positive deer have been detected in Michigan (MDNR 2016) with 21 deer occurring in DMU 452 (Personal communication, Dan O'Brien, D.V.M., MDNR). Bovine tuberculosis has persisted in this environment and become self-sustaining due to relatively high deer densities (range: 14-18 deer/km²) (O'Brien et al. 2011; Cosgrove et al. 2012), continued feeding and baiting of deer

against the baiting ban (Miller and Kaneene 2006), less-than-optimal farming/management practices (Walter et al. 2012), and ideal deer habitat quality (i.e. high thermal cover potential and availability of spring and summer food resources) (Felix et al. 2007).

Sixty-eight cattle herds in Michigan have been infected by bTB since 1994 (Bovine Tuberculosis Eradication Program Quarterly Update 2017). A major management concern for the spread of bTB is the overlap of deer and cattle herds in the area. Several farming practices have increased the risk of transmission of bTB due to wildlife access to stored cattle feed and cattle access to daytime cover used by wildlife and standing water shared by deer (Kaneene et al. 2002). The most common routes of transmission from deer to cattle are through aerosol exposure and indirect contact via shared resources (Garner 2001; Palmer and Whipple 2006). *M. bovis* is able to persist on feed for >112 days in temperatures ranging from -20°C to 23°C (Palmer and Whipple 2006). With this knowledge, current management strategies have shifted towards mitigating potential transmission from deer to cattle by shared feed. Improved farm-management practices such as protecting stored feed with hoop barns and fencing have been used to reduce interspecies transmission (Walter et al. 2012; Lavelle et al. 2015).

Past management strategies by the MDNR have been successful in reducing prevalence of bTB in the core area from 4.9% to 1.8% since 1995 (O'Brien et al. 2011, Carstensen et al. 2011; www.michigandnr.com). Two effective strategies that have been used since surveillance began include: (1) decreasing the density of white-tailed deer in the core epidemic area through increased antlerless harvest and issuing of disease control permits (DCP) and (2) implementing a feeding and baiting ban in DMU 452 and the surrounding area in 2002 (Hickling 2002; O'Brien et al. 2011). These strategies have had positive effects on reducing prevalence of

bTB, however, public acceptance of increased deer reductions has not been widely supported (O'Brien 2006).

Farm management strategies have also aimed at reducing the risk of bTB spillover from deer to cattle with farm mitigation practices such as deer-proof fencing. Lavelle et al. (2015) demonstrated a significant reduction of deer visits to livestock feed after installing deer fencing with gates around stored cattle feed on 6 farms (Lavelle et al. 2015). Researchers used radio collars to monitor the movement of deer that were known to use stored feed as a food source and deer that were not associated with cattle feed. Eleven of the deer that previously used stored feed shifted their movement patterns to more closely resemble “natural” deer in this region. This demonstrated a positive step in reducing the risk for indirect transmission of bTB from deer to cattle through shared feed.

The financial ramifications of mitigating and managing against bTB are extremely costly. Since the beginning of bTB detection in deer of NELM the MDNR has spent over \$23 million on TB-related management; Michigan Department of Agriculture has spent over \$63 million on herd testing; and the federal government has spent in excess of \$150 million (O'Brien et al. 2011). Based on the results of a modeling project simulated a trap and vaccinate approach to reduce bTB, researchers concluded it would be a labor intensive management strategy costing nearly \$50 million in the next 30 years (Cosgrove et al. 2012).

Many obstacles stand in the way of further reducing or even eradicating bTB in Michigan. With current harvest rates and a low compliance of baiting restrictions, eradication is extremely unlikely in the next 30 years (Ramsey et al. 2014). Even with a 100% compliance with the baiting ban there is only an 8% chance of bTB prevalence reduction (Ramsey et al. 2014).

Without a large increase in harvest rates (>50%) and compliance in the baiting ban, eradication is not likely to occur. Hunters in the area are unwilling or unable to increase their own personal harvest limits and an increase in hunter opportunity (i.e. additional antlerless harvest tags) is not expected to have any effect on decreasing deer density in the bTB core area (Ramsey et al. 2014). Additionally, it has been reported that hunters and farmers fail to see a major problem with the occurrence of bTB in the core area (Carstensen et al. 2011; O'Brien et al. 2011) and compliance with the baiting ban has been variable based on public perception and the desire for deer viewing, aesthetics, or hunting (Carstensen et al. 2011). With a decrease in public acceptance to further reduce deer density and the public perception of the bTB problem in the area, new management strategies must be explored.

We designed and implemented a strategy that could be used in the future for orally vaccinating deer against bTB. Our oral vaccination strategy could be another tool for wildlife managers to reduce the prevalence of bTB in NELM and other areas around the world. Our primary objective was to evaluate and optimize our ability to vaccinate the maximum number of white-tailed deer. To achieve this goal we addressed 6 objectives:

- (1) Create an oral vaccine delivery unit (VDU) and evaluate deer preferences of candidate units.
- (2) Assess several distribution methods for vaccine delivery units and their relative effect on visitation and consumption by deer.
- (3) Quantify relative deer abundance on agriculture fields during winter break-up and determine the optimal temporal scale to reach the maximum number of deer.
- (4) Quantify the visitation of possible non-target species to vaccine delivery unit grids.

- (5) Evaluate landscape and vegetation characteristics that may contribute to increased use of various agriculture fields by deer.
- (6) Quantify vaccine delivery unit consumption by deer with an effective biomarker, Rhodamine B.

CHAPTER 1

INTRODUCTION

Bovine tuberculosis (bTB) is a zoonotic disease caused by the bacteria *Mycobacterium bovis* (*M. bovis*). *Mycobacterium bovis* can cause lesions in the respiratory tract, lungs, and medial retropharyngeal lymph nodes of infected host (Domingo et al. 2014) (Fig. 1). Numerous species of wildlife around the world serve as disease reservoirs for *M. bovis*. Wildlife reservoirs of bTB include European badgers (*Meles meles*) in the United Kingdom (Delahay et al. 2007), wild boar (*Sus scrofa*) in Europe, brushtail possums (*Trichosurus vulpecula*) in New Zealand (Fitzgerald and Kaneene 2013) and cape buffalo (*Syncerus caffer*) in Kruger National Park (Miller et al. 2012). Several other species can act as hosts for bTB including red fox (*Vulpes vulpes*), grey squirrels (*Sciurus carolinensis*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and stoats (*Mustela ermine*) (Delahay et al. 2007).

Bovine tuberculosis in the white-tailed deer (*Odocoileus virginianus*) of northeastern lower Michigan (NELM) is of special importance as deer currently act as a major reservoir for the disease (Schmitt et al. 2002). Prevalence of bTB in deer in 'Deer Management Unit (DMU) 452' ranges from 1.2% - 2.0% (Michigan Department of Natural Resources, MDNR 2016). High deer densities brought on by historic supplemental feeding, baiting, and ideal habitat quality (Felix et al. 2007) have contributed to the establishment of bTB in the deer of NELM (Garner 2001). The potential for deer to transmit *M. bovis* to cattle herds in the area poses a great economic and social cost for the entire state of Michigan (O'Brien et al. 2011; Cosgrove et al. 2012; Ramsey et al. 2014) and potentially the nation.

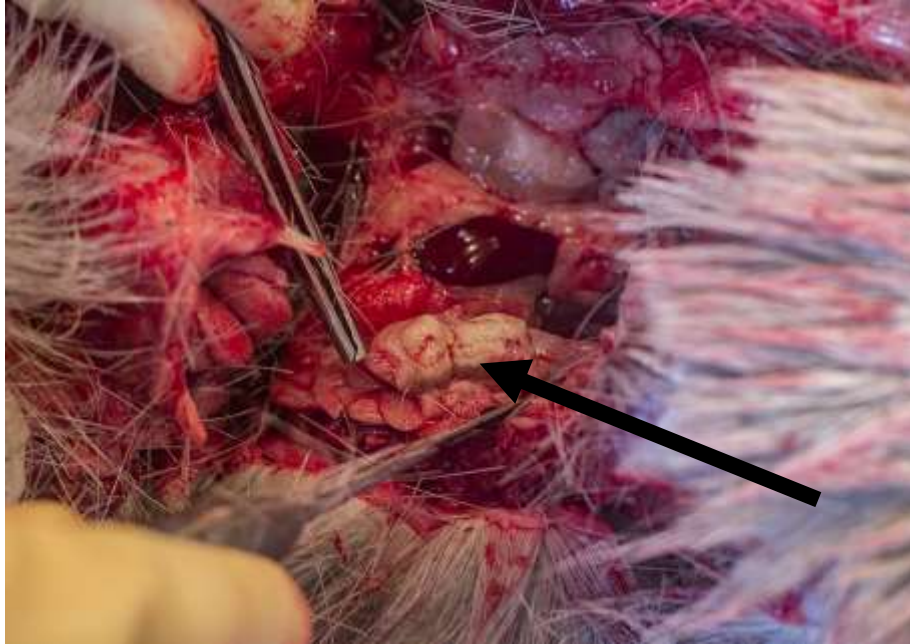


Figure 1. Enlarged retropharyngeal lymph node of white-tailed deer. One of the clinical signs of bovine tuberculosis infection in deer (Photo credit: David Dressel).

In the past, reductions in deer density (Schmitt et al. 2002) and restrictions on baiting (Garner 2001) have helped reduce bTB prevalence in the area from 4.9% to 1% -2%, however, little or no change in prevalence has occurred in the past decade (Cosgrove et al. 2012).

Current management strategies to reduce bTB in DMU 452 have concentrated on reducing deer density through disease control permits (DCP), liberal antlerless harvest (O'Brien et al. 2006; Hickling 2002), baiting restrictions (Garner 2001), and risk mitigation practices (e.g. exclusionary fences, dogs) (VerCauteren et al. 2006; VerCauteren et al. 2008, Lavelle et al. 2015). The use of a vaccine along with current management strategies may be the key to further reducing and possibly eradicating bTB in Michigan (Cosgrove et al. 2012).

Oral vaccine delivery methods have been implemented for many species including black-tailed prairie dogs (*Cynomys ludovicianus*) in South Dakota (Creekmore et al. 2002), raccoons (*Procyon lotor*) in West Virginia (Slate et al. 2014), and Eurasian wild boar (*Sus scrofa*) in Spain (Ballesteros et al. 2011). Slate et al. (2014) had success distributing an oral rabies vaccine to a population of raccoons in West Virginia. By air dropping vaccine units in intervals every 750 m (75 baits/km²) they were able to produce antibodies in 49.2% of the population, the 'highest antibody prevalence observed in raccoons by US Department of Agriculture Wildlife Services' (Slate et al. 2014: 582).

A trap/vaccinate/cull simulation study of 39 scenarios was used to evaluate the efficacy of trapping and vaccinating deer in NELM (Cosgrove et al. 2012). The estimated cost of application to the DMU 452 was \$1.5 million annually plus the cost of continued surveillance (Cosgrove et al. 2012). This management strategy would not be probable based on extensive hours of labor and extreme cost. A potentially more plausible method may be creating an oral

baiting regimen to deliver a vaccine to deer. An oral vaccine system would hopefully decrease cost and maximize the proportion of vaccinated deer in DMU 452 and the surrounding bTB area. Cosgrove's simulations suggested that vaccinating only 26% of the population may result in a >1% decrease in bTB prevalence creating a detectable decrease in prevalence.

Similar simulation models have shown that eradication or prevalence reduction of bTB in the deer population can be achieved but must take in consideration all wildlife disease management strategies (Ramsey et al. 2014). For example, compliance with the baiting ban and harvest rates have a dramatic effect on the probability of decreasing bTB in Michigan deer. With current harvest rates and a 50% compliance rate of the baiting ban, eradication of bTB in DMU 452 is very unlikely (Ramsey et al. 2014). However, with current harvest rates, researchers modeled that vaccinating 90% of the deer in DMU 452 along with a continued baiting ban could create a 95% probability of eradicating bTB within 30 years. The same model also predicted that a deer exposure rate of 50% to the bTB vaccine has an 86% probability of eradication within 30 years (Ramsey et al. 2014.) The threshold of 50% vaccination exposure to deer in DMU 452 may be a more realistic target when attempting to vaccinate the maximum number white-tailed deer.

Two initial efforts to develop an oral vaccination system for deer were conducted in the Sandhill Wildlife Area (SWA) in Wisconsin, USA and NELM. Piles of placebo alfalfa cubes (1 L) were distributed in grids with 652 m between piles inside the SWA (Fischer et al. 2016). Consumption of placebo baits was variable and 33% of baits across all bait stations were consumed in their entirety. The second study evaluated rates of consumption of vaccine delivery units (VDU) distributed to deer in Turtle Lake Hunt Club located in NELM (Phillips et al.,

USDA-National Wildlife Research Center, unpublished data). These VDUs consisted of common baking ingredients presumed palatable to deer (e.g. corn, molasses, etc.) into VDU 'cookies'. Unfortunately, researchers documented low consumption rates. The distribution of VDUs during the winter was thought to be the most efficient way of targeting deer, however, low consumption rates inspired the idea of initiating VDU distribution during winter break-up.

Prevalence of bTB in deer of NELM has not changed in the past decade (O' Brien et al. 2011), prompting the need for an additional wildlife management strategy to be implemented. The bacillus Calmette – Guerin (BCG) vaccine has shown promise in decreasing severity of bTB lesions in studies with penned deer though has not been evaluated on free-ranging deer (Palmer et al. 2014). With the decrease in public willingness of increased culling and antlerless harvest, an oral vaccination system deserves consideration as an additional disease management tool to combat bTB in DMU 452 of NELM.

Specific objectives of our study were to: (1) determine deer preference of three VDU candidates, (2) assess three different distribution methods of VDUs and their relative effect on consumption by deer, (3) evaluate a plausible oral vaccine delivery system to reach the maximum proportion of the target deer population, (4) evaluate possible non-target species visitation to VDU grids and (5) evaluate landscape and vegetation characteristics that may contribute to increased use of agriculture fields by deer. Once the BCG vaccine is ready for use and field testing, it could be placed into the optimal delivery regimen and distributed to deer.

STUDY AREA

The study was conducted in Alpena County of northeastern lower Michigan from 1 April 2015 to 18 June 2015. Alpena County consisted of 4,390 km² and is the northeast county of the associated core bTB area. Study sites consisted of 1 or 2 grids of VDUs distributed on agriculture fields of varying acreage adjacent to forest cover types or open herbaceous fields. All 17 established sites (11 separate landowners), were within Alpena County (Fig. 2). The core bTB area (DMU 452) consisted of 93% private land and 7% public land (Michigan Center for Geographic Information 2001). Deer density in Alpena County and the surrounding bTB core area ranged from 10-18 deer/km² (O'Brien et al. 2011; Cosgrove et al. 2012). Annual precipitation in the area was 71.5 cm with an annual average temperature of 6.40°C (www.ncdc.noaa.gov/cdo-web/datatools/normals). The growing season ranged from 100-130 days (Albert 1995).

Gravelly sandy loam soils were typical in this area and dominated by northern hardwoods and low lying conifer swamps. Forested land constituted 60% of the landscape in Alpena (Alpena County Recreation Plan 2009-2013) with intermittent cedar swamps and hardwood forests dispersed in between agriculture fields and private hunting lands. Lowland conifer swamps were suffused with cedar (*Thuja occidentalis*), balsam fir (*Abies balsamea*), and hemlock (*Tsuga canadensis*). Furthermore, the Alpena County area consisted of aspen (*Populus spp*), balsam poplar (*Populus balsamifera*), white birch (*Betula papyrifera*), several oak (*Quercus*) species, white pine (*Pinus strobus*), and an array of lakes and rivers (Felix et al. 2004).

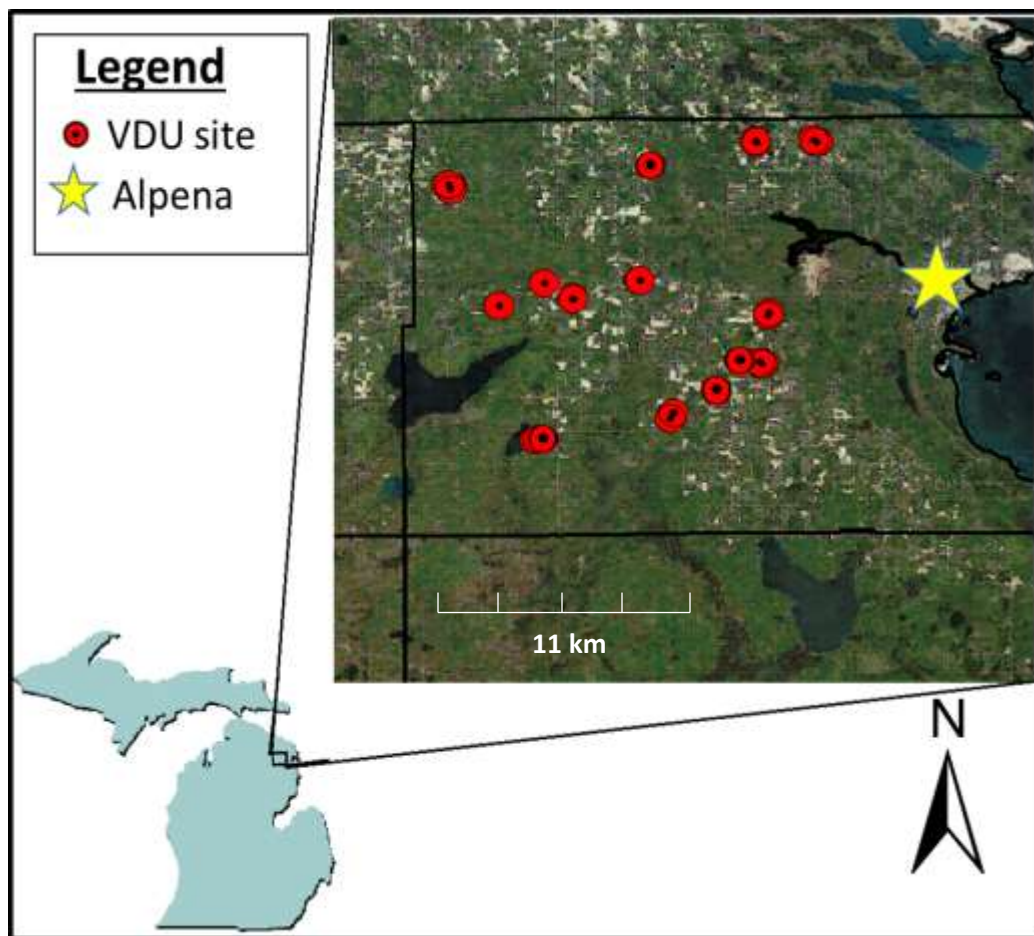


Figure 2. Alpena County with individual vaccine delivery unit (VDU) grids established throughout the County from 1 May 2015 – 18 June 2015 during VDU preference study.

Deer wintering complexes, or deer yards, are of local significance in northern Michigan and provide deer with thermal cover and winter food (Verme 1965). Cedar swamps and mixed conifer stands make up a majority of these winter complexes (Verme 1965; Van Deelan et al. 1996). The thick canopy cover provided by these vegetation types protect deer from extreme winter conditions. In NELM, deer congregate in and adjacent to winter complex areas (deer yards) depending on the severity of the winter. Deer migration has historically been limited in the northeastern portion of the bTB endemic area (range 50%-75% non-migratory) as this area has high availability of thermal cover and provides adjacent vegetation types with spring food resources (Sitar 1996; Felix et al. 2007). Deer that migrate to winter complexes begin their migration in mid to late November and depart from winter ranges in mid-March (Sitar 1996). However, variability in departure and arrival to wintering areas may depend on the amount of snow cover and the extent of spring warming events.

Thermal cover and habitat potential throughout the core bTB endemic area was described by Felix et al. (2004) Conifer dominated forests provided the highest potential thermal cover for wintering deer while early to mid-successional hardwoods provided high food suitability during fall and winter (Felix et al. 2004). The ability of this landscape or study area to contain several, or all, deer life requisites in a relatively small area provides optimal deer habitat potential.

Alpena County is also comprised of a significant amount of agriculture land with a diverse array of plant species contributing to its high habitat potential. Along with the diversity of forest types, agriculture practices on the land yield alfalfa (32%), soybeans (14.5%), corn (13.8%), wheat (7.4%), and several fruits, as well as dairy and beef production. (USDA NASS,

2012 Census of Agriculture). There are estimated to be 9,500 cattle in Alpena County (USDA Michigan Cattle County Estimates 2016). Most beef cattle operations are relatively small with 93% of farms consisting of fewer than 50 cows and an average density of one farm per 21.5 km² (Berensten et al. 2014). There are 3,400 dairy cattle in Alpena County (USDA Michigan Cattle County Estimates 2016) with 87% of dairy operations having fewer than 200 cows (Berentsen et al. 2014). Several hunting clubs also occur in this area including North Fork and Beaver Lake Hunt Club.

METHODS

Vaccine Delivery Unit Development and Consumption

From 1 May 2015 to 18 June 2015 three commercially available VDUs were evaluated for suitability for delivering an oral vaccine to deer. Vaccine delivery units were chosen based on their ability to closely match 'natural' agriculture waste grain that deer feed on during late winter-early spring. In the study area VDU products selected included 'Record Rack' corn block (Sportsman's Choice® Superior, WI), 'Dumor' alfalfa cubes (Purina® St. Louis, MO) and, 'Purina' apple flavored bites (Purina® Harrisburg, PA) (Table 1). Vaccine delivery units were created by breaking or cutting products with a hack saw, producing 17 g-20 g 'bite size' pieces to increase the probability that deer would consume the entire VDU and leave little or no residual (Fig. 3).

All three VDUs used in 2015 were evaluated for consumption by deer. Consumption rates were calculated by comparing the number of VDUs consumed in a 24-hour period with the highest number of deer and the least number of non-target visitations to a VDU grid. This method helped account for any non-target species consumption of VDUs. Average consumption was compared among all sites of the same VDU.

Vaccine Delivery Unit Distribution

Before VDUs were deployed on the landscape, all proposed VDU grid sites on agriculture fields were monitored with trail cameras (Reconyx, RC60, Holmen, WI, USA) from 31 March 2015 to 1 May 2015 to evaluate deer activity and snow cover. Once snow cover began to decrease on agriculture fields, defined in this study as 'winter break-up', and the number of deer on fields increased, VDUs were deployed beginning on 1 May 2015.

Table 1. Macro-nutrient content of commercially available vaccine delivery units (VDU) used during 2015 food preference trial. The price for each VDU commercial product as of 2015 sold in bulk is also shown.

Commercial Product	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Price per commercial Product (\$)
Apple (6.8 kg)	10	3	13	12.99
Corn (13.6 kg)	6.75	3	4	13.99
Alfalfa Cubes (18.1 kg)	16	1.5	30	11.99



Figure 3. The three vaccine delivery units deployed during deer food preference trial 1 May 2015 - 18 June 2015. From left to right; alfalfa VDU, apple VDU, corn VDU. (*Photo Credit: David Dressel*).

The VDU type used on an agriculture field was the product that most closely resembled the waste grain of the previous year's crop. For example, on agriculture fields that had corn the previous year and had waste corn left on the field, the corn VDU was used. This enabled 'natural' pre-baiting on the landscape that reduced the degree of novelty of our VDU types and we hoped would facilitate acceptance and consumption (Fischer et al. 2016). The same technique was used for the alfalfa cubes that were distributed on alfalfa fields. Due to the lack of an available suitable soybean product, the apple VDU was used on soybean fields.

Three separate VDU distribution grids were tested across all agriculture fields used in this study. Vaccine delivery unit grids used included a 100-m x 30-m grid, 50-m x 30-m grid and field edge transects. Each individual VDU was spaced either 5 m apart (e.g. 100-m x 30-m grid), 2.5 m apart (50-m x 30-m), or ~ 1 m apart (field edge transects) (Fig. 4). All VDU grids contained 140 individual VDUs. Careful consideration was used to not exceed the 'baiting restriction' of 7.6 L of foodstuff put forth by the MDNR on each agriculture field. Approval of the baiting protocol used for this study was granted by the MDNR (Appendix A).

Deer and Non-target Visitation

A total of 17 agriculture fields with 31 VDU grids were established in 2015 (Table 2). A typical agriculture field had two VDU grids (Fig. 5). Three trail cameras were placed on forest edges of each grid to assess the relative abundance of deer visiting VDU grids.

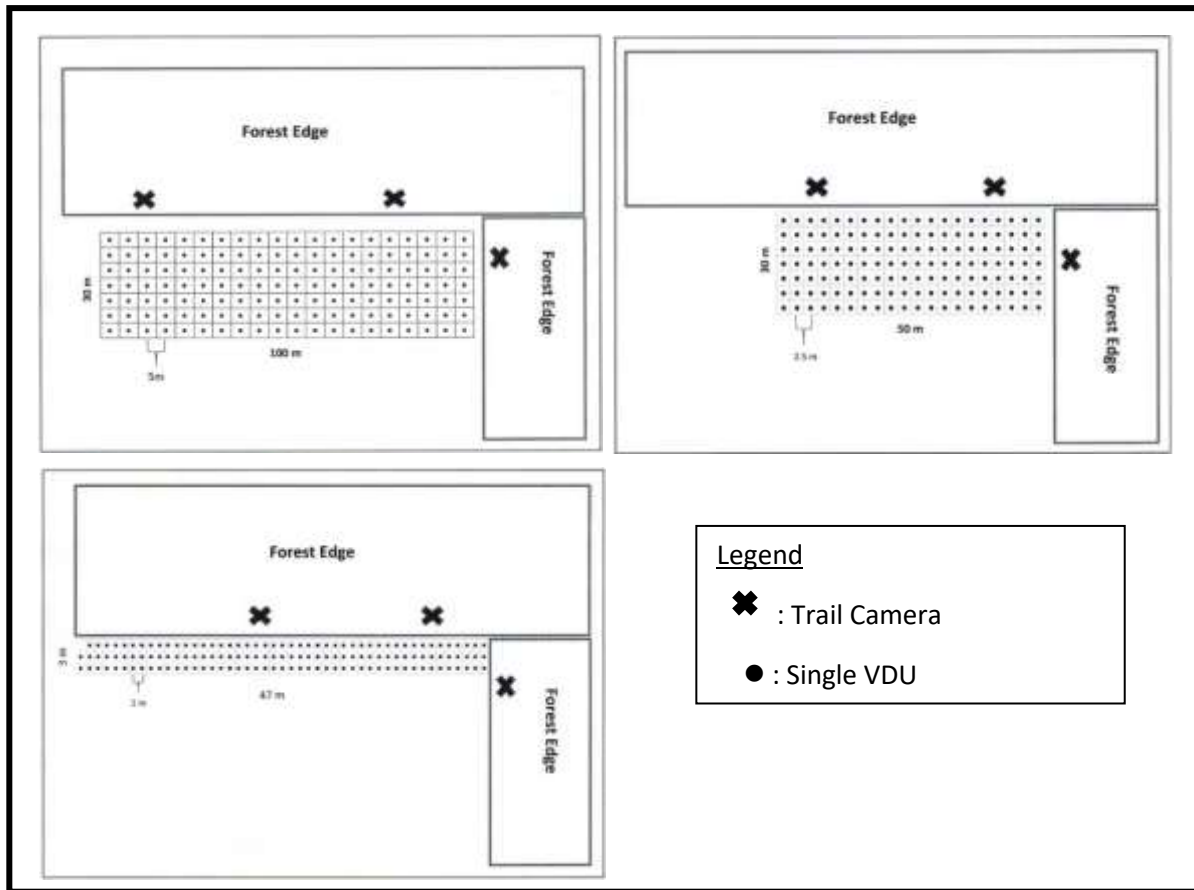


Figure 4. The design of all three vaccine delivery unit (VDU) grids placed on associated agriculture field next to forest edges. All VDU grids had 140 VDUs. In this example: 100-m x 30-m grid (top left), the 50-m x 30-m grid (top right), and the field edge grid (bottom left).

Table 2. Number of vaccine delivery unit grids and type of agriculture fields (corn, soybean, alfalfa) where vaccine delivery units were distributed from 1 May 2015 to 18 June 2015 in northeastern lower Michigan.

Vaccine Delivery Unit	# of VDU Grid Sizes			# of Agriculture Fields		
	100 m x 30 m	50 m x 30 m	Field Edge	Corn	Soybean	Alfalfa
Apple	4	7	6	2	4	2
Corn	2	4	1	3	0	2
Alfalfa	3	2	2	0	0	4
TOTAL	9	13	9	5	4	8



Figure 5. A representation of two vaccine delivery unit (VDU) grids on an agriculture field surrounded by forest edge in northeastern lower Michigan. Three trail cameras were placed on each VDU grid to evaluate deer visitation.

Trail cameras were used to further distinguish deer or non-target species visits (e.g. raccoons, turkeys (*Meleagris gallopavo*), and squirrels (*Sciurus spp.*). Cameras and VDUs were checked every 24-hours to determine consumption from the previous day. All missing VDUs were assumed to be consumed and were replaced. In 2015, all VDU grids were left on fields for five to 19 days depending on consecutive days of decreased consumption or an increase in non-target species activity.

White-tailed Deer Habitat Analysis

Following Braun's (1996) forest categories determined by MDNR 'Forest Classification', forest stands and herbaceous fields adjacent to VDU grids were categorized into five categories based on dominant overstory and/or understory plant species (Appendix B). These categories were upland hardwoods, lowland hardwoods, aspen (*Populus spp.*) and birch (*Betula spp.*), lowland conifers, and open herbaceous. Vegetation measurements were recorded in May and June 2015 and included absolute stem densities, vertical canopy cover (coniferous or deciduous), and presence of herbaceous cover.

Vegetation was measured within three 10-m x 30-m plots perpendicular to VDU grids beginning at the forest edge and stretching into adjacent cover types. All three vegetation plots were spaced evenly along the forest edge that were adjacent to the longest edge of the VDU grid (e.g. 50 m or 100 m side) (Fig. 6). All trees within the vegetation plots were counted and stratified into two size classes; >10 cm diameter at breast height (DBH) and <10 cm DBH. Measurements of the number of trees for each size class were averaged among all five cover types to calculate a stems per hectare (stems/ha) density measurement.

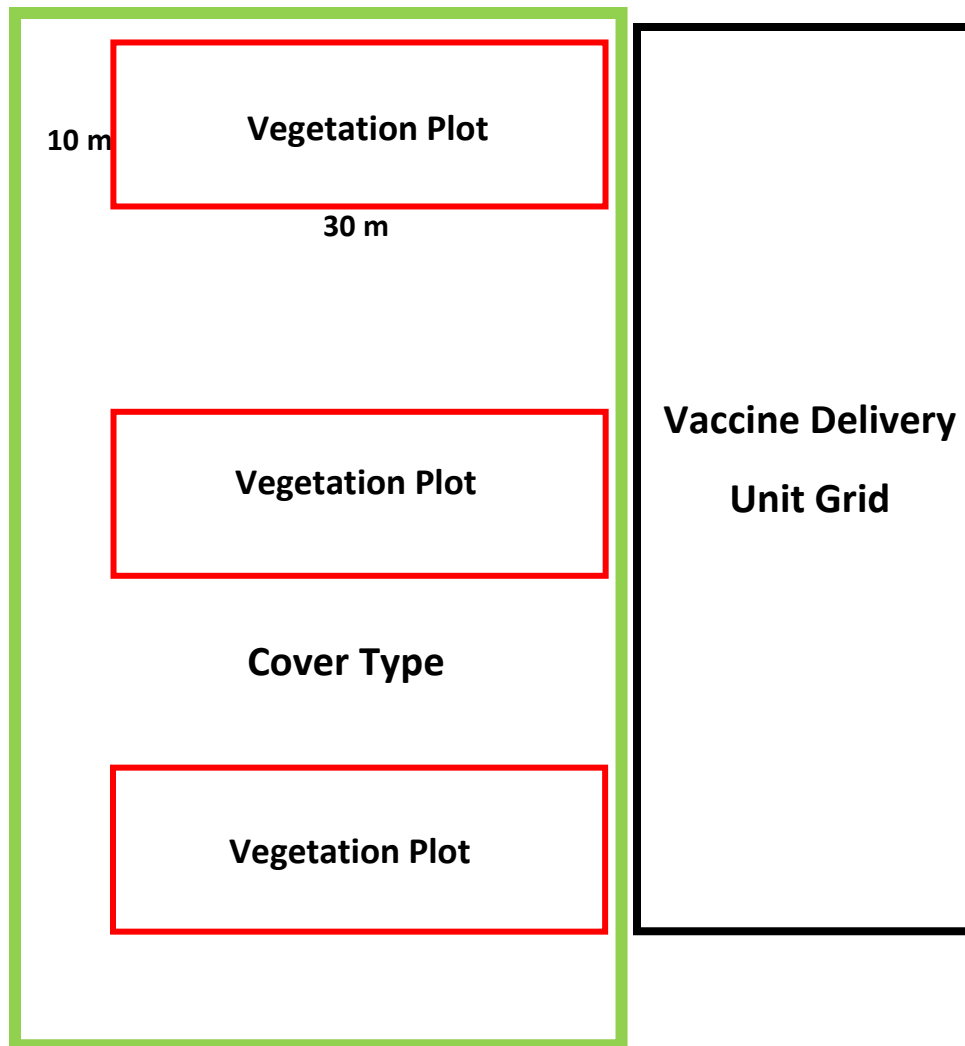


Figure 6. Diagram of three 10-m x 30-m vegetation plots in cover types next to vaccine delivery unit grids in agricultural fields.

Vertical forest canopy cover and herbaceous cover presence/absence were measured using three evenly spaced 30-m transects along forest edges adjacent to VDU grids. Presence or absence of herbaceous and overstory forest canopy cover were recorded every 5-m along transects. The types of cover (coniferous, deciduous, or none) were also recorded. All VDU development, deployment, and data collection were reviewed and approved by the Michigan State University Animal Care and Use Committee (AUF # 05/15-084-00; 29 April 2015).

Statistical Analysis

All statistical analyses were conducted using computing software R 3.2.2. A Kruskal-Wallis test (significance based on $\alpha = 0.05$) was used to compare differences in average consumption among VDU types and if any significant differences occurred in consumption of varying VDU grid sizes. Dunn's post hoc test was performed to determine where significant differences occurred among VDUs and grid sizes.

In addition, differences in deer consumption rates for each of the three VDU grid sizes were compared to distinguish the optimal grid size resulting in increased consumption. A Kruskal-Wallis test was used to determine differences in consumption between and among grid sizes for all three VDUs. Dunn's post hoc test with Bonferroni adjustment was used to distinguish where differences in consumption existed between the three VDU grid sizes.

Visitation by deer and non-target species (e.g. raccoons, turkeys, and squirrels) were compared by examining presence and absence data for each operable bait station night. An operable bait station night was defined as a 24-hour period on an individual VDU grid where VDUs were deployed. Visitation was determined by using trail camera data and comparing the number of operable bait station nights that had visitation by deer, raccoons, turkeys, and

squirrels. Deer abundance on a single VDU grid in 24-hours was estimated by assessing the highest number of deer photographed in a single trail camera frame. This method resulted in a conservative estimate of the highest number of unique deer to visit a VDU grid in 24-hours. The average of these conservative estimates was used to evaluate the mean number of deer present on agriculture fields next to all five cover types.

RESULTS

Vaccine Delivery Unit Development and Consumption

Average VDU consumption by deer throughout the field season differed significantly ($P \leq 0.001$) among the three VDUs (Fig. 7). The mean ($\pm$ SE) consumption of apple VDUs by deer was greater ($47.6 \pm 8.01\%$, $P \leq 0.001$) than alfalfa VDUs ($0.611 \pm 0.61\%$). However, there was no significant difference in consumption between corn VDU ($16.4 \pm 5.76\%$) and apple or alfalfa VDUs.

Vaccine Delivery Unit Distribution

The mean number of apple VDUs consumed by deer was significantly different among the three grid designs ($P \leq 0.05$). However, no significant difference occurred among grid designs of corn ($P > 0.05$) and alfalfa ($P > 0.1$) VDUs (Table 3). The mean consumption ($\pm$ SE) of apple VDUs by deer was significantly greater on the 50-m x 30-m grids (84.7 VDUs ± 16.9 , $P \leq 0.05$) than the 100-m x 30-m grids. However, there was no significant difference between apple VDU consumption on the 50-m x 30-m and field edge grids ($P > 0.3$). Among apple, corn, and alfalfa VDUs, the 50-m x 30-m grid had the greatest average number of VDUs consumed per 24 hours of 84.7 VDUs, 40.0 VDUs, and 4.3 VDUs, respectively, when compared to the 100-m x 30-m and the field edge grids (1 May 2015 – 18 June 2015). On the 50-m x 30-m grid the consumption rate for apple VDU was 32% and 57.4% higher than corn and alfalfa VDUs, respectively (Table 3).



Figure 7. Mean percent consumption ($\pm$ SE) by white-tailed deer for apple, corn, and alfalfa vaccine delivery units from 1 May 2015 to 18 June 2015 in northeastern lower Michigan. Different letter notations above SE bars indicate significant differences ($P \leq 0.05$) among VDU types using Kruskal-Wallis test with Dunn's tests including a Bonferroni adjustment.

Table 3. Average number of vaccine delivery units ($\pm$ SE) consumed in 24-hours by white-tailed deer per grid of 140 VDUs for all three grid designs during VDU distribution (1 May 2015 – 18 June 2015) in northeastern lower Michigan.

VDU	100 x 30-m Grid		Field Edge		50 x 30-m Grid		Chi Square χ^2	P value
	VDUs $\bar{X}$ (SE)	# Fields	VDUs $\bar{X}$ (SE)	# Fields	VDUs $\bar{X}$ (SE)	# Fields		
Apple	<u>15 ($\pm$ 3.87) ab¹</u>	4	<u>58.1 ($\pm$ 17.8)</u>	6	84.7 ($\pm$ 16.9) a	7	6.6	0.037
Corn	23 ($\pm$ 6.0) a	2	7	1	40 ($\pm$ 5.5) ab	4	4.82	0.089
Alfalfa	0 b	3	0	2	4.33 ($\pm$ 2.5) b	2	5.83	0.12
<i>P</i> -value	0.049		0.075		0.033			

*Underlining (within row only) denotes groups that are not significantly different at $\alpha = 0.05$.

¹Different letters (within column only) indicate significant difference ($\alpha < 0.05$) using Kruskal-Wallis test with Dunn test for multiple comparisons using Bonferroni adjustment.

Significant differences in consumption among all three VDUs were seen on the 100-m x 30-m grid ($P \leq 0.05$) and the 50-m x 30-m grid ($P \leq 0.05$) (Table 3). No significant differences occurred among VDUs in the field edge grids ($P > 0.05$). However, the highest mean consumption on the field edge grids was observed for apple VDUs (58.1 ± 17.8 VDUs). The highest average VDU consumption in 24-hours among all three grids was observed for apple VDUs on the 50-m x 30-m grids (84.7 ± 16.9 VDUs).

Deer and Non-target Visitation

During the surveillance and VDU distribution on our VDU grids (1 April to 18 June), the greatest number of total deer photographed each night across all VDU grids occurred in April ($n = 1,259$) with a decline in total deer photographed in May ($n = 608$) and June ($n = 195$) (Fig. 8). Among all grids, the highest number of individual deer seen within a 24-hour period was 108 deer in April with a range of 17-deer/24-hour to 108-deer/24-hour (median = 41) (Fig. 9). A decrease in individual deer photographed on VDU grids occurred in May ranging from 4-deer/24-hour to 39-deer/24-hour (median = 21) and in June ranging from 3-deer/24-hour to 27-deer/24-hour (median = 11). The mean number of deer observed per 24-hours over all VDU grids during the sampling period ranged from 0.3-deer/24-hour to 7.7-deer/24-hour (Fig. 9). The total number of unique deer observed over all VDU grids was 319 individuals. Relative individual deer abundance per VDU grids ranged from 2-deer to 22-deer/24-hour.

From 1 May 2015 to 18 June 2015, 11,098 single VDUs were distributed on 17 agriculture fields in Alpena County, MI. There were 274 operable bait station nights with visitation by deer, raccoons, turkeys and squirrels (*Sciurus* spp.).

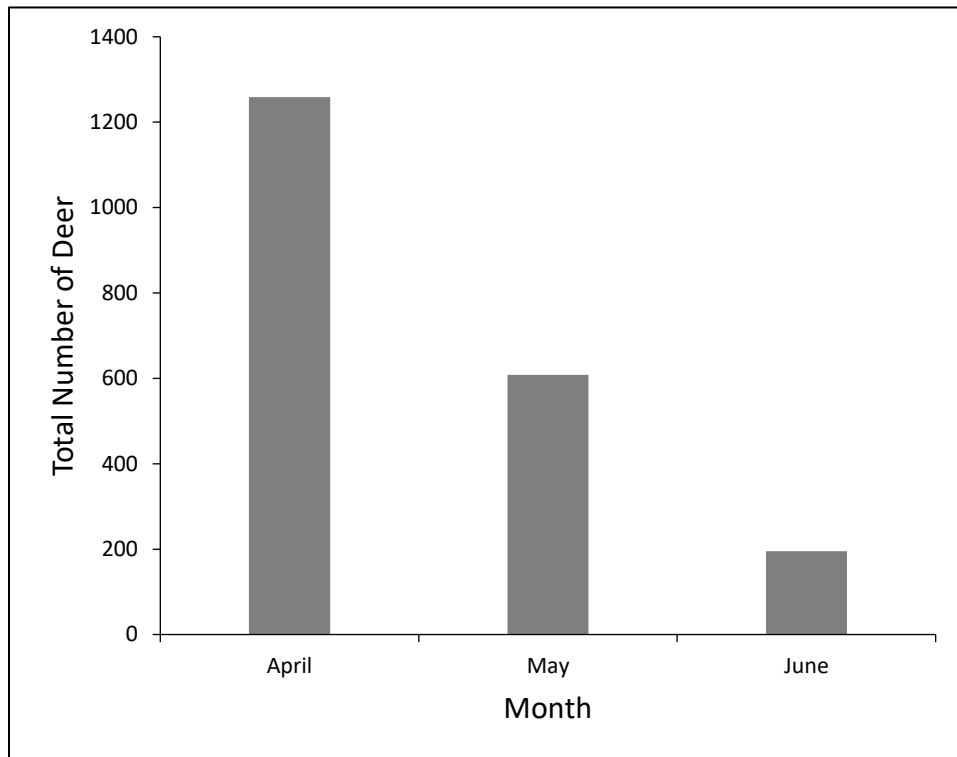


Figure 8. Total number of white-tailed deer photographed by trail cameras across all vaccine delivery unit grids from 1 April 2015 to 18 June 2015 in northeastern lower Michigan.

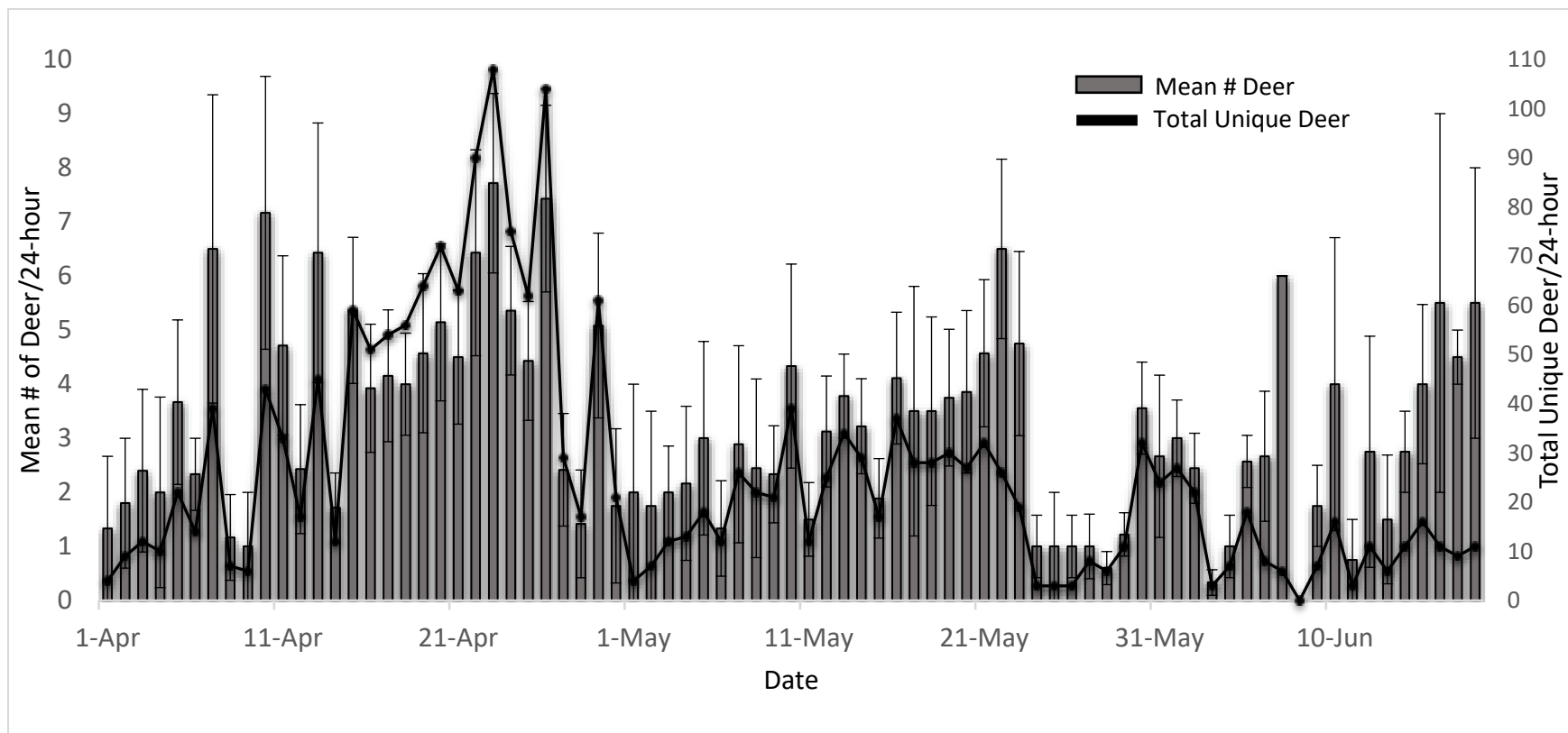


Figure 9. Mean number of deer ($\pm$ SE) photographed and number of unique deer seen in a 24-hour period across all vaccine delivery unit grids from 1 April 2015 to 18 June 2015 in northeastern lower Michigan.

An operable bait station night was defined by a 24-hour period of VDU deployment on one VDU grid. Visitation was defined by the presence of a species recorded with trail cameras during VDU deployment. Deer were the most frequently observed species accounting for 63.9% of the visits to VDU grids, with raccoons being the second most prevalent species visiting 52.6% of the total visits (Table 4). There was no significant difference in visitation between deer and raccoons ($P \geq 0.4$). Turkeys and squirrels accounted for 10.6% and 3.6% of the visits to VDU grids, respectively.

White-tailed Deer Habitat Analysis

Stands of lowland conifers had the lowest mean ($\pm$ SE) herbaceous cover ($38.1 \pm 4.7\%$) among the five cover types (Table 5). Woody stem density for trees <10 cm DBH for the lowland conifer stands ranged from 133.2 stems/ha to 577.2 stems/ha ($P \leq 0.03$) (Appendix C). Mean coniferous tree canopy cover in the lowland conifers was $87.3 \pm 16.8\%$ and was significantly different than the four other cover types ($P \leq 0.02$). Lowland conifer stands had the highest mean ($\pm$ SE) number of stems/ha >10 cm DBH (277.4 ± 69.3 stems/ha) when compared to the four other cover types.

The stem densities (i.e. stems/ha) of aspen/birch stands demonstrated a significant amount of variation among stands for trees <10 cm DBH ($P \leq 0.03$) and trees >10 cm DBH ($P \leq 0.03$), and for percent deciduous canopy cover ($P \leq 0.03$) (Appendix D). Aspen/Birch stands had the highest mean number of stems/ha <10 cm DBH (703.7 ± 179.1 stems/ha) when compared to the four other cover types.

Table 4. Visitation of target and non-target species on vaccine delivery unit (VDU) grids during vaccine delivery unit deployment (1 May 2015 to 18 June 2015). There was a total of 274 operable bait station (OBS) nights from 1 May 2015 to 18 June 2015.

Species	ALL VDUs		APPLE VDU		CORN VDU		ALFALFA VDU	
	# Nights	% Visited	# Nights	% Visited	# Nights	% Visited	# Nights	% Visited
Deer	175 a ¹	63.87	100 a	66.67	50 a	60.98	25 a	59.52
Raccoons	144 a	52.55	103 a	68.67	33 ab	40.24	8 ab	19.05
Turkeys	29 b	10.58	11 b	7.33	18 ab	21.95	0 b	0.00
Squirrels	10 b	3.65	0 b	0.00	10 b	12.20	0 b	0.00
Total (OBS)	274		150		82		42	
<i>P</i> -Value	<0.001		<0.001		<0.05		<0.01	

¹Different letters within a column only indicate significant difference ($\alpha < 0.05$) using Kruskal-Wallis test with Dunn test for multiple comparisons using Holm adjustment.

Table 5. Mean vegetation characteristics ($\pm$ SE) and average deer visitation per 24-hours associated with five cover types next to vaccine delivery unit grids in northeastern lower Michigan; 2015.

Cover Types	Average deer/24-hr	# of Fields	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous canopy cover	% Deciduous canopy cover	% Coniferous canopy cover
Aspen/Birch	16.4 c ¹	5	703.74 ($\pm$ 179.1)	57.72 ($\pm$ 27.9) a	62.9 ($\pm$ 4.6) ab	77.2 ($\pm$ 7.6) a	9.5 ($\pm$ 4.5) a
Lowland conifer	12.7 bc	3	362.6 ($\pm$ 128.4)	277.4 ($\pm$ 69.3) b	38.1 ($\pm$ 4.7) a	20 ($\pm$ 6.9) b	87.3 ($\pm$ 16.8) c
Open herbaceous	5 a	3	151.7 ($\pm$ 35.3)	25.9 ($\pm$ 3.7) a	100 d	15.9 ($\pm$ 6.5) b	11.1 ($\pm$ 7.8) ab
Lowland hardwood	7.38 ab	8	663.2 ($\pm$ 88.6)	160.9 ($\pm$ 30.4) bc	88.7 ($\pm$ 4.6) cd	80.9 ($\pm$ 3.1) a	35.1 ($\pm$ 8.2) b
Upland hardwood	6.2 a	5	643.8 ($\pm$ 105.1)	99.9 ($\pm$ 19.5) ac	81.9 ($\pm$ 6.3) bc	85.7 ($\pm$ 4.3) a	21.9 ($\pm$ 9.8) ab
<i>P</i> -value	0.036*		0.061	0.005*	0.002*	0.007*	0.021*

¹Means in a column with a different letter denotes a significant difference (post-hoc Dunn's test after Kruskal-Wallis test, $P < 0.05$)

*Significance denoted at $\alpha < 0.05$

Among all five cover types the lowest mean percentage of coniferous canopy cover was observed for the aspen birch cover type ($9.5 \pm 4.5\%$). Mean percent herbaceous cover was $62.9 \pm 4.6\%$ for the aspen/birch stands.

The highest percentage of herbaceous cover occurred in the open herbaceous fields (100%). Open herbaceous fields adjacent to VDU grids had significant variation in the percent coniferous canopy cover ($P \leq 0.02$) among individual stands (Appendix E). Besides open herbaceous fields, the highest mean ($\pm$ SE) percentage of herbaceous cover occurred in the lowland hardwood stands ($88.7 \pm 4.6\%$). There was a significant amount of variation among lowland hardwood stands for the density of trees <10 cm DBH ($P \leq 0.03$) and percent coniferous canopy cover ($P \leq 0.02$). (Appendix F). Upland hardwood stands had the highest mean ($\pm$ SE) percentage of deciduous canopy cover ($85.7 \pm 4.3\%$) among all five cover types. Upland hardwoods also showed a significant amount of variation among stands for the stems/ha <10 cm DBH ($P \leq 0.02$) and percent coniferous canopy cover ($P \leq 0.04$) (Appendix G).

Four out of five vegetation characteristics showed significant differences among all five cover types; density of trees >10 cm DBH ($P \leq 0.005$), percent herbaceous cover ($P \leq 0.002$), percent deciduous cover ($P \leq 0.008$), and percent coniferous cover ($P \leq 0.03$) (Table 5). The density of trees <10 cm DBH among the five cover types ranged from 151.7 ± 35.3 stems/ha (open herbaceous) to 703.74 ± 179.1 stems/ha (aspen/birch) did not vary significantly ($P > 0.05$).

The mean number of deer documented per 24-hours differed significantly ($P \leq 0.05$) among the five cover types. Vaccine delivery unit grids that showed the greatest use by deer

were adjacent to aspen/birch stands (mean = 16.4 deer/24-hour) and lowland conifer stands (mean = 12.7 deer/24-hour) (Table 5). The lowest mean number of deer were recorded on grids next to upland hardwood stands (6.2 deer/24-hour) and open herbaceous stands (5 deer/24-hour) (Table 5). The highest number of unique deer in 24-hours was observed for the aspen/birch stand (n = 22) and the lowland conifer stand (n = 17).

DISCUSSION

The incidence of bTB spillover from deer to cattle in NELM drives the research need for a potential oral vaccine distribution method in DMU 452. With the availability of BCG vaccine to combat bTB in wildlife, the design and application of an oral vaccine system that could reach a large proportion of deer remains the primary objective. Cross et al. (2007) explained two major obstacles in successful oral vaccination programs for free-ranging wildlife. First, the development of a vaccine delivery strategy that is able to reach the maximum number of the target population can be difficult. Second, managers need to develop a suitable VDU that is as species-specific as possible.

The highest mean consumption rate by deer in 24-hours was observed for the apple VDU ($47.6 \pm 8.01\%$) (Figure 9) which suggests it may be a suitable VDU to deliver the BCG vaccine to white-tailed deer. The relatively high consumption rate of the apple VDU contradicted our hypothesis that a VDU resembling the waste grain on agriculture fields would be consumed at a greater rate by deer. Several explanations may account for this higher consumption rate when compared to the alfalfa or corn VDUs: (1) the deployment of VDUs was after the initial thawing events of winter break-up (March and April) causing an increase in the availability of residual corn and alfalfa remaining in fields competing directly with our corn and alfalfa VDUs, (2) the increasing availability of natural forage in forests may also have competed with our VDUs as spring progressed (Table 5), (3) increasing spring temperatures degraded and dried out the alfalfa VDUs within 24-hours and (4) non-target species, specifically raccoons, were photographed visiting apple VDU grids (Table 4). However, when analyzing consumption

rates we relied on trail camera data to exempt daily consumption rates from VDU grids that were visited by raccoons.

The VDU distribution grids on agriculture fields influenced consumption rates by deer. The 50-m x 30-m VDU grid had the highest consumption rates for all three VDUs distributed (Table 3). These results can be attributed to three factors. First, the 50-m x 30-m grid most closely reflected the observed feeding pattern of deer on agriculture fields before VDU distribution. Before VDU distribution, deer were recorded with trail cameras filtering out of forest edges during winter break-up and moving toward thawed patches on agriculture fields. Deer remained close together as they moved to these small thawed patches. The 50-m x 30-m grid had VDUs spread 2.5-m apart, allowing deer to find several VDUs in a relatively small area. Second, on the contrary, the larger 100-m x 30-m grid had VDUs 5-m apart and was perhaps too far spread out for the deer to easily find. The 100-m x 30-m grid had the lowest consumption rate of VDUs for the apple and alfalfa VDUs (Table 3). Only a few individual deer were able to locate VDUs with the rest of the group unaware or unable to discover VDUs as they moved about the grid. Lastly, the field edge VDU layout was too easily accessible to non-target species as raccoons were frequently photographed consuming VDUs close to field edges. We recommend a modest liberal spreading of VDUs on the landscape, no more than 2.5-m apart, to avoid bait piling and to reflect the normal feeding behavior of deer on agriculture fields. Bait piling as defined by MDNR is the piling of baits in a 3-m by 3-m area (MDNR; 12 April 2017).

It is vital not to pile VDUs on the landscape as bait piling can lead to increased disease transmission (Garner 2001). Additionally, bait piling may decrease the probability deer would encounter VDUs in their daily movements during the winter break-up period (Fischer et al.

2016). Our VDU grids utilized a greater proportion of the agriculture field when compared to simply piling baits in a smaller area, perhaps resulting in increased probability of deer locating our VDUs. In addition, by having our VDUs liberally spread out across agriculture fields, we deterred deer from unnaturally congregating in one area. Our vaccine system also targeted deer that were already feeding on the available waste grain on agriculture fields. The construction of our VDUs also helped decrease the risk of disease transmission associated with bait piling. All VDUs were 'bite size' 17 g – 20 g units that enabled deer to consume the entire VDU. The small size of the VDU resulted in leaving little to no residual after consumption by deer. Only 6.3% of all VDUs that were consumed had left-over residue after consumption of that VDU (Dressel, unpublished data).

Ecology of deer in northern climates must be taken into great consideration as the objective of our placebo vaccine system was to reach the maximum proportion of deer. The degree of winter severity in NELM influences deer yarding and site fidelity activity. During the winter break-up period in the Upper Peninsula of Michigan, deer have high site fidelity to lowland conifer stands (Verme 1973) and this is also true during winters in NELM (Sitar 1996), resulting in increased use of agriculture fields adjacent to these cover types. The greatest number of deer overall were observed in April ($n = 1,259$) with a decrease in deer numbers as spring progressed (Fig. 7). Other researchers have observed the same trend leading to highest deer numbers on agriculture fields in the months of March and April in NELM (Sitar 1996). However, annual variations in temperature and snowfall will impact timing of dispersal during winter break-up. During April 2015 average temperatures in NELM were observed at 5.2°C with a below average snowfall of 5.1 cm. These mild winter conditions may have decreased deer site

fidelity to stands of lowland conifers and resulted in the first initial thawing events occurring in April 2015. It is possible that an earlier distribution of our VDU system in the months of March and April would have resulted in higher deer abundance being available to ingest VDUs on agriculture fields. This natural congregation of deer enables us a unique opportunity to target nutrient stressed deer (Delgiudice et al. 1990) coming out of lowland conifer stands with VDUs. In addition, implementing our vaccine system during the initial thawing periods of March and April may result in less competition of our VDUs with natural forage and non-target species, resulting in higher consumption by deer.

As spring progressed in our study there was a significant decline in the number of deer observed on agriculture fields and our VDU grids (Fig. 8). The decline in deer use of agriculture fields in late spring and summer has been observed in past studies (Sitar 1996). During late spring, snow cover decreases substantially, while the availability of natural forage increases. The addition of spring food resources influences migratory movement and dispersal of deer in NELM (Felix et al. 2007).

During our VDU distribution (1 May 2015 to 18 June 2015) animal visitation to our VDU grids did not significantly differ among deer and raccoons (Table 4). Raccoons visited 52.6% of the nights VDUs were distributed on fields potentially resulting in some consumption of our VDUs. Non-target species consumption may have a detrimental effect on the success of orally vaccinating the white-tailed deer in DMU 452 by decreasing the availability of vaccine units for deer. Deployment of a vaccination regimen during the first initial thawing events in NELM would decrease the probability of non-target species encountering the VDU grids. During our study, VDUs were distributed and monitored for five to 19 days depending on deer and non-

target species activity. Decreasing the number of consecutive days the VDU grids are deployed on fields may decrease the amount of time non-target species, specifically raccoons, have to discover and continually return to VDU grids. Non-target consumption of VDUs have been observed in several studies (Fletcher et al. 1990, Olson et al. 2000, Steelman et al. 2000, Campbell and Long 2007, Fischer et al. 2016) and continue to be a challenge for successfully distributing oral vaccines to free-ranging wildlife. Turkeys, squirrels, skunks (*Mephitis mephitis*), and coyotes (*Canis latrans*) were also observed on VDU grids but it is unlikely they substantially reduced availability of our VDUs.

Lethal removal of non-target species before a vaccination regimen has not been a plausible method to reduce non-target species consumption in previous studies. Removal of raccoons was attempted during an oral rabies vaccination trial for gray foxes (*Urocyon cinereoargenteus*) and resulted in a 33.3% increase in the visitation of raccoons after a seven day lethal removal period (Steelman et al. 2000). After removal of raccoons, surrounding males advanced to the vacant area increasing raccoon activity and presence. A more plausible method to reduce non-target visitation to VDU grids would be earlier seasonal deployment of the vaccine system when snow still remains and nightly temperatures are below -4.4°C. Raccoons may enter a state of 'semi-hibernation' or reduced activity level during these colder temperatures which will greatly reduce their activity on VDU grids (Sharp and Sharp 1956).

Deer abundance and consumption rates were greatly influenced by vegetation characteristics. The greatest number of deer observed occurred next to lowland conifer and aspen/birch forests. During winter months deer associate with lowland conifer stands (Nelson 1995). Lowland conifers provide thermal cover for deer during winter months (Felix et al. 2007)

and offer protection from wind and snowfall, helping deer conserve energy during harsh winter months (Verme 1965). In NELM 58% of deer are migratory, initiating winter migration in November and departing winter grounds in March and April (Sitar 1996). Deer may become nutrient stressed during these months (Delgiudice et al. 1990) and increase their use of agriculture fields during March or April, feeding on residual waste grain exposed by the melting of snow (Sparrowe and Springer 1970). The availability of residual crops may account for the increase in deer use of agriculture fields observed early in the month of April in this study. In addition, in NELM, Sitar (1996) observed higher than expected deer use of agriculture fields (40%) during early spring (March and April) due to the proximity of lowland conifer swamps. Our vaccine system can be used more efficiently by initially targeting agriculture fields that are adjacent to lowland conifers in providing sufficient thermal cover. Targeting these areas will enable wildlife managers to vaccinate the highest proportion of deer in an area during winter break-up.

Winter severity also plays a large role in the intensity of deer selecting lowland conifers during harsh winter conditions (Morrison et al. 2003). In NELM winter severity can influence the intensity of deer congregation in lowland conifer stands. However, a more flexible response by deer to winter conditions may result in less yarding activities than what is observed in the Upper Peninsula of Michigan (Van Deelan et al. 1998). In NELM lowland conifers still provide adequate thermal cover for deer during the winter and will influence site fidelity to these forest types. A severe winter that produces more snow (>214.12 cm) and lower temperatures (< -5.7°C) than normal (NOAA National Weather Service) may force a heavier concentration of deer

in lowland conifers and, therefore, increase the number of deer accessing agriculture fields during winter break-up.

Young aspen/birch stands (<10 years) provide abundant browse for deer during the spring (Felix et al. 2002). Migration to spring and summer ranges tend to be toward heavily forested areas and away from agriculture lands (Sitar 1996). High deer selection for aspen/birch stands were shown for previous studies as those forest types provide the necessary life requisites for spring food resources (Sitar 1996, Felix et al. 2002). The available spring foods may have contributed to relatively high deer numbers on agriculture fields associated with aspen/birch stands in this study. Targeting agriculture fields next to aspen/birch stands after the initial thawing periods of late winter will enable this vaccine system to reach migratory deer moving to spring food resources.

The lack of availability of herbaceous material measured in the lowland conifers ($38.1 \pm 4.7\%$) and aspen/birch stands ($62.9 \pm 4.6\%$) contributed to increased deer use of adjacent agriculture fields. In contrast, the higher herbaceous material measured for the upland hardwood stands ($81.9 \pm 6.3\%$) and open herbaceous fields (100%) contributed to minimal deer activity on associated agriculture fields. The increase of herbaceous material in forests had a large effect on decreasing consumption rates of our VDUs by deer.

By distributing VDUs on agriculture fields next to forest types with limited forage and increased deer activity, we can minimize waste and cost associated with production of VDUs. Careful consideration must be taken to develop VDUs that can be easily mass produced in a cost effective method. All VDU types in this study consisted of manipulating commercially available food products, limiting the cost and labor of VDU construction. A total of 11,072 VDUs

were distributed during the 2015 field season. The total cost of our vaccine deployment system was estimated at \$7,000 (operating cost, equipment, and supplies). We are aware that this cost estimate is only for our placebo vaccine system and will undoubtedly increase substantially when a vaccine is added into the system. Furthermore, the application of our vaccine system may occur at a larger spatial scale than our trial. Cost and physical labor can be greatly reduced if our vaccine system was mechanized and non-target species consumption was minimized.

Oral vaccination with BCG does represent the most cost effective method to administer the vaccine to a large proportion of white-tailed deer. There are several advantages to orally delivering BCG to the deer of NELM: (1) the ability to vaccinate the host from consumption of one VDU, (2) the relatively low cost in production of BCG (Waters et al. 2012) and (3) ease of incorporation of BCG into foodstuff. Compared to the trap and vaccinate method estimated cost of \$1.5 million annually (Cosgrove et al. 2012), an oral vaccine distribution method is extremely cost effective. However, a cost-benefit analysis should be performed to ensure the cost of an oral vaccine system outweighs the current social and economic cost of continued surveillance and testing of bTB in the area.

Vaccine unit waste did occur during our study as heavy rainfall was responsible for degrading VDUs, requiring a significant amount of replacement (~1,200 VDUs) on VDU grids. Deer avoided VDUs once they were exposed to heavy daily rainfall. After exposure to heavy rainfall, VDUs expanded, became very porous and fell apart. In May 2015 several heavy rainfall events were observed with a total monthly precipitation of 9.3 cm (NOAA National Weather Service). This deterioration may cause an issue with the use of an encapsulated vaccine and

result in significant waste. A VDU that can withstand weather events more efficiently would help reduce waste associated with weather.

Significant advances in the use of the BCG vaccine to protect deer against bovine tuberculosis have been demonstrated in numerous studies (Waters et al. 2004, Palmer et al. 2007, Nol et al. 2008, Palmer et al. 2009). *Bacillus Calmette-Guerin* vaccine has been shown to reduce disease severity by decreasing gross lesions and sites of infection (Palmer et al. 2007). In addition, deer vaccinated with BCG may be able to transmit BCG to non-vaccinated deer (Palmer et al. 2009). Vaccination of deer against bTB is a plausible method to reduce inter- and intra-species disease transmission.

Researchers have modeled that a vaccination coverage of 50% in deer of DMU 452 would have an 86% probability of eradicating bovine tuberculosis in the area (Ramsey et al. 2014). An increase in exposure rate of the vaccine would increase the probability of eradication; however, the success of a vaccination program relies on the continued use of additional management strategies; restrictions on baiting, liberal antlerless harvest, disease control permits, and potentially fencing around stored feed sources. These current management strategies have all aimed at reducing transmission, while simultaneously reducing the prevalence of bTB. The availability of an oral vaccine system would be another wildlife management tool to decrease prevalence of bTB in the deer of NELM.

Additional research should investigate and optimize the proportion of deer that could be inoculated with a vaccine using this oral delivery method. Further studies should include the use of a biomarker or vaccine to evaluate total coverage of the vaccine system. In addition, researchers should explore the viability of BCG in the environment and different strategies to

encapsulate and assimilate BCG into VDU products. Our study demonstrated that targeting nutrient stressed deer during the winter break-up period in a northern climate may effectively reach a large percentage of deer with an oral vaccine unit. Oral vaccination has the potential to become the most cost effective and practical method to inoculate the greatest proportion of deer with a vaccine.

CHAPTER 2

INTRODUCTION

Bovine tuberculosis (bTB) is an infectious disease caused by *Mycobacterium bovis* (Karlson and Lessel 1970) and is maintained in several wildlife reservoirs including European badgers (*Meles meles*) in the United Kingdom (Delahay et al. 2007), brushtail possums (*Trichosurus vulpecula*) in New Zealand (Coleman and Caley 2000), and wild boar (*Sus scrofa*) in Spain (Naranjo et al. 2008). In the United States, a wildlife reservoir of bTB is present in free-ranging white-tailed deer (*Odocoileus virginianus*) (hereafter referred to as 'deer') of northeastern lower Michigan (NELM) (Schmitt et al. 2007). The transmission of bTB from deer to cattle in NELM is a primary concern for wildlife managers, the agriculture industry and the public. Transmission can occur through direct cattle to deer contact and indirect contact of shared feed (Garner 2001).

Wildlife managers use several strategies to decrease the prevalence of bTB in deer of NELM and decrease exposure risk to cattle. Wildlife mitigation methods such as fences (Lavelle et al. 2015), increased antlerless harvest, restrictions on baiting (Hickling 2002), and disease control permits (DCP) issued to landowners and United States Department of Agriculture-Wildlife Services (USDA-WS) by MDNR have been used to decrease bTB transmission. The Michigan Department of Natural Resources (MDNR) has established Deer Management Unit 452 (DMU 452) to include the core area of bTB infection in deer (Fig. 10). Prevalence rate of bTB in DMU 452 fluctuated between 1% - 2% over the past 15 years (Okafor et al. 2011).

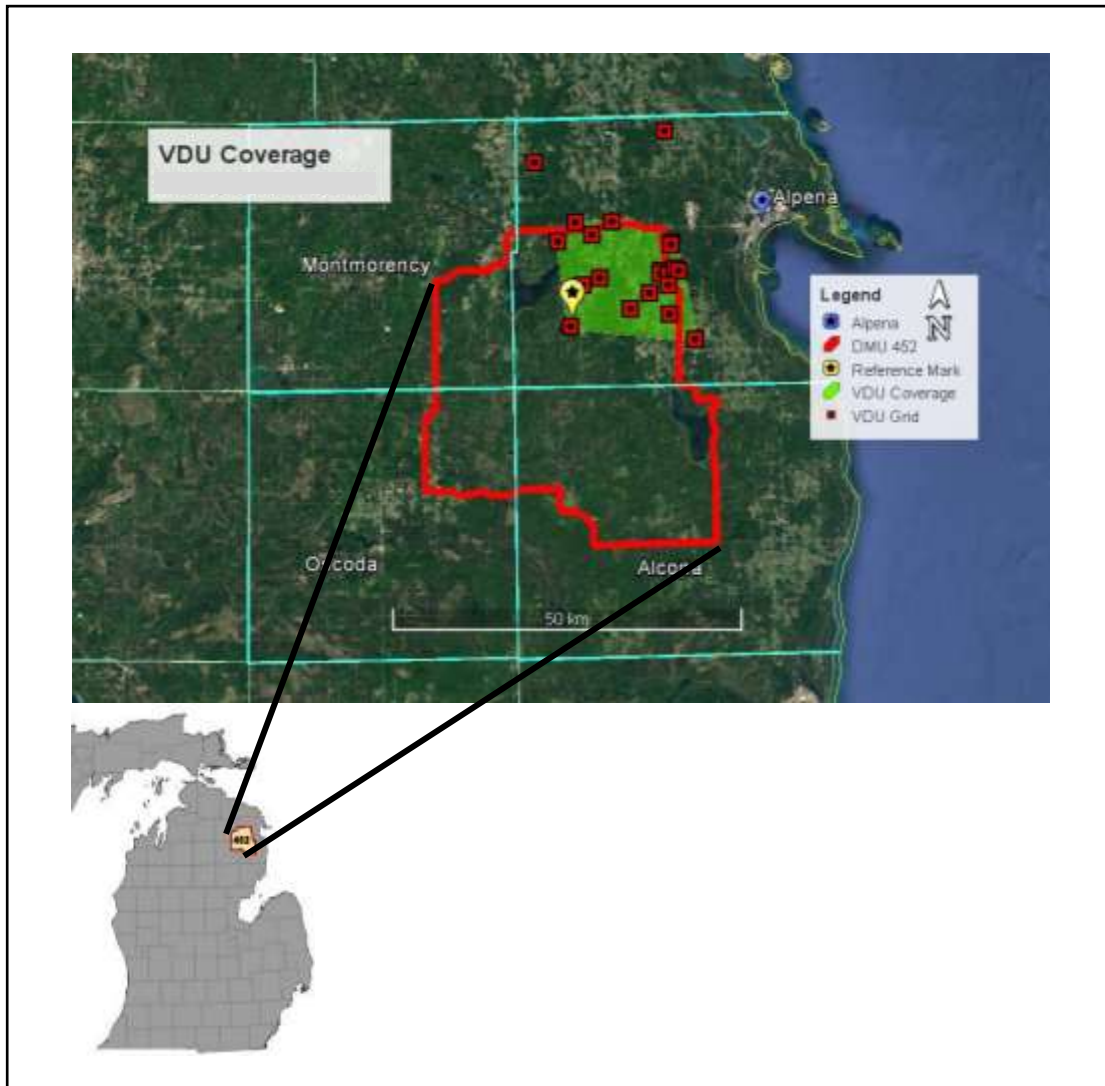


Figure 10. The 2016 placebo vaccination trial was performed in Alpena County, MI; the northeastern county of the core Deer Management Unit 452 from 7 February 2016 to 26 May 2016. Red outline represents deer management unit 452. Green polygon represents the majority of all placebo vaccine distribution sites deployed in 2016 (273 km²).

The continued transmission of bTB from deer to cattle and the stalled prevalence rate has given precedent for an additional management strategy to combat bTB in NELM.

Oral vaccination systems for wildlife may be a viable strategy for disease management and are becoming increasingly used to protect wildlife, livestock and people against disease transmission. The Oral Rabies Vaccination (ORV) program targeting raccoons (*Procyon lotor*) distributed over 50 million vaccine-laden baits across 15 eastern states of USA 1993-2003 and has been successful at preventing the spread of rabies (Slate et al. 2005). Current oral vaccination systems for bTB reservoirs are underway for badgers in south-east Ireland (Gormley et al. 2017) and brushtail possums (Nugent et al. 2016).

The bacillus Calmette-Guerin (BCG) vaccine has been successful at reducing bTB disease severity in penned white-tailed deer (Palmer et al. 2007). Deer that were intratonsilarly challenged with BCG had reduced gross lesions and persistence of BCG for 249 days. In addition, there is some evidence that deer are able to transmit BCG to unvaccinated deer. Oral vaccination of deer with BCG has also been shown to be superior in increasing antibody production when compared to administering the vaccine by injection (Miller et al. 1999). The efficacy of BCG to be administered orally allows for the opportunity to create a vaccination program for bTB in the deer of NELM.

With the availability of a vaccine to inoculate deer against bTB, the primary obstacle for a successful oral vaccination system is the design and application of a species-specific vaccine delivery unit that can be distributed to deer. Additionally, an oral delivery method may be the most cost-effective and feasible method to deliver a vaccine to a large number of deer. Before a bTB vaccination program in NELM can be initiated, understanding key components such as

palatability of VDUs, the proportion of deer that can be inoculated using a specific VDU and any non-target species consumption and impact must be investigated.

The use of a biomarker can enable researchers to address a number of these uncertainties. Rhodamine B (RB) has been used as an effective biomarker for several oral vaccination studies due to (1) the ability of RB as a systemic marker in whiskers and claws, (2) the instantaneous absorption of RB into keratinous tissues, (3) the ease of detection of fluorescent bands on whiskers with the use of a fluorescence microscope and (4) it is commercially available and relatively affordable. Rhodamine b has been used extensively in bait uptake studies of black-tailed prairie dogs (*Cynomys ludovicianus*) (Fernandez and Rocke 2011), raccoons (Fry 2010), mountain beavers (*Aplodontia rufa*) (Lindsey 1983), stoats (*Mustela ermine*) (Spurr 2002) and wild pigs (*Sus scrofa*) (Beasley 2015).

By distributing biomarker-laden vaccine units to free-ranging deer in NELM it may be possible to investigate the potential efficacy of a large scale vaccination program to combat bTB. Rhodamine B provides an opportunity to investigate the possible vaccine coverage of an oral vaccine system for this target population of deer. With the assimilation of RB into keratinous tissue we hope to detect individuals or the percentage of deer that consume biomarker-laden VDUs.

With current deer harvest rates and the baiting ban, eradication of bTB is extremely unlikely in the next 30 years (Ramsey et al. 2014). Even with a 100% compliance rate of the baiting ban there is only an 8% chance of bTB prevalence reduction without further intervention strategies (Ramsey et al. 2014). However, models have demonstrated a vaccine coverage of 50% in the deer of DMU 452 would have an 86% probability of bTB eradication

within 30 years (Ramsey et al. 2014). Thus, if further reduction or eradication of bTB in NELM is truly desired new management strategies must be explored. The objectives of this study were to investigate (1) the preference of deer for three VDU candidates, (2) an oral vaccine delivery system to reach the maximum proportion of the target deer population, (3) the efficacy of RB as an effective biomarker to quantify VDU uptake by deer, (4) the influence of vegetation characteristics on deer abundance on agriculture fields in late winter to early spring, (5) non-target species visitation to VDU grids and (6) the cost of a vaccine distribution system for deer.

STUDY AREA

We implemented our placebo vaccination trial from 7 February 2016 to 26 May 2016 in Alpena County of northeastern lower Michigan (Fig. 10). Alpena County is the northeast county of DMU 452, the established core bTB area with the highest prevalence of bTB in Michigan deer. To date, bTB has affected 68 cattle herds in the area (Legislative Report bTB program Jan 2017 Qtrly Update). Vaccine delivery units were distributed on 25 agriculture fields to evaluate VDU consumption and coverage of our placebo vaccine system.

Agriculture fields where VDUs were deployed consisted of harvestable crops containing either corn, wheat, alfalfa, or soybean. Average farm size in Alpena County was 61.1 ha with a total of 458 farms located in Alpena County (USDA Census of Agriculture). Top crop items produced in Alpena County consisted of hay and grass silage (8,030 ha), soybeans (2,258 ha), corn (2,146 ha) and wheat (1,152 ha). There were 189 farms in Alpena County with cattle and a total of 8,838 head of cattle. One-hundred and eleven of these farms were primarily beef cattle operations and another 37 farms contain mostly dairy cows.

Alpena County consists of 61% forested land and 24% agriculture land (USDA Natural Resources Conservation Service). Density of deer in Alpena County ranged from 10-14 deer/km² (O'Brien et al. 2011). Historically, deer density in this area has been as high as 18 deer/km² (Cosgrove et al. 2002). Normal average temperatures in the area range from -10° C to 13° C with an annual precipitation and snowfall of 71.3 cm and 203.9 cm, respectively. Elevation ranged from 175.9 m to 336.8 m above sea level. Moderately well drained, fine sandy loam soils make up a majority of the landscape and support northern red oak (*Quercus rubra*), sugar maple (*Acer saccharum*), American basswood (*Tilia americana*), white ash (*Fraxinus*

americanas), bigtooth aspen (*Populus grandidentata*), red maple (*Acer rubrum*) and eastern hemlock (*Tsuga canadensis*) (USDA Natural Resources Conservation Service). Lowland conifer stands are an important resource in providing deer necessary thermal cover during winter and are comprised of northern white cedar (*Thuja occidentalis*), black spruce (*Picea mariana*), tamarack (*Larix laricina*), white spruce (*Abies balsamea*-*Picea glauca*), balsam fir (*Abies balsamea*) and jack pine (*Pinus banksiana*) (Appendix B; MDNR Forest Classification).

According to Sitar (1996), approximately 58% of the deer in this region of Michigan are migratory. The majority of migratory deer (>80%) leave winter ranges by 1 May depending on environmental conditions (Sitar 1996). During spring migration, migratory deer typically move to more heavily forested areas and away from open-agriculture lands, however, 45% of deer may establish summer ranges near agriculture areas (Sitar 1996). In addition, non-migratory deer tend to establish home ranges in agriculture areas of NELM. Alfalfa fields remain an important food resource for deer during the spring and can contribute to significant loss of alfalfa production within 90 m of field edges (Braun 1996). Less crop damage was recorded for agriculture fields when spring food quality was higher in surrounding forest edges (Braun 1996).

METHODS

Turtle Lake Preference Trial

Development of Vaccine Delivery Units

From 30 January 2016 to 1 February 2016 preference testing of five VDUs was conducted at the Turtle Lake Hunt Club in NELM. VDUs consisted of three commercially available food products; (1) a corn-based VDU (Sportsman's Choice® Superior, WI), (2) an apple-based VDU (Purina® Harrisburg, PA) and (3) an alfalfa/molasses-based VDU (Chaffhaye® Dell City, TX) and two custom VDUs consisting of an apple VDU and a corn VDU (Fig. 11 and Appendix H). Big Tine Fortified Deer Blend (Scott Pet Products® Rockville, IN), a mixture of shell corn, blackoil sunflower seeds, cracked corn, Imperial 30-06 mineral/vitamin supplement, milo, dry molasses, cherry flavoring, and mineral oil was used as the base for both custom products. The base was broken up into smaller pieces by adding water and mixing with a food processor. Whole corn kernels and xanthan gum were added to the Big Tine Fortified deer blend to create the custom corn VDU. Ripe Apple Buck Jam (Evolved® Baton Rouge, LA), water, and xanthan gum was added to make the apple custom VDUs. All VDUs were then molded with a muffin baking sheet to create 17 g -20 g VDUs. All VDUs were dried with an oven dryer at 51.67 °C for 6-12 hours or until moisture dissipated out of the VDUs. Vaccine delivery units were then stored inside a chest freezer until use.



Figure 11. Five vaccine delivery units were tested for preference by deer in Turtle Lake Hunt Club from 30 January 2016 to 1 February 2016. *Left Column*: Commercial apple VDU (top left), commercial corn (middle left), and commercial alfalfa/molasses (bottom left). *Right column*: Custom corn (top right) and custom apple (middle right). (Photo Credit: David Dressel).

Deployment of Vaccine Delivery Units

Snow plows were used to remove snow and expose underlying vegetation from field edges. Vaccine delivery units were then distributed next to field edges of hardwoods in three 10-m x 45-m transects consisting of 45 VDUs spaced 1-m apart (Fig. 12). A competing VDU was deployed adjacent to the first grid along the same edge. This method was repeated for 10 separate sites allowing for all VDUs to compete for preference by deer. Additional single VDU grids were established next to hardwood forests without competing VDUs. Three trail cameras (Reconyx, RC60, Holmen, WI, USA) were installed on trees at each grid to record deer activity on VDU grids. Consumed or missing VDUs were recorded and replaced every 24-hrs.

Consumption rates were compared among all five VDUs (custom and commercial) to determine the most suitable VDU candidate for use across Alpena County, MI during winter break-up. Consumption rates were evaluated by comparing the total consumption among all three days of VDU deployment and if any VDUs were consumed more readily than others.

Vaccine System for White-tailed Deer in NELM

Vaccine Delivery Unit Development and Cost

From 6 March 2016 to 26 May 2016 three VDU products were evaluated as suitable vaccine delivery mechanisms for deer in NELM. The three commercially available food products consisted of an alfalfa/molasses-based VDU (Chaffhayes® Dell City, TX), a corn-based VDU (Sportsman's Choice® Superior, WI), and an apple-based VDU (Purina® Harrisburg, PA) (Fig. 13). All three VDU products were deconstructed with a ribbon mixer and then reconstructed around an encapsulated biomarker; rhodamine B. Manipulated VDUs consisted of the dry commercial food product, water, and xanthan gum as a binding agent (Table 6).

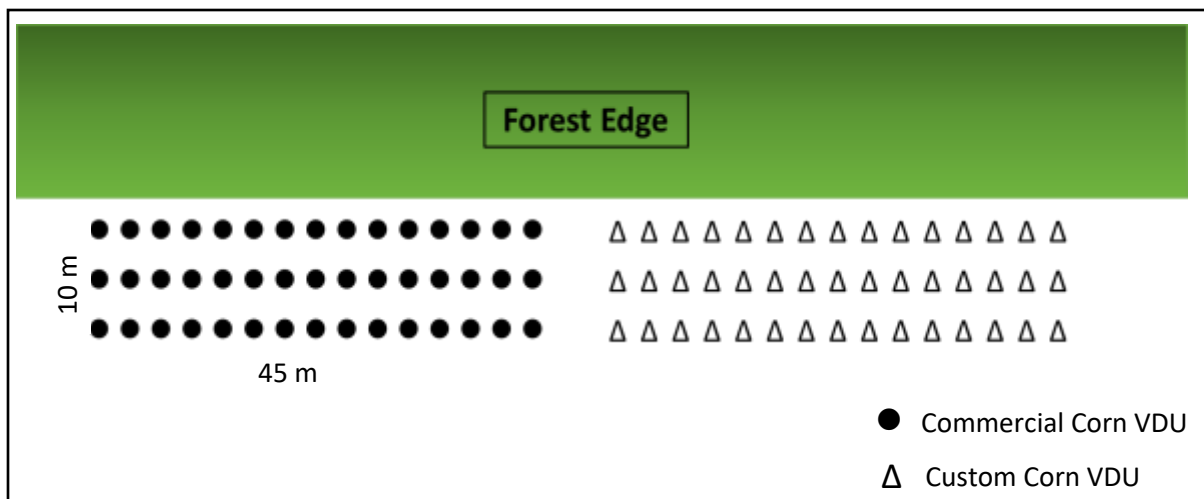


Figure 12. Distribution method for two competing vaccine delivery units deployed at Turtle Lake Hunt Club from 30 January 2016 to 1 February 1 2016.



Figure 13. The three vaccine delivery units (alfalfa/molasses-based, corn-based, apple-based VDUs) distributed to free-ranging white-tailed deer from 6 March 2016 to 26 May 2016 in Alpena County, MI. *(Photo Credit: David Dressel).*

Table 6. Recipe for modified commercially available vaccine delivery units (VDU). Xanthan gum was added as the binding agent to preserve consistency and shape of the final VDU. Single VDUs weighed between 17 and 20 grams.

Commercial Product VDU	Dry Ingredient (kg)	Corn Added (kg)	H2O Added (L)	Xanthan Gum Added (kg)	Ribbon Mixture Time (Min)
Apple	20.41	N/A	7.57	0.68	4
Corn	27.21	3.63	5.69	0.23	4
Alfalfa/molasses	22.68	N/A	5.69	0.68	4

A ribbon mixer was initially used to break up and mix all ingredients together. All ingredients were combined into the ribbon mixer and mixed for 4 minutes to ensure even distribution of ingredients (Table 6). After VDUs and ingredients were combined, each VDU was manually molded by hand into 17 g – 20 g “bite size” VDUs to reduce any residue after consumption by deer. Total time (min) and cost (\$) to construct VDUs were also recorded.

Rhodamine B was encapsulated in 00 size capsules (1.17cm x 2.02 cm) with the use of a manual capsule filling machine (CN-100CL, CapsulCN International CO. LTD, Ruian, Zhejiang, China). All capsules contained 476.5 mg of RB and were kept in gallon size Ziploc bags at room temperature. A single RB capsule was manually inserted into each VDU before deployment on agriculture fields (Fig. 14). Our method was chosen intentionally to mimic the future use of one dose of the BCG vaccine in a VDU. Ingestion of RB-laden VDU by deer will cause two staining events; (1) the oral (mouth, tongue) and internal cavity (rumen, intestine and digestive tract) of deer will be stained a fluorescent pink for 24-36 hours after consumption and (2) a fluorescent band will appear on deer vibrissae for up to 8 weeks (Phillips et al., USDA, unpublished data). The presence of oral, internal, or vibrissae staining will allow us to evaluate how many deer consumed at least one VDU.

Vaccine Delivery Unit Distribution and Consumption

Vaccine delivery units were distributed on 25 agriculture fields from 6 March 2016 to 26 May 2016 (Fig. 15). Proposed VDU sites were first chosen by using data from deer road surveys conducted in 2014 by USDA-Wildlife Services.

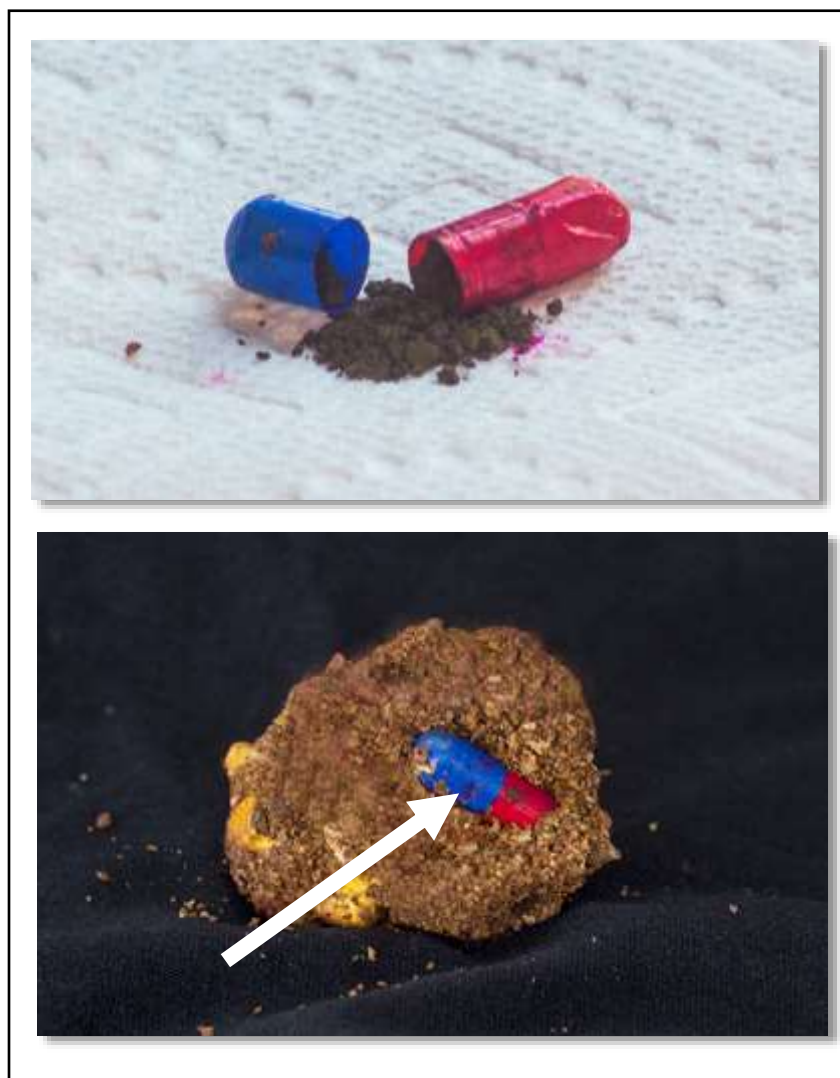


Figure 14. Rhodamine B (476.5 mg) was encapsulated into empty gelatin capsules (top). Rhodamine B capsules were then manually placed inside each vaccine delivery unit (bottom). *(Photo Credit: David Dressel).*

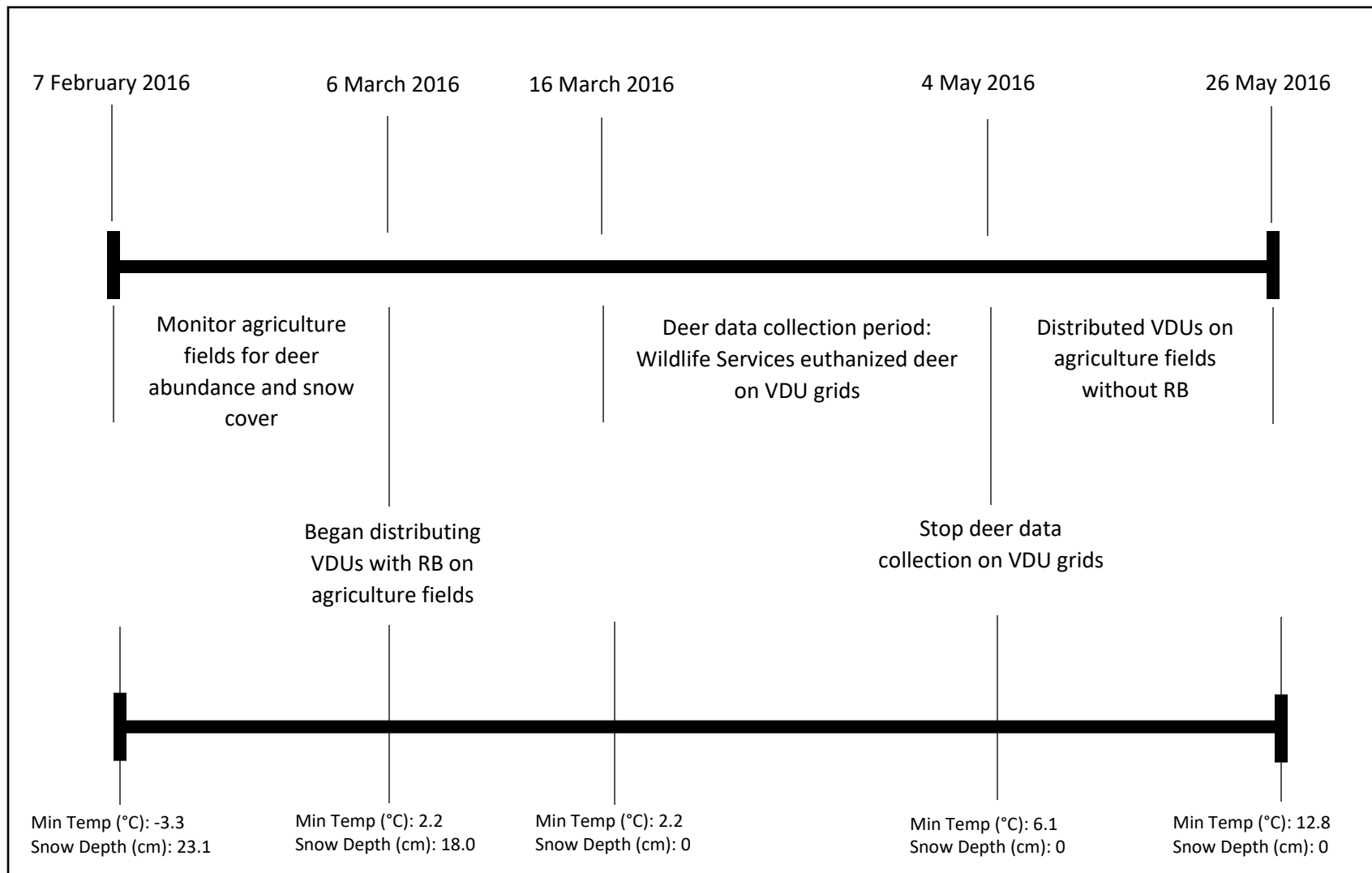


Figure 15. Timeline of 2016 placebo vaccination trial from 7 February 2016 to 26 May 2016 in northeastern lower Michigan and associated environmental conditions (i.e. minimum temperature and snow depth).

Vaccine delivery unit sites were further chosen based on landowners who had previously participated in USDA and National Wildlife Research Center (NWRC) deer bTB projects. Finally, specific agriculture fields were chosen based on the type of crop grown on the land during the previous year and anticipated deer activity by personal conversations with landowners and previous habitat quality research done in the area (Felix et al 2004).

Before VDUs were distributed on agriculture fields, all proposed fields were monitored with trail cameras (Reconyx, RC60, Holmen, WI, USA) from 7 February 2016 – 6 March 2016 (Fig. 15) to evaluate deer abundance. In addition, trail cameras and personal observations were used to assess snow cover and thawing events on agriculture fields. For this study, a thawing event was defined as an increase in temperature resulting in the thawing of snow covered fields, exposing patches of residual crop from the previous growing season. Vaccine delivery units were distributed on fields when the first initial thawing event was observed for Alpena County (28 February 2016; Fig. 16).

Vaccine delivery unit sites consisted of an agriculture field (wheat, soybean, alfalfa, or corn) with two 50-m x 20-m grids of VDUs with each single VDU spaced 2.5-m apart (Fig. 17). Each VDU grid had a total of 100 VDUs and was placed near associated forest or herbaceous stands. Three trail cameras were placed on field edges and took motion-activated and time-lapse (every 30 minutes) photographs. All VDU grids were deployed for 7 consecutive days on each agriculture field. During the first three days/nights VDUs without RB were distributed on agriculture fields. Subsequently, the following three days/nights consisted of VDUs with RB. Every 24 hours all VDUs that were missing were recorded and assumed eaten. At this time missing VDUs were then replaced.



Figure 16. The first thawing event in Alpena County, MI during 2016 winter break-up was observed on 28 February 2016. The natural congregation of deer on a thawed patch on an agriculture field initiated the distribution of vaccine delivery units in this study.

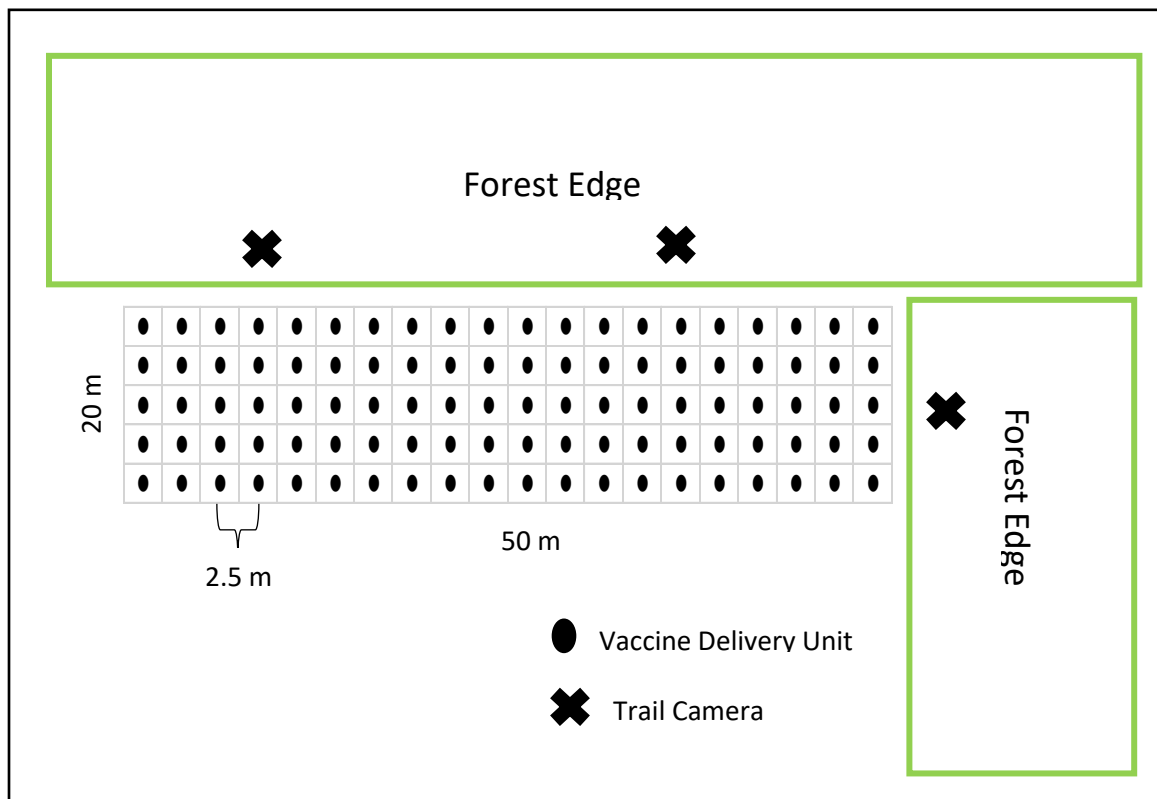


Figure 17. The design of the 50-m by 20-m vaccine delivery unit (VDU) grid placed on associated agriculture field next to forest or herbaceous edges. All VDU grids had 100 VDUs.

White-tailed Deer and Non-target Visitation

Images with the highest number of deer in a single frame during a 24-hour period were used to record the minimum number of individual deer visiting VDU grids. Vaccine delivery unit grid visits were recorded for all non-target species (raccoons, turkeys and squirrels) and deer by trail cameras. A VDU grid visit was defined as a 24-hour period on an individual VDU grid where VDUs were deployed. Visitations to VDU grids were compared using trail camera data and the percent of nights/days visited by deer, raccoons, turkeys and squirrels.

Biomarker Analysis

During the seventh night of VDU distribution, USDA-Wildlife Services personnel euthanized 1-13 deer on 17 of the agriculture fields with the use of MDNR Disease Control permits. Deer collection on VDU grids would continue each night until our target number of deer was met (target number of deer depended on landowner discretion and success rate). A total of 116 deer were euthanized on VDU grids from 16 March to 4 May 2016. We performed necropsies on all deer to assess the presence or absence of oral or internal cavity staining by RB. Rhodamine B was recorded present when staining was observed in the oral cavity or the digestive tract of deer.

Additionally, six maxillary vibrissae were pulled from each deer (three from each side of the mouth) using tweezers and immediately put into #7 coin envelopes. Deer number and VDU site were recorded on envelopes. Within 6 hours of initial vibrissae collection, all vibrissae were removed from the envelope, cleaned with a wet tissue and then transferred to a envelope. All vibrissae were then stored at room temperature away from direct sunlight.

All vibrissae analysis was conducted at the USDA/APHIS/Wildlife Services – National Wildlife Research Center (NWRC, Fort Collins, CO). Vibrissae were first mounted on a 75 mm x 25 mm Corning® microscope slide (three vibrissae on each slide) using a fluoromount™ aqueous mounting medium. A fluorescent microscope with a 100W mercury bulb and rhodamine B filter block (TRITC, Leica, Germany) was used to search for the presence of a fluorescent band on each vibrissae. If a fluorescent band was observed on ≥ 1 vibrissae, that deer was marked positive for VDU consumption. A total of 726 deer vibrissae were analyzed for RB presence/absence. All vibrissae analysis was conducted after consultation and training with USDA-NWRC laboratory specialist (20 June 2016; Heather Sullivan).

White-tailed Deer Habitat Analysis

Vaccine delivery units were distributed on agriculture fields adjacent to one of five cover types. Cover types were classified as upland hardwoods, lowland hardwoods, aspen (*Populus spp.*) and birch (*Betula spp.*), lowland conifers, and open herbaceous fields (Appendix B; MDNR Forest Classification). Three evenly spaced 30-m transects were established within deer habitat adjacent to fields perpendicular to VDU grids to evaluate (1) percent herbaceous cover, (2) percent deciduous canopy cover, and (3) percent coniferous canopy cover. Presence or absence of vegetation type (herbaceous, deciduous, coniferous) was recorded every 5-m along transects.

Furthermore, three 10-m x 30-m evenly spaced vegetation plots were placed in adjacent cover types perpendicular to VDU grids, and were used to record the number of trees present and stratified into two size classes; (1) stem density of trees with diameter at breast height (DBH) <10 cm and (2) stem density of trees with DBH >10 cm (Fig. 6). Tree counts were then

calculated to estimate stems per hectare in both size classes for forests next to VDU grids. Deer habitat use has been shown to be influenced by tree size class characteristics (Kearney and Gilbert 1976). All VDU development, deployment, and data collection were reviewed and approved by the Michigan State University Animal Care and Use Committee (AUF # 05/15-084-00; 29 April 2015; Amended 4 January 2016)

Statistical Analysis

Consumption by deer for all three VDUs was evaluated by comparing the average number of VDUs consumed during the 24 hours with the highest number of deer present on the agriculture field and the least number of non-target species (Dressel 2016; unpublished data). This method was used to best evaluate average consumption by deer without the influence of non-target species consumption.

Visitations between species were compared using a Kruskal-Wallis test (significance based on $\alpha < 0.05$) to determine the primary species visiting our VDU grids. A Dunn's post hoc test for multiple comparisons with holm adjustment was used subsequently to determine if/where differences occurred.

A Kruskal-Wallis test (significance based on $\alpha < 0.05$) with Dunn's post hoc test was used to compare differences in vegetation characteristic of the five cover types and mean number of deer/24-hrs visiting VDU grids adjacent to cover types. Differences in the number of VDUs consumed during non-RB nights and RB nights were conducted using a Wilcoxon Rank-Sum test (significance based on $\alpha < 0.05$) to determine if consumption was deterred by the presence of RB. Male and female deer differences in marked vibrissae were tested using a Pearson's Chi-

square test. All previous statistical analyses described were conducted using computing software R 3.2.2.

A multiple linear regression was used using SAS/STAT® software to evaluate the effects of (1) the number of individual deer observed in 24-hrs, (2) the percent (%) of herbaceous cover in adjacent cover types, (3) the percent deciduous canopy cover, (4) the percent coniferous canopy cover, (5) the stems/ha <10 cm DBH, (6) the stems/ha >10 cm DBH and (7) the date of VDU distribution on the consumption of VDUs in 24 hours (response variable). The significance of individual parameters (based on $\alpha < 0.05$) and the Akaike Information Criterion (AIC) were used in model selection. A separate regression analysis performed on each predictor variable suggested a log transformation on the number of VDUs consumed in 24-hrs (response variable) and the number of individual deer/24-hrs was appropriate based on higher R^2 values (Draper and Smith 1998). A constant of one was added to all response variable observations to allow for the log transformation of VDUs consumed. The final multiple linear regression equation was used to predict the number of VDUs that may be consumed by deer in 24-hrs given the individual variables measured.

RESULTS

Turtle Lake Preference Trial

A total of 1,789 VDUs were distributed during the three-day trial in Turtle Lake Hunt Club from 30 January 2016 to 1 February 2016. Vaccine delivery units were distributed adjacent to forest edges on 18 fields. On day one (30 January) the highest consumption of any VDU by deer was observed for the corn commercial VDU (56.1%) when compared to the other four VDU candidates (Table 7). On day two, the corn, apple and alfalfa/molasses commercial VDUs were consumed at 100.0%, 95.0% and 88.3%, respectively and were greater than the observed consumption for our two custom VDUs. On day three of the trial, all commercially available VDUs were consumed at 100% with the corn custom and apple custom VDUs consumed at 93.3% and 88.8%, respectively (Table 7). The number of individual deer observed per 24 hours on each site ranged from 9 - 27 deer/24-hours.

Vaccine System for White-tailed Deer in NELM

Vaccine Delivery Unit Development and Cost

The total time to produce 800 VDUs with RB ranged from 199 minutes (alfalfa/molasses VDU) to 239 minutes (apple and corn VDU; Table 8). Time estimates included the deconstruction of commercial food products, mixing ingredients, encapsulating RB, and adding RB capsules into VDUs. The total cost to construct 800 VDUs with RB of the alfalfa/molasses, corn and apple VDUs was \$140.71, \$153.70 and \$167.69, respectively (Table 9). There was an average of 417 VDUs distributed over the 7-day period on each agriculture field.

Table 7. Preference trial of five vaccine delivery units (VDU) distributed to deer on fields in Turtle Lake Hunt Club of northeastern lower Michigan from 30 January 2016 to 1 February 2016. Number of fields (n) where each VDU candidate was distributed is also shown.

VDU Candidate	30-Jan			31-Jan			1-Feb		
	n	# VDUs consumed/ # available	% VDUs Consumed	n	# VDUs consumed/ # available	% VDUs Consumed	n	# VDUs consumed/ # available	% VDUs Consumed
Alfalfa/Molasses	4	43/180	23.8%	4	159/180	88.3%	3	135/135	100.0%
Corn Commercial	4	101/180	56.1%	4	180/180	100.0%	3	135/135	100.0%
Apple Commercial	4	63/180	35.0%	4	171/180	95.0%	3	135/135	100.0%
Corn Custom	3	57/135	42.2%	3	96/135	71.1%	2	84/90	93.3%
Apple Custom	3	35/135	25.9%	3	85/135	62.9%	3	120/135	88.8%

Table 8. The amount of time (mins) for production of 800 vaccine delivery units (VDU) for the three VDU candidates tested in 2016. Time estimates include VDU production with and without rhodamine B.

VDU/Steps	Break Baits (min)	Ribbon Mixer/ add Ingredients (min)	RB Capsules (min) (800)	Construct VDU/ add Capsule (min)	VDUs Produced	Total Time (min)	Total Time w/o RB (min)
Alflafa/molasses (22.68 kgs)	5	4	60	130	800	199	139
Apple (20.4 kgs)	45	4	60	130	800	239	179
Corn (27.2 kgs)	45	4	60	130	800	239	179

Table 9. The total cost^a of production of 800 vaccine delivery units (VDU) for all three VDU candidates tested in 2016. Cost estimates include with and without rhodamine B and average cost per agriculture field (two 50-m x 20-m VDU grids) with VDUs distributed for seven consecutive days.

VDU/Ingredients	Product Cost (\$)	Xanthan Gum (0.68 kg) (\$)	Empty Gelatin Capsule (800) (\$)	Rhodamine B (800 capsules) (\$)	Cost w/o RB (\$)	Cost w/ RB (\$)	Average ^b VDUs per Field	Average ^b Cost (\$) per Field w/o RB	Average ^b Cost (\$) per Field w/ RB
Alfalfa/molasses (22.68 kgs) (1 Bag)	14.99	33.00	11.12	81.6	47.99	140.71	417	25.01	73.35
Apple (20.4 kgs) (3 Bags)	41.97	33.00	11.12	81.6	74.97	167.69	417	39.08	87.41
Corn (27.2 kgs) (2 blocks)	27.98	33.00	11.12	81.6	60.98	153.7	417	31.79	80.11

^aDoes not include labor costs.

^bDependent on VDU consumption by deer.

The average number of VDUs distributed on, though, was influenced by varying consumption rates of VDUs and thus has the potential to increase or decrease operationally. Agriculture fields that had alfalfa/molasses VDUs distributed cost an average of \$73.35 per field and was the least expensive when compared to the average cost for the corn VDU (\$80.11) and apple VDU (\$87.41) per field.

Vaccine delivery units were distributed on 16.9% of the total available agriculture land in our study area (273 km²). The total cost to distribute our VDUs on those agriculture fields from 6 March 2016 to 26 May 2016 was \$8,567.89 (VDU construction, salary, travel and operating supplies). Since the VDUs were placebos, our estimate does not include the cost of BCG vaccine.

Vaccine Delivery Unit Distribution and Consumption

A total of 8,636 VDUs were distributed to free-ranging deer in NELM during the 2016 field season. Twenty – one of the 25 agriculture fields were baited with the alfalfa/molasses VDU (Table 10). Four agriculture fields were baited with the apple VDU (two fields) and corn VDU (two fields) as it became evident early in the trial that the alfalfa/molasses VDU was preferred by deer (Table 10). The highest mean consumption per 24-hours was observed for the alfalfa/molasses VDU (48.2%, SE = 4.1) when compared to the corn (19%, SE = 9.0) and apple VDUs (25.5%, SE = 2.5; Fig. 18). All further analysis reported include only the VDU grids consisting of the alfalfa/molasses VDUs from 6 March 2016 to 26 May 2016. Fifteen of the 21 agriculture fields baited with the alfalfa/molasses VDU included the use of RB (Table 10).

Table 10. Number of agriculture fields and vaccine delivery unit grids deployed from 6 March 2016 to 26 May 2016 for all three vaccine delivery unit candidates.

VDU	# Agriculture Fields	# Agriculture Fields w/ RB	# VDU Grids	# VDU Grids w/ RB
Alfalfa/molasses	21	15	38	27
Corn	2	1	4	2
Apple	2	1	4	2
Total	25	17	46	31

Table 11. Total number of vaccine delivery units (VDU) distributed on 15 alfalfa/molasses biomarker fields from 6 March 2016 to 4 May 2016. The number of VDUs consumed on rhodamine B (RB) and non-RB nights are compared. The number of RB-laden VDUs consumed and then regurgitated by deer is also shown.

	# VDUs Distributed	# VDUs Consumed	% Consumed	# Capsules Consumed	% Capsules Not Ingested
W/RB	3511	2011	57.28	1314	34.7
W/o RB	3112	1811	58.19	-	-
Total	6623	3979	60.08	-	-
P-value	-	0.78	-	-	-

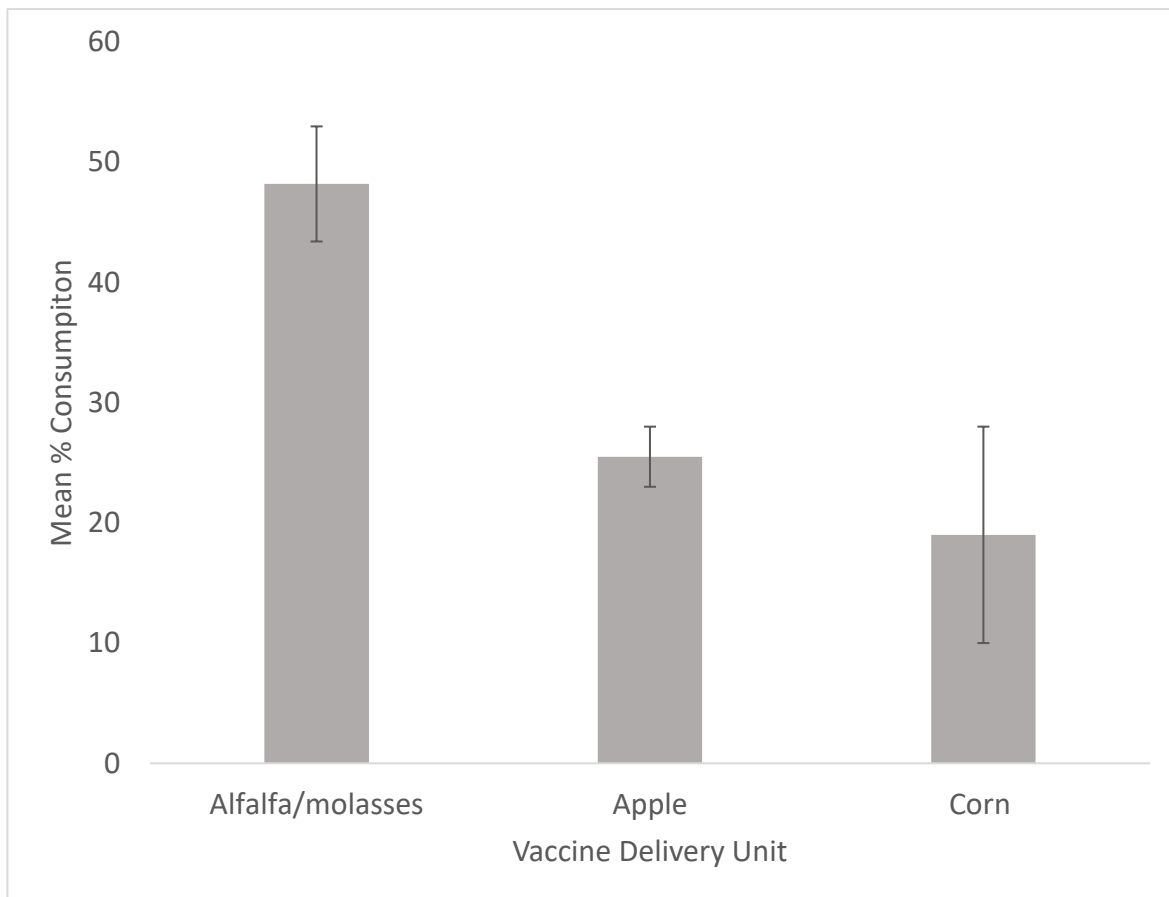


Figure 18. Mean consumption ($\pm$ SE) by white-tailed deer for the alfalfa/molasses, apple and corn vaccine delivery units from 6 March 2016 to 26 May 2016 in northeastern lower Michigan.

A total of 6,623 alfalfa/molasses VDUs were distributed on 15 agriculture fields with 3,511 VDUs containing the RB (Table 11). Deer consumed 2,011 VDUs containing RB (57.28%) and there was no significant difference between the number of VDUs consumed during non-RB and RB nights ($W = 152.5$, $P = 0.78$; Table 11). However, deer did spit out 34.7% of the consumed RB-VDUs; identified by the consumption of the VDU and not the RB-capsule.

White-tailed Deer and Non-target Visitation

From 7 February 2016 to 26 May 2016, twenty-five agriculture fields in Alpena County, MI were monitored for deer abundance using trail cameras. Deployment of VDUs on agriculture fields began on 6 March 2016. From 6 March 2016 to 19 March 2016 (early – mid March) the highest mean number of deer ($\pm$ SE) per 24-hours photographed on VDU grids was 13.8 ± 2.4 deer (Fig. 19). The highest total number of deer ($n = 543$) across all VDU grids were photographed from 20 March 2016 to 8 April 2016 (late March – early April; Fig. 20) with a mean of 9.9 deer/24 hrs (SE = 1.8; Fig. 19). A decrease in the total number of deer ($n = 361$) was photographed across all VDU grids from 9 April 2016 to 22 April 2016 (mid – late May) with a mean of 6.7 deer/24 hrs (SE = 1.9). As the season progressed from 23 April 2016 to 12 May 2016 (late April – early May) an increase in the total number of deer ($n = 478$; Fig. 20) and mean number of deer/24 hrs (9.1 deer, SE = 1.9; Fig. 19) were observed across VDU grids. From 13 May 2016 to 26 May 2016 (mid – late May) a drastic decrease in the total number of deer ($n = 132$) and mean number of deer (2.6 deer, SE = 0.4) was observed across VDU grids. A total of 404 individual deer were observed across all 25 agriculture fields (range = 2 – 45 deer/24 hrs). On alfalfa/molasses VDU fields only, there was a total of 361 individual deer photographed with 299 deer occurring on fields with RB (Table 12).

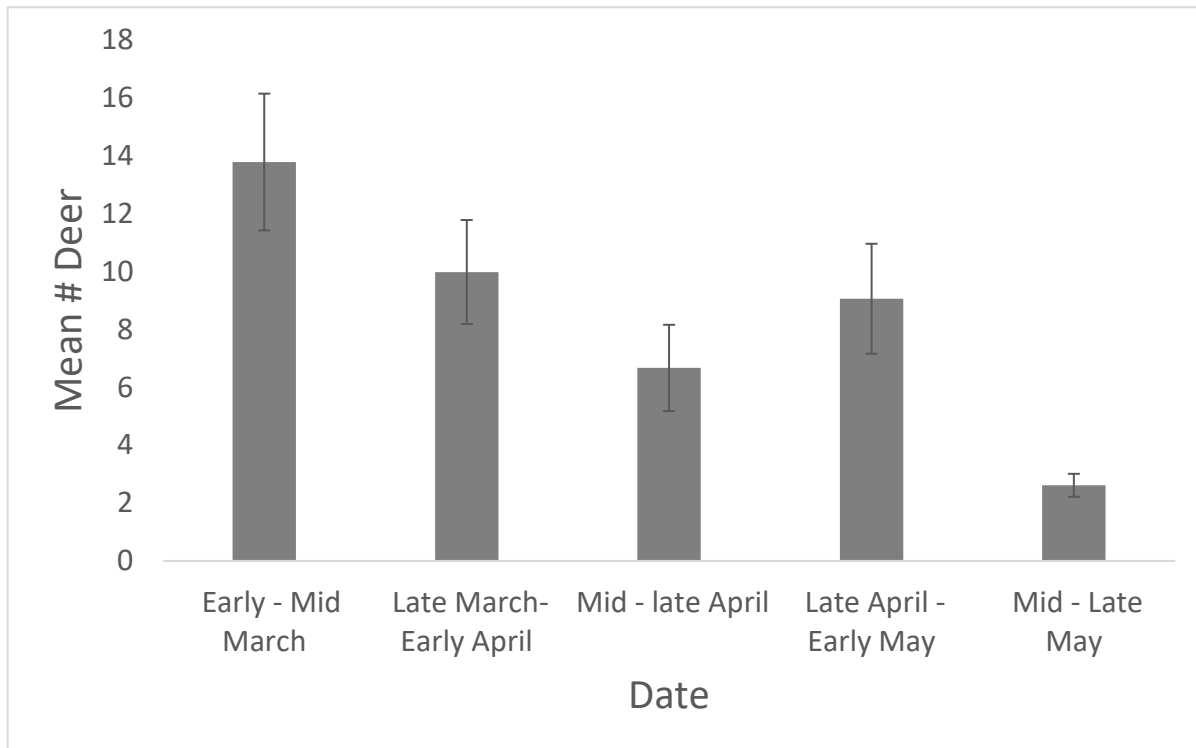


Figure 19. Mean number of deer ($\pm$ SE) photographed across all vaccine delivery unit grids from 6 March 2016 to 26 May 2016.

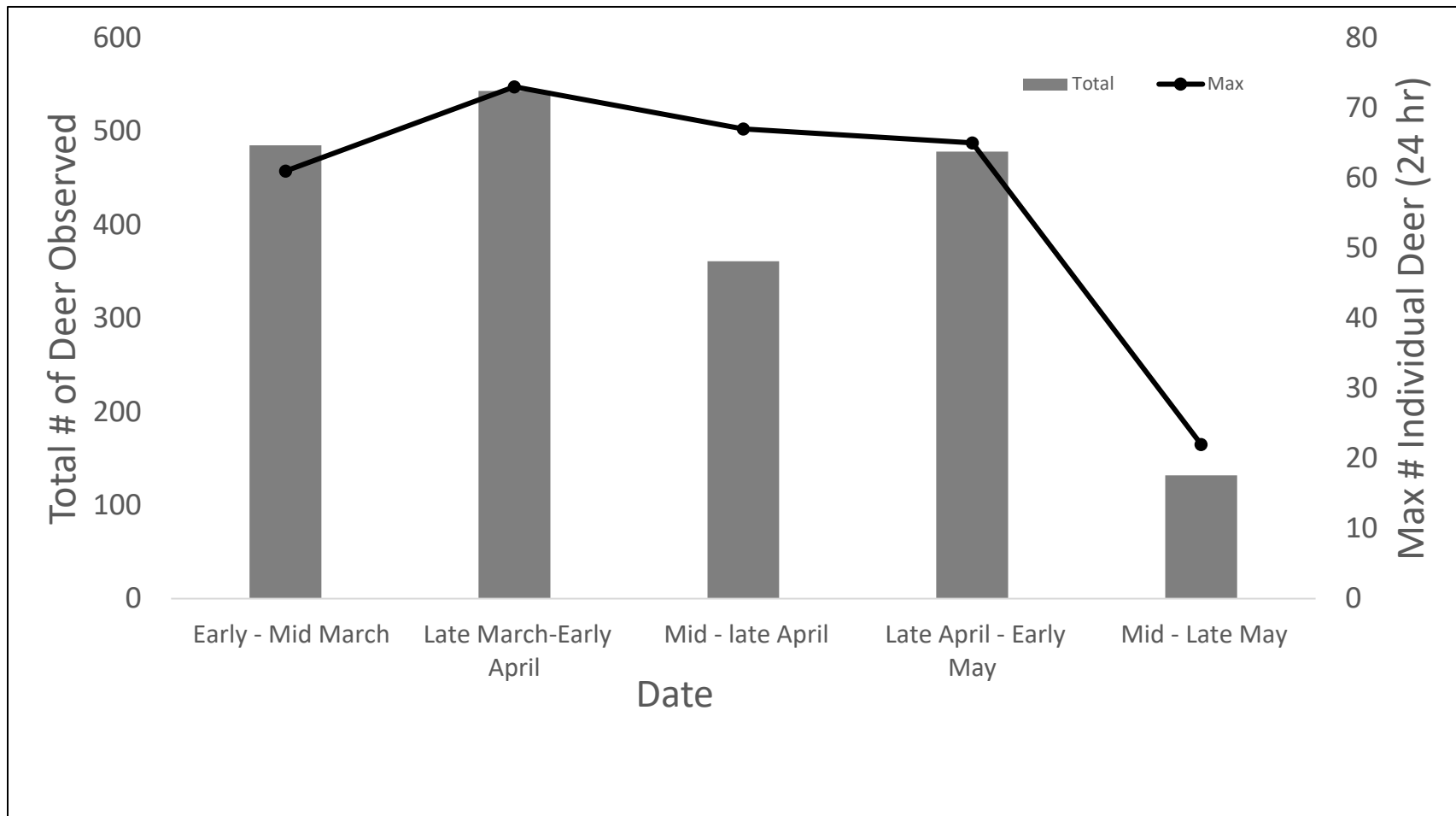


Figure 20. Total number of deer photographed on vaccine delivery unit (VDU) grids from 6 March 2016 to 26 May 2016 in northeastern lower Michigan. The highest number of individual deer photographed in 24 hours across all VDU grids is also represented.

Table 12. Number of individual deer photographed across alfalfa/molasses vaccine delivery unit grids with and without rhodamine B from 6 March 2016 to 26 May 2016 in northeastern lower Michigan.

	# of Fields	# Individual Deer
W/o Rhodamine B	6	62
With Rhodamine B	21	299
Total	27	361

There were 273 operable bait station nights on alfalfa/molasses VDU grids from 6 March 2016 to 26 May 2016. Deer were photographed during 87.9% of the operable bait station nights and were the primary species visiting alfalfa/molasses VDU grids ($P < 0.001$; Table 13). Raccoons were the second most prevalent species visiting 17.9% of the nights. Turkeys and squirrels were also photographed on alfalfa/molasses VDU grids visiting 10.9% and 1.8% of the operable bait station nights, respectively. There was no significant difference in the number of nights visited by raccoons and turkeys (Table 13).

Biomarker Analysis

From 16 March 2016 to 4 May 2016 (Fig. 15) 107 deer were euthanized by USDA personnel (males = 34, females = 73) on 15 agriculture fields containing alfalfa/molasses VDUs (Table 14). Internal cavity RB-marking was observed in 26.1% ($n = 28$) of the deer during necropsy (Fig. 21) and vibrissae RB-marking (Fig. 22) was evident in 64.5% ($n = 69$) of the deer (Table 14). A total of 74 deer (69.2%) were RB-marked either by internal cavity staining or vibrissae staining. There was no difference in RB-marking between males and females ($\chi^2 = 0.053$, $P = 0.82$; Table 14). There were five deer on two separate fields that showed two separated fluorescent bands on their vibrissae, indicating consumption during multiple RB-VDU days (Fig. 23). Comparing the number of individual deer photographed ($n = 299$) with the percentage of deer RB-marked on each field resulted in a conservative estimate of 198 deer (66.2%) consuming the RB-alfalfa/molasses VDUs (Appendix I). Vibrissae from five road killed deer in Alpena County were also analyzed for RB-marking with no deer showing RB staining.

Table 13. Species visitation to alfalfa/molasses vaccine delivery unit grids from 6 March 2016 to 26 May 2016. There were a total of 273 operable bait station nights with visitation by deer, raccoons, turkeys and squirrels.

Alfalfa/molasses VDUs		
Species	# Nights	% Visited
Deer	240 a ¹	87.91
Raccoons	49 b	17.95
Turkeys	30 bc	10.99
Squirrels	5 c	1.83
Total	273	
<i>P</i> -Value	<0.001	

¹Number in a column with a different letter denotes a significant difference (post-hoc Dunn's test with Holm adjustment after Kruskal-Wallis test, $P < 0.05$)

Table 14. The number of male and female deer euthanized on alfalfa/molasses vaccine delivery unit grids with rhodamine B marking in the internal cavity and vibrissae of deer.

	# Deer Euthanized	# Deer w/ Internal Marking	# Deer w/ Vibrissae Marking	# Deer marked (vibrissae or internal)	Total % Deer Marked
Male	34	10	22	23	67.6
Females	73	18	47	51	69.9
Total	107	28	69	74	69.2
Chi-Square (χ^2)	-	2.54	0.001	0.053	-
<i>P</i> - value	-	0.11	0.97	0.82	-



Figure 21. After a deer consumed a rhodamine B – laden vaccine delivery unit their oral cavity (left) and digestive tract (right) became stained pink. Deer internal cavity staining will remain for 24 – 36 hours after consumption. *(Photo Credit: David Dressel)*.

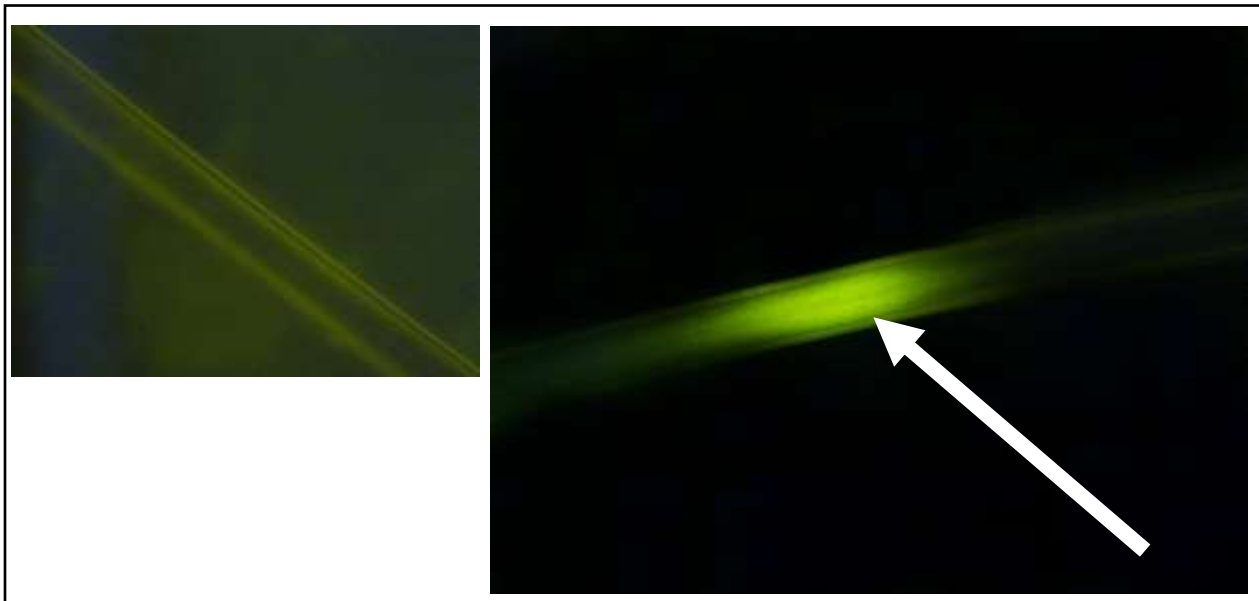


Figure 22. After consumption of a rhodamine B (RB) – laden vaccine delivery unit (VDU) a white-tailed deer's vibrissae contained a microscopic fluorescent band that was visible using a fluorescent microscope. The presence of a fluorescent band indicates that individual deer consumed at least one of the VDUs. *Left: non-stained vibrissae of deer; Right: stained vibrissae of deer indicated by fluorescent band. (Photo Credit: David Dressel)*.

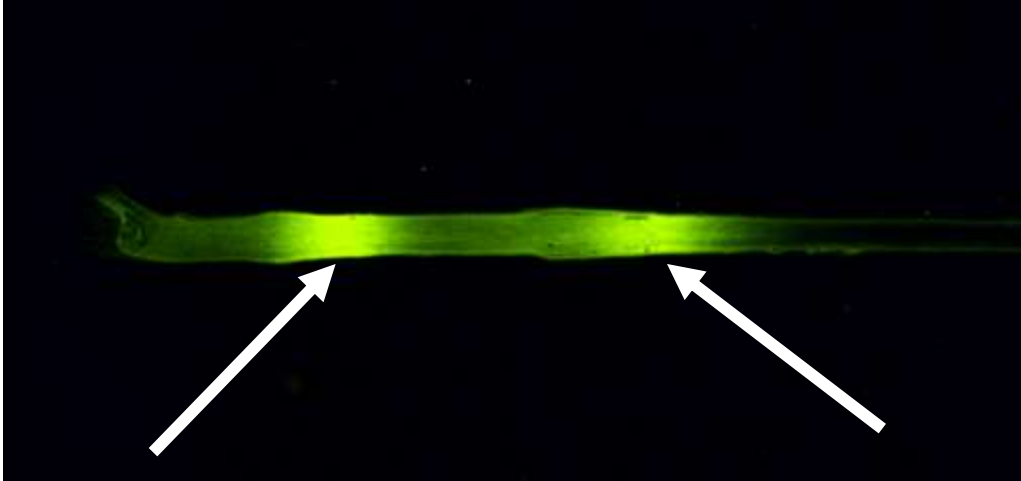


Figure 23. Five deer were observed with multiple fluorescent bands on vibrissae caused by consumption of more than one rhodamine B vaccine delivery units on separate days. (*Photo Credit: David Dressel*).

White-tailed Deer Habitat Analysis

Lowland conifer stands (Appendix J) had the lowest mean percentage of herbaceous cover of 20.2% (SE = 3.2) among all five cover types (Table 15). The mean number of deer visiting VDU grids adjacent to lowland conifer stands was 25.5 deer/24-hrs and was significantly higher than the four other cover types ($P < 0.03$). Vaccine delivery unit grids next to lowland conifers had the highest mean VDU consumption at 68.8% per 24-hrs and was significantly higher than VDU grids adjacent to the open herbaceous, lowland hardwood and upland hardwood cover types ($P < 0.02$). However, no significant difference of mean VDU consumption by deer occurred between lowland conifer and aspen/birch forests (Table 15).

Aspen/Birch (Appendix K) stands had a higher percentage of deciduous cover (84.5%, SE = 4.9) and number of stems/ha <10 cm DBH (666.0, SE = 63.2; Table 15) than the four other cover types. Mean number of deer photographed adjacent to aspen birch stands was 13.3 deer/24-hrs. Vaccine delivery unit grids next to upland hardwood stands (Appendix L) had the fewest mean deer visitation per 24-hrs (7.8 deer) and the lowest VDU consumption rate for 24 hrs (15.2%; Table 15). Open herbaceous fields (Appendix M) adjacent to VDU grids had a higher percentage of herbaceous material (88.1%, SE = 7.1) than the four other cover types and an average of 11 deer/24-hrs (Table 15). Vaccine delivery unit grids adjacent to lowland hardwood stands (Appendix N) had 11.3 deer/24-hrs and a mean consumption rate of 24.5% per 24-hrs (Table 15).

Table 15. Mean vegetation characteristics ($\pm$ SE), mean deer visitation per 24 hours, and mean vaccine delivery unit consumption per 24 hours associated with five cover types next to vaccine delivery unit grids in northeastern lower Michigan; 2016.

Cover Type	Mean # deer/24 hr	% Mean Consumption (24 hour)	# Fields	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover
Aspen/Birch	13.3 a ¹	43.5 ab	4	666.0 $\pm$ 63.2 a	80.5 $\pm$ 5.3 ab	51.2 $\pm$ 5.3 ab	84.5 $\pm$ 4.9 a	14.3 $\pm$ 1.7 a
Lowland Conifer	25.5 b	68.8 a	8	246.7 $\pm$ 35.0 b	431.5 $\pm$ 16.7 c	20.2 $\pm$ 3.2 b	22.0 $\pm$ 2.8 b	91.7 $\pm$ 1.7 b
Open Herbaceous	11 a	19.5 bc	2	210.9 $\pm$ 22.2 b	33.3 $\pm$ 11.1 a	88.1 $\pm$ 7.1 c	16.7 $\pm$ 2.4 b	26.19 $\pm$ 2.4 ac
Lowland Hardwood	11.3 a	24.5 bc	6	630.8 $\pm$ 37.2 a	159.1 $\pm$ 6.8 b	84.1 $\pm$ 3.2 c	83.3 $\pm$ 2.0 a	40.5 $\pm$ 4.0 c
Upland Hardwood	7.8 a	15.2 c	5	574.9 $\pm$ 65.3 a	170.94 $\pm$ 35.4 b	77.2 $\pm$ 2.3 ac	79.9 $\pm$ 5.1 a	37.1 $\pm$ 3.2 c
P-value	<0.03	<0.02	-	<0.01	<0.001	<0.001	<0.002	<0.001

¹Means in a column with a different letter denotes a significant difference (post-hoc Dunn's test after Kruskal-Wallis test, $P < 0.05$)

The regression model assessing the number of deer/24-hrs, the date of VDU distribution and vegetation characteristics (% herbaceous cover, % deciduous canopy cover, % coniferous canopy cover, stems/ha in size class <10 cm DBH and stems/ha in size class >10 cm DBH) showed a linear relationship to the number of VDUs consumed in 24-hrs ($R^2 = 0.67$). Four predictor variables were significant predictors of the number of VDUs consumed by deer in 24-hrs; (1) number of deer/24-hrs observed ($t = 14.53$, $P < 0.0001$), (2) percent coniferous canopy cover ($t = -2.97$, $P = 0.0033$), (3) stems/ha >10 cm DBH ($t = 2.76$, $P = 0.0063$) and (4) date of VDU distribution ($t = -2.67$, $P = 0.0080$) (Table 16). Based on the lowest AIC score (-102.38) and highest R^2 value ($r^2 = 0.68$) (Appendix O) for all subsequent models the best fitting model was chosen:

$$[1] \quad Y = 2.355 + 2.68(X1) - 0.514(X2) - 0.762(X3) - 1.924(X4) + 0.00321(X6) - 0.00732(X7)$$

Y = predicted number of vaccine delivery units consumed by deer
X1 = number of deer observed in 24 hours
X2 = % herbaceous cover in adjacent cover type
X3 = % deciduous canopy cover
X4 = % coniferous canopy cover
X5 = stems/ha in tree size class <10 cm DBH (not present in model)
X6 = stems/ha in tree size class >10 cm DBH
X7 = date of vaccine delivery unit distribution

The predictor variable stems/ha <10 cm DBH was not included in the best fitting model as suggested by AIC and R^2 values. Holding all predictor variables constant except for the number of deer observed (X1) we estimate that one deer consumed 2.68 VDUs on average.

Table 16. Regression analysis on the number of vaccine delivery units consumed in 24 hours by deer based on seven predictor variables. Analysis consisted of 234 operable bait station nights (OBS) and corresponding vegetation characteristics. All log transformed data is shown on observed scale.

Variable	Coefficient	SE	t Value	P-Value
Intercept	2.36	0.51	4.62	<0.0001*
Deer	2.68	0.18	14.53	<0.0001*
% Herbaceous Cover	-0.51	0.34	-1.52	0.13
% Deciduous Canopy Cover	-0.76	0.39	-1.94	0.054
% Coniferous Canopy Cover	-1.92	0.65	-2.97	0.003*
Stems/ha <10 cm DBH	-0.00011	0.00063	-0.17	0.87
Stems/ha >10 cm DBH	0.0032	0.0012	2.76	0.0063*
Date	-0.0073	0.0027	-2.67	0.008*
Full Model P = <0.0001				
N = 234 OBS				
Adj R ² = 0.68				

*Significance indicated at $\alpha < 0.05$

DISCUSSION

The Turtle Lake preference trial gave us a unique opportunity to explore deer preference for several different VDUs away from our main study area. The three-day trial demonstrated substantial consumption (>88%) by day three for all five VDUs emphasizing the value of pre-baiting to maximize consumption prior to introducing a vaccine. However, taking into consideration the increased labor to produce custom VDUs, we chose to use the three commercially available VDUs for further testing with RB to free-ranging deer in NELM.

Wildlife managers must take into consideration the efficacy of the methods and the cost associated with a vaccine distribution strategy in NELM. The alfalfa/molasses VDU was relatively inexpensive to produce and cost (\$) the least of all VDUs tested. With an average cost for the alfalfa/molasses VDUs per field of \$73.25 (for six days), the use of this vaccine system on the entirety of DMU 452 is a real possibility. The cost of deploying this oral vaccine system across DMU 452 (1,476 km²) would need to take into consideration the number of VDUs to distribute, the cost of the BCG vaccine, the spatial scale at which distribution would occur and the cost of specialized training needed to handle the BCG vaccine. The cost of an oral vaccine distribution system across DMU 452 would be substantially lower than the estimated cost for other management strategies of vaccine delivery (i.e. trap/vaccinate methods, \$1.5 million annually) (Cosgrove et al. 2012). We are aware that the cost estimate may increase when BCG is added to the VDUs but may still be very cost effective at 0.36 cents/dose (Cosgrove et al. 2012)

An alfalfa/molasses VDU distributed to deer in NELM during winter break-up has been shown to be a suitable VDU candidate to deliver a biomarker and potentially the BCG vaccine to

free-ranging deer. With a mean consumption rate of 48.2%/24 hrs by deer, the new alfalfa/molasses VDU had the highest average consumption rate when compared to the three VDUs tested in a 2015 preference trial (Dressel, unpublished data). In our study there was no difference in the number of non-RB and RB-laden vaccine units consumed by deer. Taste aversion to RB by deer, though, has been shown in other studies (Webb et al. 2000). However, 34.7% of RB-VDUs that were consumed by deer were regurgitated, indicating there may have been some taste or sense aversion to the RB or the capsule itself. It is possible that deer may have broken the capsule and received a minor dosage indicating obvious implications for an encapsulated BCG vaccine. Possibly, distributing RB-VDUs to nutrient stressed deer directly after winter resulted in the indiscriminative consumption of vaccine units with or without RB. After a heavy snowfall, there were several instances of deer returning to VDU grids and attempting to dig in the snow to possibly reach VDUs (Fig. 24). Higher consumption of the alfalfa/molasses VDUs than the corn or apple VDUs and apparent preference by deer makes the alfalfa/molasses VDU a suitable candidate to deliver a vaccine to deer if desired in the future.

Deployment of our vaccine delivery system during the initial thawing events in the area (6 March 2016) had drastic effects on the number of total and individual deer that visited our VDU grids. By deploying VDUs earlier in the winter break-up period (March and April), as opposed to May and June, we observed relatively more individual deer on our VDU grids compared to a 2015 trial (Dressel, unpublished data). As the season progressed fewer deer were observed on agriculture fields. The increase in alternative foods with warmer weather is responsible for increased deer dispersal.



Figure 24. White-tailed deer photographed by trail cameras returning to vaccine delivery unit (VDU) grids after heavy snowfall in April 2016. Deer are pictured digging through snow, apparently searching out alfalfa/molasses VDUs that are buried. This VDU grid had been operational for five days prior to this photo.

Deer target new sprouts for foraging, specifically in aspen/birch stands and upland mixed forest stands (Kohn and Mooty 1971). Warm weather provides an increase in herbaceous forage that is highly desirable and digestible by deer. The availability of highly nutritional browse will result in relatively less food needed by deer causing a decrease in forage intake (Moen 1978). The high nutrition value of new herbaceous growth may have competed directly with our VDUs in late spring. Deer also disperse from agriculture fields into more heavily forested areas by late spring and early summer (Sitar 1996) driven by vegetation types providing more food resources and requirements for fawning areas (Bahnak et al 1981; Felix 2004). This deer movement may signal the appropriate time to reduce or stop the proposed vaccination system on the landscape, as fewer deer will come into contact with VDU grids.

Substantial VDU loss by non-target VDU consumption was recorded in the 2015 vaccine delivery unit trials (Dressel, unpublished data) and would undoubtedly add to the cost of a vaccine delivery system. However, in this study, deploying our vaccine system with the alfalfa/molasses VDUs during the winter break-up period (March) significantly decreased the number of non-target species visiting our VDU grids ($P < 0.05$).

Possible vaccine coverage to free-ranging deer of NELM was demonstrated with using RB as a biomarker. Rhodamine B was an effective biomarker that allowed for easy detection of deer that consumed ≥ 1 VDU. As indicated with the use of RB-laden VDUs, 69.2% of the deer consumed a VDU from this placebo vaccine system. A vaccination coverage of 50% of the deer in NELM has been modeled to have an 86% probability of eradicating the disease in 30 years (Ramsey et al. 2014). This vaccination method demonstrates a possible vaccine coverage above 50% with the use of the alfalfa/molasses VDU during winter break-up in NELM.

Vegetation characteristics played a crucial role in the number of deer observed on adjacent agriculture fields and the mean consumption rates of VDUs by deer. Vaccine delivery unit grids adjacent to lowland conifer stands had the highest mean number of deer and average consumption of VDUs per 24-hrs relative to the four other cover types. The high winter thermal cover potential of lowland conifer stands may explain the increased abundance of deer adjacent to these cover types during winter break-up (Felix et al. 2007). The selection of lowland conifer stands by deer in NELM tends to increase during the winter months (Sitar 1996) and the intensity of site fidelity to these cover types may be driven by environmental conditions. Deer in Michigan have demonstrated high site fidelity to yarding areas associated with lowland conifer stands (Ozoga 1969; Verme 1973). As soon as environmental conditions permit, (i.e. decrease in snow cover and depth) deer will leave their associated yarding areas in search of food resources (Verme 1973). Deer metabolism also begins to increase with the initiation of spring (March and April) (Moen 1978), resulting in deer leaving yarding areas to feed on agriculture waste grain and alternative food resources. In addition, an increase in deer abundance on agriculture fields has been observed during spring months in NELM and may be a condition of the proximity of agriculture lands to lowland conifer stands (Sitar 1996).

A priority for developing a vaccine distribution system is creating a VDU that will be sought out and readily consumed by the target species. By understanding what conditions affect the number of VDUs consumed by deer, we can create a practical and cost-effective vaccine delivery system. Our regression analysis indicated that the number of deer and date of VDU distribution play important roles in determining how many VDUs will be consumed in a given 24 hours. Additionally, the percent of coniferous canopy cover and stems/ha >10 cm DBH

were also statistically significant predictor variables when attempting to predict the number of VDUs that will be consumed by deer. Our model predicts a negative relationship with the amount of coniferous canopy cover and the number of VDUs consumed by deer. Kohn and Mooty (1971) observed a similar relationship as deer avoided coniferous cover types in late spring and early summer and selected more deciduous and aspen/birch stands. The positive relationship between trees >10 cm DBH and increase VDU consumption is most likely a product of increased deer abundance adjacent to lowland conifer stands in early spring. Trees >10cm DBH provide more protection for deer during winter conditions than smaller trees (i.e. <10cm DBH) (Verme 1965, Felix et al. 2004). Deer were relatively more abundant on agriculture fields that were adjacent to these larger trees in this study.

When choosing the best model for VDU consumption prediction we included the percent of herbaceous cover in the adjacent cover type since this predictor variable did add to the overall accuracy of the model equation. As represented by the negative relationship of herbaceous cover and VDU consumption in this model, herbaceous cover is a good indicator if VDUs would be readily consumed by deer. A forest type with a high percentage of herbaceous cover would compete heavily with VDUs and would not be an efficient way of distributing VDUs if increased consumption by deer is the goal.

Perhaps the most useful component and most significant predictor variable on VDU consumption was the number of deer observed on an agriculture field in 24 hours. Our model predicts that for each increase in the number of deer, 2.68 VDUs are consumed. This ratio of 2.68 VDUs consumed by each deer is important for three reasons; (1) this ensures that deer are not over-consuming the VDUs and contributing to significant waste, (2) the majority of VDU

consumption is not done by one or two deer but the majority of deer visiting VDU grids and (3) a double dosage of the BCG vaccine has been shown to decrease disease severity (Palmer et al. 2007).

The relatively low cost of production, the relatively high consumption rates by deer, and the limited non-target visitation makes the alfalfa/molasses VDU an ideal candidate to deliver the BCG vaccine to free-ranging deer of NELM. With the use of a biomarker (RB) we demonstrated that by targeting deer on agriculture fields during winter break-up, it is possible to vaccinate a relatively large proportion of deer in a given landscape. Further research should aim to evaluate the efficacy of BCG vaccine insertion into these VDUs and the viability of distributing BCG to deer of NELM. Developing this vaccination system has shown it may be the most cost-effective strategy to deliver the vaccine when compared to other labor intensive strategies (i.e. trap and vaccinate) and should be used in collaboration with current wildlife disease mitigation strategies implemented in the area.

MANAGEMENT IMPLICATIONS

The oral vaccination of free-ranging white-tailed deer against bTB may prove to be the most ecological and cost effective method to deliver a vaccine to the greatest proportion of deer in NELM. The ability of wildlife managers to inoculate the highest number of deer remains a major obstacle in the successful application of a vaccine system. By using the strategies discussed in this study it may be possible to distribute a species-specific vaccine unit to deer. However, as we have shown, timing and location are essential to the success of the oral vaccine distribution system. Beginning the oral vaccination system during the initial winter break-up period will help ensure that the highest number of deer will come into contact with the vaccine system and consume VDUs. The start of winter break-up in NELM will undoubtedly vary annually given the environmental conditions but is easily observed by the snow melt on agriculture fields and by monitoring weather conditions.

As shown by this study, wildlife managers should initially target agriculture fields next to lowland conifer stands at the end of winter-early spring (March). Distributing VDUs next to lowland conifer stands will result in the highest consumption rates of VDUs by deer allowing for high numbers to be inoculated with the oral vaccine. In addition, this oral vaccination system may be used in a more precise and targeted application for specific areas or 'hot-zones'. These 'hot-zones' are defined by four characteristics (1) farms of multiple bTB infections, (2) locations of bTB positive deer, (3) lowland conifer stands in the surrounding area of bTB farms, and (4) agriculture areas that are adjacent to these lowland conifer stands. By specifically targeting areas that satisfy these four characteristics it is possible to use this oral vaccine system in a more deliberate and systematic nature to protect areas of multiple infection against bTB.

As spring progresses and herbaceous cover increases, deer move to deciduous forest cover types, specifically near aspen/birch stands to feed on browse and ultimately new herbaceous growth and leaves brought on by warmer temperatures (Kohn and Mooty 1971; Moen 1978; Sitar 1996; Felix et al. 2004). If wildlife managers wish to extend the duration of this oral vaccine system into late-spring (May), they should switch their focus to targeting agriculture fields near aspen/birch stands. This continuous and adaptive method will allow the oral vaccine system to target those deer with high site fidelity to lowland conifer stands and migratory deer moving to spring food resources in late spring.

The alfalfa/molasses VDU used in this study would be an effective and efficient VDU that could be used to distribute BCG to free-ranging deer in NELM. By following the recipes for alfalfa/molasses VDU construction, these VDUs can be easily mass produced within 24 hours of being distributed onto agriculture fields. Distributing alfalfa/molasses VDUs during the initial thawing events in NELM will enable the highest number of deer to consume VDUs. Specifically, during these thawing events agriculture fields adjacent to lowland conifer stands should be targeted. A two person team is capable of distributing VDUs on agriculture fields within 45 minutes. This systematic distribution of VDUs used in this study has the capacity to be mechanized with the use of ATVs or other forms of VDU distribution that would allow for several fields to be “baited” in 24 hours.

Bovine tuberculosis is a pervasive issue in NELM and the continued spillover to the cattle operations in the area poses a great economic and social constraint for many stakeholders. Management strategies have aimed at reducing deer numbers and decreasing contact rates of deer and cattle. Our proposed vaccine system could be an additional

management strategy that can be used to combat bTB in NELM and further reduce or eradicate the disease.

APPENDICES

Appendix A



RICK SNYDER
GOVERNOR

Bait Permit

STATE OF MICHIGAN
DEPARTMENT OF NATURAL RESOURCES
LANSING



KEITH CREAGH
DIRECTOR

March 31, 2015

Dr. Henry (Rique) Campa, III
Associate Dean, The Graduate School and
Professor of Wildlife Ecology
Michigan State University

Dear Dr. Campa:

This is a letter of authorization for your graduate students to conduct baiting for deer in the below specified area outside of normal baiting dates in the state of Michigan.

Specified Area:

Northeast lower peninsula, specifically Alpena County.

This baiting is for research purposes regarding the use of vaccine delivery systems for deer. This project will help determine a reliable, non-invasive biomarker to assess the uptake of bait and potentially a future bovine tuberculosis vaccine delivery system. Baiting for this project will be done between March 31, 2015 and June 30, 2016.

Please abide by statewide baiting regulations for bait limits (a maximum of two gallons of bait per location).

If you have any questions or concerns regarding this letter of authorization, please contact Dr. Steve Schmitt at the DNR Wildlife Disease Laboratory at 517-336-5040.

Sincerely,

Russ Mason, Ph.D., Chief
Wildlife Division
517-284-WILD

Cc: Mr. Gary Hagler, LED
Lt. James Gorno, LED
Mr. Rex Ainslie, WLD
Mr. Brian Mastenbrook, WLD
Ms. Jennifer Kleitch, WLD
Dr. Steve Schmitt, WLD

Appendix B

Vegetation Classifications

Vegetation Classification of Northeastern Lower Michigan created by Michigan Department of Natural Resources

Vegetation Category

Vegetation Species by Category

Open Herbaceous

Pasture, herbaceous plants

Upland Hardwoods

SUGAR MAPLE (*Acer saccharum*), RED MAPLE (*Acer rubrum*),
ELM (*Ulmus spp*), BEECH (*Fagus grandifolia*), YELLOW BIRCH (*Betula lutea*),
CHERRY (*Prunus spp*), BASSWOOD (*Tilia americana*), WHITE ASH (*Fraxinus americanas*),
and Oak (*Quercus spp*)

Aspen/Birch

TREMBLING ASPEN (*Populus tremuloides*),
BIGTOOTH ASPEN (*Populus grandidentata*),
WHITE BIRCH (*Betula papyrifera*)

Lowland Hardwoods

ASH (*Fraxinus spp*), ELM (*Ulmus spp*), SOFT MAPLE (*Acer saccharinum*), COTTONWOOD (*Populus deltoides*), BALM-OF-GILEAD (*Populus gileadensis*), QUAKING ASPEN (*Populus tremuloides*), WHITE BIRCH (*Betula papyrifera*),
OTHER LOWLAND HARDWOODS

Lowland Conifer

NORTHERN WHITE CEDAR (*Thuja occidentalis*),
BLACK SPRUCE (*Picea mariana*), TAMARACK (*Larix laricina*), BALSAM FIR-WHITE SPRUCE (*Abies balsamea-Picea glauca*), BALSAM FIR (*Abies balsamea*), JACK PINE (*Pinus bandsiana*)

Appendix C

Lowland Conifer 2015

Table 17. Vegetation characteristics for lowland conifer stands next to individual vaccine delivery unit grid sites. Also noted is the maximum individual deer for each VDU grid site observed by trail cameras and maximum consumption rate observed during a 24-hr period.

Site Name	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous Canopy Cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover	Max Individual Deer (24- hr)	Max Consumption (%) (24-hr)
CWG3	133.2 a ¹	177.6	42.8	33.3	80.9	17	100.0
SHH1	577.2 b	244.2	28.5	9.5	100	10	18.6
RozF1	377.4 ab	410.7	42.8	19	42.8	11	41.4
<i>Mean</i>	362.6	277.5	38.0	20.6	74.6	12.67	53.3
<i>P-value</i>	0.034*	0.073	0.293	0.104	0.558	-	-

Significant difference at $\alpha < 0.05$ denoted with ().

¹Different letter within column denote significant differences using post-hoc Dunn's test after Kruskal-Wallis test.

Study Site names available from authors: David Dressel, Henry Campa III

Appendix D

Aspen/Birch 2015

Table 18. Vegetation characteristics for aspen/birch stands next to individual vaccine delivery unit grid sites. Also noted is the maximum individual deer for each VDU grid site observed by trail cameras and maximum consumption rate observed during a 24-hr period.

Site Name	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous Canopy Cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover	Max Individual Deer (24- hr)	Max Consumption (%) (24-hr)
LSE2	421.8 a ¹	22.2 a	61.9	61.9 a	0	22	0.0
LSE3	421.8 a	22.2 a	61.9	61.9 a	0	13	75.7
CWG1	854.7 bc	55.5 ab	66.7	95.2 b	9.5	12	0.0
CWG5	477.3 ab	166.5 b	47.6	95.2 b	14.3	19	68.6
CWG6	1343.1 a	22.2 a	76.2	71.4 b	23.8	16	55.0
<i>Mean</i>	703.7	57.7	62.9	77.1 b	9.5	16.4	39.9
<i>P-value</i>	0.031*	0.035*	0.639	0.037*	0.09	-	-

Significant difference at $\alpha < 0.05$ denoted with ().

¹Different letter within column denote significant differences using post-hoc Dunn's test after Kruskal-Wallis test.

Study Site names available from authors: David Dressel, Henry Campa III

Appendix E

Open Herbaceous 2015

Table 19. Vegetation characteristics for open herbaceous stands next to individual vaccine delivery unit grid sites. Also noted is the maximum individual deer for each VDU grid site observed by trail cameras and maximum consumption rate observed during a 24-hr period.

Site Name	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous Canopy Cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover	Max Individual Deer (24- hr)	Max Consumption (%) (24-hr)
BradC2	222	22.2	100	9.5	33.3 a ¹	4	14.3
LSE5	122.1	33.3	100	4.7	0 b	3	23.6
LSE6	111	22.2	100	33.3	0 b	8	10.7
<i>Mean</i>	151.7	25.9	100.0	15.8	11.1	5	16.2
<i>P-value</i>	0.11	0.846	NaN	0.102	0.021*	-	-

Significant difference at $\alpha < 0.05$ denoted with ().

¹Different letter within column denote significant differences using post-hoc Dunn's test after Kruskal-Wallis test.

Study Site names available from authors: David Dressel, Henry Campa III

Appendix F

Lowland Hardwood 2015

Table 20. Vegetation characteristics for lowland hardwood stands next to individual vaccine delivery unit grid sites. Also noted is the maximum individual deer for each VDU grid site observed by trail cameras and maximum consumption rate observed during a 24-hr period.

Site Name	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous Canopy Cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover	Max Individual Deer (24- hr)	Max Consumption (%) (24-hr)
Bev J1	621.6 ab ¹	155.4	95.2	80.9	23.8 a	7	46.4
Bev J4	865.8 b	55.5	95.2	90.4	28.6 a	3	20.0
Bev J5	899.1 b	111	95.2	90.5	28.5 a	4	48.6
Bev J6	710.4 ab	277.5	76.2	71.4	52.3 ab	12	23.6
Bev J7	488.4 a	144.3	100	71.4	19 ac	8	29.3
LSE4	410.7 a	199.8	90.4	90.4	0 c	14	93.6
SH2	854.7 b	133.2	95.2	71.4	71.4 b	7	11.4
WB2	455.1 a	210.9	61.9	80.9	57.1 b	4	20.7
<i>Mean</i>	663.2	161.0	88.7	80.9	35.1	7.4	36.7
<i>P-value</i>	0.037*	0.06	0.122	0.264	0.028*	-	-

Significant difference at $\alpha < 0.05$ denoted with ().

¹Different letter within column denote significant differences using post-hoc Dunn's test after Kruskal-Wallis test.

Study Site names available from authors: David Dressel, Henry Campa III

Appendix G

Upland Hardwood 2015

Table 21. Vegetation characteristics for upland hardwood stands next to individual vaccine delivery unit grid sites. Also noted is the maximum individual deer for each VDU grid site observed by trail cameras and maximum consumption rate observed during a 24-hr period.

Site Name	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous Canopy Cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover	Max Individual Deer (24- hr)	Max Consumption (%) (24-hr)
Now I1	865.8 a ¹	77.7	95.2	90.4	28.6 a	3	12.9
Now I2	865.8 a	66.6	80.9	90.4	23.8 a	5	29.3
Now I3	421.8 b	166.5	76.2	80.9	52.4 a	3	17.1
Bev J2	377.4 b	66.6	95.2	71.4	0 b	6	100.0
Hemm D1	688.2 ab	122.1	61.9	95.2	0 b	14	39.3
<i>Mean</i>	643.8	99.9	81.9	85.7	21.0	6.2	39.7
<i>P-value</i>	0.025*	0.221	0.162	0.443	0.040*	-	-

Significant difference at $\alpha < 0.05$ denoted with ().

¹Different letter within column denote significant differences using post-hoc Dunn's test after Kruskal-Wallis test.

Study Site names available from authors: David Dressel, Henry Campa III

Appendix H

Recipes

VDU Candidate 1

Commercial Corn product

0.45 kg corn block
197.4 g whole deer corn
0.32 l H₂O
24.84 g Xanthan gum

Directions: Break up corn block with sledgehammer. Add water to broken up corn pieces and blend evenly. Pour mixture into ribbon mixer, add whole deer corn and Xanthan gum. Mix until even. Dump mixture into plastic bin and hand mold or use a muffin baking sheet to make 17 g – 20 g vaccine delivery units. Place in oven dryer at 51.67°C for 6-12 hours or zip lock bags and store in chest freezer until use.

VDU Candidate 2

Commercial Apple product

0.45 kg apple treats
0.47 l H₂O
24.84 g Xanthan gum

Directions: Grind up horse treats to small pieces with sledgehammer. Add broken apple pieces to plastic bin and add water. Place mixture into ribbon mixer for 4 minutes. Add xanthan gum and mix until even. Dump mixture into plastic bin and hand mold or use a muffin baking sheet to make 17 g – 20 g vaccine delivery units. Place in oven dryer at 51.67°C for 6-12 hours or into zip lock bags. Place into chest freezer until use.

VDU Candidate 3

Commercial Alfalfa/molasses product

0.45 alfalfa/molasses
0.47 l H₂O
24.84 g Xanthan gum

Mix all ingredients (H₂O, xanthan gum, alfalfa) in plastic bin until xanthan gum is evenly distributed. Pour mixture into ribbon mixer and mix for 4 minutes. Pour mixture into plastic bin. Hand mold or use a muffin baking sheet to make 17 g – 20 g vaccine delivery units. Store in chest freezer until use.

Appendix H (cont...)

VDU candidate 4

Corn Custom product

0.45 kg Big Tine deer blend*
0.24 l H₂O
149.1 g whole deer corn
24.84 g Xanthan gum

Directions: Add deer blend and water into food processor and mix until even. Pour mixture into ribbon mixer and add corn and xanthan gum. Mix until even. Pour out mixture and hand mold or use a muffin baking sheet to produce 17 g – 20 g vaccine delivery units. Place into drying oven at 51.67° C for 6 – 12 hours or until hardening. Store end product in freezer until use.

VDU candidate 5

Apple custom product

0.45 kg Big Tine deer blend*
0.32 l H₂O
0.17 l Evolved habitats buck jam
(Ripe Apple)
24.84 g Xanthan gum

Directions: Blend deer blend and water in food processor. Pour mixture into ribbon mixer and add buck jam and Xanthan Gum. Mix evenly. Pour out mixture and hand mold or use a muffin baking sheet to produce 17 g – 20 g vaccine delivery units. Place into drying oven at 51.67° C for 6 – 12 hours or until hardening. Store end product in freezer until use.

***Big Tine Fortified deer blend:** Shell Corn, Blackoil Sunflower, Cracked Corn, Imperial 30-06 Mineral/vitamin Supplement, Milo, Dry Molasses, Cherry Flavoring, Mineral Oil.

Appendix I

Percent Deer Marked

Table 22. The maximum number of individual deer photographed in 24 hours on each individual site where alfalfa/molasses vaccine delivery units were distributed. The percentage of euthanized deer showing rhodamine B staining was used to calculate a conservative estimate of the number of individual deer that may have consumed alfalfa/molasses VDUs on each site.

Site name	Max Individual deer	# Deer Euthanized	% Deer w/ RB-marking	Max # Deer RB-marked
SH1B	32	3	66.7	21.34
CWG3B	22	10	70	15.40
CWG1B	6	10	70	4.20
Dok1B	21	13	61.54	12.92
DOK2B	17	13	69.23	11.77
CWG5B	9	10	70	6.30
LSE1B	25	10	70	17.50
HemmD1B	45	10	80	36.00
GHL1B	27	5	60	16.20
CWG6B	7	6	83.3	5.83
JWB1B	10	2	0	0.00
CWG7B	9	5	80	7.20
RTN1B	14	3	100	14.00
LSE7B	11	1	0	0.00
FTP1B	44	6	66.67	29.33
Total	299	107	-	198.00

Appendix J

Lowland Conifer 2016

Table 23. Vegetation characteristics for lowland conifer stands next to individual vaccine delivery unit grid sites during 2016. Also noted is the maximum individual deer for each VDU grid site observed by trail cameras and maximum consumption rate observed during a 24 hr period.

Site Name	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous Canopy Cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover	Individual Deer/24 hr	Max Consumption % (24 hr)
CWG7B	288.6	432.9	28.5	14.3	85.7	9	25
HemmD1	333	466.2	9.5	23.8	1	45	81
SH1	199.8	366.3	19	28.5	95.2	32	86
CWG3B	111	388.5	14.3	14.3	95.2	22	58
Dok2	264.4	455.1	14.3	28.5	85.7	17	100
Dok1	244.2	455.1	19	9.5	90.4	21	58
FTP1B	188.7	466.2	19	28.5	90.4	44	45
CWG8B	344.1	421.8	38.1	28.5	90.4	14	97
Mean	246.73	431.51	20.21	21.99	79.25	25.50	68.75
P-Value	0.1	0.72	0.63	0.52	0.38	-	-

Appendix K

Aspen/Birch 2016

Table 24. Vegetation characteristics for aspen/birch stands next to individual vaccine delivery unit grid sites in 2016. Also noted is the maximum individual deer for each VDU grid site observed by trail cameras and maximum consumption rate observed during a 24 hr period.

Site Name	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous Canopy Cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover	Individual Deer/24 hr	Max Consumption % (24 hr)
LSE1B	521.7	66.6	57.1	71.4	14.3	25	48
CWG1B	788.1	88.8	61.9	85.7	9.5	6	43
CWG9B	599.4	88.8	38.1	95.2	19.1	11	65
BevJ2B	754.8	77.7	47.6	85.7	14.2	11	18
Mean	666.0	80.48	51.18	84.50	14.28	13.25	43.50
P-Value	0.36	0.8	0.45	0.18	0.86	-	-

Appendix L

Upland Hardwood 2016

Table 25. Vegetation characteristics for upland hardwood stands next to individual vaccine delivery unit grid sites in 2016. Also noted is the maximum individual deer for each VDU grid site observed by trail cameras and maximum consumption rate observed during a 24 hr period.

Site Name	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous Canopy Cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover	Individual Deer/24 hr	Max Consumption % (24 hr)
CWG6B	743.7 a ¹	288.6	81	71	33.1	7	26
JW1B	388.5 b	88.8	80.9	66.7	38.1	10	15
JWA3B	699.3 ab	133.2	80.9	85.7	28.5	9	12
JWA5B	499.5 ab	133.2	71.4	80.9	47.6	4	1
CWG5B	543.9 ab	210.9	71.4	95.2	38.1	9	22
Mean	574.98	170.94	77.12	79.9	37.08	7.8	15.2
P-value	0.03*	0.056	0.68	0.24	0.59	-	-

Significant difference at $\alpha < 0.05$ denoted with (*).

¹Different letter within column denote significant differences using post-hoc Dunn's test after Kruskal-Wallis test.

Appendix M

Open Herbaceous 2016

Table 26. Vegetation characteristics for open herbaceous stands next to individual vaccine delivery unit grid sites in 2016. Also noted is the maximum individual deer for each VDU grid site observed by trail cameras and maximum consumption rate observed during a 24 hr period.

Site Name	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous Canopy Cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover	Individual Deer/24 hr	Max Consumption % (24 hr)
RTN1B	188.7	22.2	95.2	19	23.8	14	29
BC1B	233.1	44.4	80.9	14.3	28.5	8	10
Mean	210.9	33.3	88.05	16.65	26.15	11	19.5
P-Value	0.26	0.2	0.09	0.64	0.82	-	-

Appendix N

Lowland Hardwood 2016

Table 27. Vegetation characteristics for lowland hardwood stands next to individual vaccine delivery unit grid sites in 2016. Also noted is the maximum individual deer for each VDU grid site observed by trail cameras and maximum consumption rate observed during a 24 hr period.

Site Name	Stems/ha <10 cm DBH	Stems/ha >10 cm DBH	% Herbaceous Canopy Cover	% Deciduous Canopy Cover	% Coniferous Canopy Cover	Individual Deer/24 hr	Max Consumption % 24 hr
GHL1B	599.4	155.4	80.1	85.7	23.8	27	61
BevJ1B	610.5	166.5	95.2	90.4	47.6	17	39
LSE7	799.2	166.5	90.4	85.7	42.8	11	26
JWA4	599.4	177.6	85.7	80.9	52.3	2	1
BevJ3B	577.2	133.2	76.2	80.9	38.1	5	5
JWB2B	599.4	155.4	76.2	76.2	38.1	6	15
Mean	630.85	159.1	83.97	83.3	40.45	11.33	24.5
P-Value	0.23	0.87	0.51	0.76	0.08	-	-

Appendix O

Regression Models

Table 28. The top five models with the lowest AIC scores and associated variables are listed in order of lowest AIC score to highest. The model with the lowest AIC score (-102.38) was chosen as the best model that most accurately represents the prediction of the number of VDUs consumed by deer.

Variables In Model	R-Squared	Adj R-Squared	AIC
Deer, Herbaceous, Deciduous, Coniferous, >10cm DBH, Date	0.687	0.678	-102.38*
Deer, Deciduous, Coniferous, >10cm DBH, Date	0.683	0.676	-101.83
Deer, Herbaceous, Deciduous, Coniferous, <10cm DBH, >10cm DBH, Date	0.687	0.677	-100.41
Deer, Deciduous, Coniferous, <10cm DBH, >10cm DBH, Date	0.684	0.675	-100.01
Deer, Herbaceous, Coniferous, <10cm DBH, >10cm DBH, Date	0.682	0.674	-98.56

*Chosen model based on lowest AIC score between all models

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