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SAFETY AND IMMUNOGENICITY OF ONRAB® IN RACCOONS AND SKUNKS IN WEST VIRGINIA – 2011 FIELD TRIAL REPORT

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FIELD TRIAL REPORT

United States Department of Agriculture

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TABLE OF CONTENTS

ABSTRACT 1
INTRODUCTION 2
BRIEF BACKGROUND 2
FIELD TRIAL DESIGN 5
MATERIALS AND METHODS 5
Animal Sampling 6
Target species biological and serological sampling 6
Target species swab sampling 6
Target and nontarget species histopathology sampling 6
Rabies Virus Antibody Determination 7
Human Adenovirus (Serotype-5) Antibody Determination 7
Tetracycline Biomarker 7
ONRAB® Detection from Oral Swab Samples 8
Histopathology 8
Field studies 8
Captive studies 8
ONRAB® Bait Contact 8
Statistical Methods 9
RESULTS AND DISCUSSION 10
Target Species Serology 10
Histopathology 13
Field study 13
Captive study 13
ONRAB®Bait Contacts 13
RECOMMEDNATIONS 15
TABLES 17
FIGURES 22
REFERENCES 30
APPENDICES A1

Appendix A: Final Outline for Proposed ONRAB® Oral Rabies Vaccine Field Trial in West Virginia..... A-1

Appendix B: Post-ORV Sampling to Detect Human Adenovirus Serotype-5 as a Part of the September 2011 ONRAB Field Trial, Southeastern West Virginia..... B-1

Appendix C: Materials and Methods Used in qPCR Screening of Human Adenovirus Serotype-5 Rabies Virus Glycoprotein Recombinant Vaccine (ONRAB®) C-1

Appendix D: Safety of ONRAB® in Select Captive Non-target Species..... D-1

Appendix E: Human Adenovirus Serotype-5 Titers Pre-ONRAB® E-1

Appendix F: Human Adenovirus Serotype-5 Titers Post-ONRAB® F-1

Appendix G: Histopathology Results from Captive Studies at Wildlife Services, NWRC.... G-1

ABSTRACT

In 2011, a field trial was conducted in southeastern West Virginia to evaluate the safety and immunogenicity of a live recombinant human adenovirus (serotype 5)-rabies glycoprotein vaccine (ONRAB®) in wild raccoons and skunks. ONRAB® was selected for evaluation based on the success achieved in eliminating raccoon rabies from Ontario and Quebec, Canada, through the integration of this vaccine into rabies management strategies and significantly higher sero-positivity in comparison to Raboral V-RG® in studies along the U.S.-Canada border. The field trial site was composed of forested and agricultural habitats in an area where raccoon rabies is enzootic and oral rabies vaccine (ORV) has never been used as a management strategy. The ULTRALITE bait is composed of a small blister pack that contains the ONRAB® vaccine with a waxy coating matrix of sweet attractants, impregnated with tetracycline biomarker, camouflaged by a khaki green dye. Approximately 80,000 baits were distributed at 75 baits/km^2 mostly by fixed wing aircraft along parallel flight lines spaced at 750 m intervals. There were no phone calls related to human or pet bait contacts reported through the affixed, legible toll free phone number on each bait. Relatively low human population density in the study area and bait attributes that may make them less attractive to dogs could have been contributing factors resulting in no reported bait contacts. Histopathology results from up to 23 tissue types from diverse target and nontarget wildlife species samples pre (n=290) and post-ORV (n=300) with ONRAB® will be provided under a separate cover once the analysis is completed. No differences were observed between control and treatment captive animals, including cottontail rabbit (*Sylvilagus floridanus*), opossum (*Didelphis virginiana*), fox squirrel (*Sciurus niger*), and eastern wild turkey (*Meleagris gallopavo silvestri*) that received a 10x ONRAB® dose in a companion histopathology study conducted at the Wildlife Services (WS), National Wildlife Research Center. These species that have not been previously evaluated for potential ONRAB® effects are common to the West Virginia field trial site. The proportion of raccoons categorized as sero-positive was significantly higher in ($P < 0.05$) the post-ORV ONRAB® baiting period (49.4%, n=296) in comparison to the pre-ORV (9.6%, n=395)) period (naïve to ORV either with ONRAB® or Raboral V-RG® but where raccoon rabies is enzootic). The 49.4% sero-positivity in raccoons was the highest observed by WS for areas with similar management histories that were baited once at 75 baits/km^2 with Raboral V-RG®. Biomarker presence was significantly ($P<0.05$) higher among sero-positive raccoons post-ORV and similar ($P>0.05$) among raccoons during the pre-ORV period, an indication of vaccine-induced rabies virus neutralizing antibodies (RVNA) following bait consumption. Skunk sample size was inadequate to assess ONRAB® effects, but other studies have not shown a strong RVNA response at 75 baits/km^2. Overall, the safety and immunogenicity outcomes from this field trial support replicating the West Virginia field trial to determine if raccoon population immunity would increase as a function of a second annual ORV campaign with ONRAB®. Expansion of field trials into other strategic areas such as the Ohio contingency action zone, the Niagara Frontier and along the Quebec border with New York, Vermont and New Hampshire is recommended.

INTRODUCTION

In 2011, APHIS, Wildlife Services (WS) coordinated a collaborative oral rabies vaccine field trial to evaluate the safety and immunogenicity of ONRAB® (Artemis Technologies, Guelph, Ontario, Canada) in raccoons and skunks in West Virginia (Appendix A). It was the first field trial of this type in the U.S. in over 20 years (Roscoe et al. 1998). This field trial was conducted to evaluate a vaccine that has the potential to be licensed and applied strategically to more aggressively meet raccoon rabies management goals, including elimination. ONRAB® is a live recombinant vaccine that uses human adenovirus (serotype 5) to express the rabies virus glycoprotein gene (Charlton et al., 1992). Over 6 million doses of ONRAB® have been distributed in Ontario since 2006 for skunk and raccoon rabies control (D. Donovan, personal communication) and Quebec since 2007 for raccoon rabies control (J. Mainguy, personal communication). Both Provinces appear to have eliminated raccoon rabies virus variant through the integration of ONRAB® into broader rabies control strategies (Rosatte et al. 2009a, Rees et al. 2011).

Highly collaborative processes were put in place to meet regulatory, technical, environmental and logistical demands associated with the vaccine-bait field trial conducted in West Virginia. Collaborators included county, state, federal and international partners from government and non-government organizations, as well as Artemis Technologies, ONRAB® manufacturer (Table 1). Artemis Technologies was responsible for providing the requisite data to APHIS, Veterinary Services, Center for Veterinary Biologics (CVB), with formal public health input from CDC to facilitate a comprehensive ONRAB® risk assessment. Published information on ONRAB® and data on file were also part of the foundation for a WS Environmental Assessment that evaluated the potential impacts of releasing this vaccine into the wild in West Virginia. A joint CVB and WS Federal Register Notice was issued to notify the public of plans for an ONRAB® field trial and to solicit input and comments (U. S. Department of Agriculture, 2011a).

This report covers key aspects and findings of the ONRAB® field trial and provides recommendations for future field evaluations.

BRIEF BACKGROUND

The concept of oral rabies vaccination (ORV) to control rabies in free-ranging wildlife originated in the U.S. (Baer et al., 1971; Baer, 1988). ORV has been widely used in Europe beginning in the late 1970's and Canada in the late 1980's with success in controlling and eliminating rabies in foxes from several European countries (Blancou, 2008; Wandeler, 2008) and all but a few residual foci of an arctic variant of rabies in skunks in Ontario (Nadin-Davis et al., 2006; Rosatte et al., 2011), as well as raccoon rabies from Ontario (Rosatte et al., 2009b) and Quebec (Rees et al., 2011)(no cases since 2009). The rapid spread of raccoon rabies along the eastern seaboard of

the U.S. and west to eastern Ohio as the probable result of translocations from Florida to Virginia and West Virginia during the 1970's (Nettles et al., 1979) served as a major stimulus for intervention with ORV to prevent raccoon rabies from gaining an even broader geographic footprint in the U.S.

Due to the risk, albeit it low, of vaccine-induced rabies associated with residual pathogenicity in the Evelyn Rockitnicki Abelseth (ERA) strain of attenuated rabies virus (Fehlner-Gradiner et al., 2008), a live recombinant *Vaccinia*-rabies glycoprotein (V-RG) vaccine became the prime candidate for testing in the U.S. (Rupprecht et al., 1986). This vaccine cannot cause rabies, although low levels of risk may be associated with exposure to the virus vector (Roess et al., 2012). However, V-RG proved to be safe in more than 50 vertebrate species (Artois et al., 1990; Rupprecht et al., 1992a; Rupprecht et al., 1992b). As a result of field safety and immunogenicity testing in the early 1990s (Hanlon et al., 1998; Hanlon and Rupprecht, 1998; Roscoe et al., 1998), V-RG was conditionally licensed in 1995, and fully licensed in1997, in the U.S. for vaccination of free-ranging raccoons by Merial Ltd., Athens, Georgia under the registered name Raboral V-RG®. It was also fully licensed in 2002 for coyotes in the U.S. and for raccoons in Canada (Rosatte et al., 2001). It has been approved for experimental use in wild gray foxes and has been used for this purpose in Arizona, New Mexico, and Texas (J. Maki, personal communication). Raboral V-RG® currently remains the only licensed oral rabies vaccine for use in the U.S.

Following the first field trial with V-RG in 1990, the number of vaccine-laden baits distributed annually in the U.S. rose exponentially through 2006, but has decreased to about 8 million baits in 2011 due to implementation of efficiencies such as consistency in ORV zone width throughout most areas and 750m flight line spacing, as well as level WS funding for rabies management (Figure 1). Since WS involvement in rabies management, APHIS has contributed about 120 million Raboral V-RG® baits for distribution in the U.S. between 1995 and 2010 (U.S. Department of Agriculture, 2012). More than 75% of those baits have been used in campaigns to prevent the spread of raccoon rabies beyond the eastern U.S. (Figure 2) and the remainder has been applied in Texas, New Mexico and Arizona for rabies control and elimination efforts in coyotes and gray foxes (U.S. Department of Agriculture, 2008; U.S. Department of Agriculture, 2009; U.S. Department of Agriculture, 2010a).

Coordinated ORV with Raboral V-RG® has resulted in three major rabies management accomplishments in the U.S. Canine rabies from sources in Mexico, which had spilled over and spread among coyotes in south Texas, has been eliminated through the integration of ORV into conventional rabies prevention and control programs (Fearneyhough et al., 1998; Sidwa et al., 2005). This success led to the U.S. being declared canine rabies free in 2008 (Velasco-Villa et al., 2008). ORV has also been successfully applied to control a unique variant of gray fox rabies in western Texas, with no reported cases since May 2009 (Blanton et al., 2011). Intervention

with ORV beginning in 1997 has also prevented appreciable spread of raccoon rabies from its new-found stronghold in the eastern U.S. (Slate et al., 2009).

These accomplishments are noteworthy, yet the inability to eliminate raccoon rabies in high risk spread corridors has prompted the need to evaluate vaccines that may be capable of producing higher levels of population immunity to achieve this programmatic goal. Ideally, vaccines deemed safe, inexpensive and immunogenic in all meso-carnivore rabies reservoir species under field conditions are desired. To date, little RVNA response has been observed among skunks captured from ORV zones established with Raboral V-RG® to raccoon rabies control (Slate et al., 2009). WS and collaborators identified the need to test new vaccine-baits as a priority in the Vaccine/ Bait/Biomarker Team as part of discussions at the annual WS National Rabies Management Team meeting during the past decade. In addition, a comprehensive review of the WS, National Rabies Management Program recommended that new or improved vaccine-baits be brought on line as swiftly as possible to better ensure program sustainability and success (U.S. Department of Agriculture, 2010b).

After considering four candidate vaccines (Table 2), WS and collaborators decided to pursue a field trial with ONRAB® in 2011. That decision was largely based on extensive use of ONRAB® in Ontario and Quebec along the U.S border without untoward events and success in reducing and eliminating raccoon rabies. Other contributing factors included the inability to access other candidate vaccines for field testing in a timely fashion. Also, no skunks infected with the raccoon rabies virus variant have emerged in Ontario since 2004, or Quebec since 2009, suggesting that the occurrence of skunks infected with raccoon rabies is dependent on spillover from the raccoon reservoir. In addition, under operational rabies control strategies using high ORV bait densities (300 baits/km^2) and narrow flight lines (250m), meaningful levels of immunity have been achieved in skunk populations in an attempt to eliminate residual foci of an arctic rabies variant from Ontario (Rosatte et al., 2011). While these bait densities would not be sustainable at the landscape level, perhaps bait modifications could enhance future performance of ONRAB® in skunks at lower bait densities.

ONRAB® uses a human adenovirus type 5 (HAd5) as a vector to express the rabies virus glycoprotein gene. The virus vector has a well-characterized molecular structure, genomic stability, and possesses the ability to grow high titers in a wide spectrum of cells (Graham and Prevec, 1992). Each ONRAB® bait contains 1.8 ±0.1 ml of HAd5 ONRAB® vaccine (titer of not $<10^{9.5}$ cell culture infectious dose 50%[$CCID_{50}$]/ml) in an elongated plastic blister pack coated with an attractant-bait matrix (Figure 3).The matrix is composed of partially hydrogenated vegetable shortening (34%), Microbond® wax (International Wax Ltd., Agincourt, Ontario, Canada) (30%), stearine (12.5%), icingsugar (20%), vegetable oil (1%), artificial marshmallow flavor (1%), artificial sweet flavor (1%), and a fat-soluble food dye (0.5% khaki green) to camouflage the baits. Each vaccine-bait weighs approximately 4 g. The body of the blister pack is an elongated oval with dimensions of 1.81 x 0.55 x 0.39 in (30 x 14 x 10 mm) and a

rectangular lip extending to 1.57 x 0.79 in (40x20 mm) (Figure 3). The blister pack contains an identifying label as to the contents of the bait and a toll-free phone number where people can obtain information about the baits (Rosatte et al., 2009a).

The ONRAB® bait matrix also can deliver 100 mg of tetracycline hydrochloride as a biomarker to aid in evaluating bait ingestion. Tetracycline deposits in metabolically active portions of bones and teeth of animals, which can be viewed in slide preparations under fluorescent light microscopy. Tetracycline-positive tooth or bone indicates the likelihood that an animal has eaten at least one bait (Johnston and Voight, 1982; Rupprecht et al., 1987; Rosatte et al., 2009a). The presence of tetracycline does not equate to immunity because it is possible for an animal to eat the outer bait matrix without rupturing the blister pack, resulting in no oral exposure to the vaccine. Potential sources of "background" tetracycline may include consumption of medicated feeds such as those sometimes used for production animals, intentional treatment by humans with tetracycline, and non-specific fluorescence that may be found naturally (Chopra and Roberts, 2001).

FIELD TRIAL DESIGN

The ONRAB® ORV zone was 1,460 km^2 composed of hardwood and mixed coniferous-hardwood forest, interspersed with livestock agriculture and small towns (U.S. Department of Agriculture, 2011a). The spatial field trial design contained four 127 km^2 sampling cells, separated from each other by a 5 km buffer (Figure 2). The distance from the western edge of the ONRAB® sampling cells to the existing ORV zone baited at 75 baits/km^2 since 2001 (U.S. Department of Agriculture 2005) with Raboral V-RG® was 11km. The buffer was provided to reduce the chance of confounded serological results. The distance from the edges of the sampling cells and the outer perimeter of ONRAB® baited area was 5 km to reduce dilution effect from raccoon home ranges that may overlap from ONRAB® naive areas, where raccoons and skunks would not have had equal opportunity to consume baits as compared to animals with home ranges totally within the ONRAB® zone. A total of 79,027 ONRAB® baits with tetracycline biomarker in the bait matrix were distributed within the field trial zone, with 77,448 baits aerially distributed along parallel flight lines at a 750 m spacing. A total of 1,580 ONRAB® baits were distributed through ground baiting in the vicinity of towns in the field trial zone where aerial baiting could present a safety concern.

MATERIALS AND METHODS

The pre and post ONRAB® sampling profile for assessing safety and immunogenicity of ONRAB® at 75 baits/km^2 is summarized in Table 3.

Animal Sampling

Target species biological and serological sampling

Animal sampling included live (cage) trapping (Tomahawk model 608) and release of target species that were apparently not rabid based on behavior and gross examination for lesions. Suspect animals were tested for rabies using a direct rapid immunohistochemistry test (dRIT), with diagnostic confirmation and rabies virus variant typing performed at CDC. Non-target species were either released or included among the required samples for histopathological analysis. The same trapping approach was applied for pre and post-ONRAB® sampling.

About 150 live traps were set within 800 m of 25 preselected random points in each sampling cell to ensure adequate spatial coverage (Figure 4). The same random points were used both pre and post-ORV ONRAB® sampling periods, which were August 2-26, 2011 and September 21-31, respectively. Traps were checked once every 24 hours. All rabies reservoir species captured during pre and post-ORV with ONRAB® were anesthetized using an IM injection of a 5:1 mixture of ketamine and xylazine (Kreeger, 1999) so that blood as well as first premolar tooth samples could be collected for aging and biomarker presence, and other pertinent biological information such as sex, reproductive status, and the general condition of each animal. Serum samples were collected from blood centrifuged the same day of capture, with aliquots briefly stored in labeled cyrovials at minus 25-70° C before shipment to the appropriate laboratory for analysis. After full recovery from anesthesia, all raccoons and skunks were released at their site of capture.

Target species swab sampling

The same animal capture and handling procedures were followed from day 1-6 post-ORV with ONRAB® to collect oral swab samples (Appendix B). However, live trapping was confined to the intercellular spaces between the four primary sampling cells 1-2, 2-3, and 3-4. This sampling scheme was used to reduce the chance of trap shyness among raccoons and skunks post-ORV with ONRAB® to collect blood sera and other samples, which began the recommended 5-weeks after bait distribution (C. Fehlner-Gardiner, personal communication) and would include recaptures from the pre-ORV sampling period. Swab samples were labeled and stored in universal viral transport tubes (Becton Dickinson, #220222) containing 3 ml of transport medium and shipped in near real time to the Animal Health Diagnostic Center, Cornell University for analysis.

Target and nontarget species histopathology sampling

Victor Rat (model BM205) and mouse (model BM154) traps were used to capture small mammals for tissue sampling from major organs. Trapping began on July 21, 2011, two weeks prior to serological baselines samples, and continued opportunistically up to the distribution of ONRAB® baits September 16, 2011. These samples were supplemented with live trapped

nontarget captures to assure a diverse representation of faunal samples for histopathological investigation. Larger mammals and birds were donated from WV, Division of Natural Resources or collected by live trapping or shooting.

Histopathological samples were also collected from target and nontarget species within the ONRAB® ORV zone after the 6-day swab sampling period. No target species were collected for histopathology from the four primary sampling cells within the field trial zone (Figure 2). Necropsies were performed on animals on the day of capture and tissues were harvested, placed in labeled vials in 10 percent buffered formalin, stored for a short period, and shipped for laboratory analysis to MD Anderson Cancer Center (Houston, Texas) according to the protocol in Appendix B.

RVNA Antibody Determination

Serum was shipped frozen in labeled, cryovials within containers of dry ice to the CDC Rabies Program Laboratory upon completion of the Pre-ONRAB® sampling period and again after the Post-ONRAB® sampling period. The RVNA were determined at CDC using the Rapid Fluorescent Focus Inhibition Test (Centers for Disease Control and Prevention, 2011). Samples with I.U.≥0.06 were deemed indicative of positive RVNA activity.

Human Adenovirus (Serotype-5) Antibody Determination

Serum was also shipped frozen in labeled, cryovials within containers of dry ice to the Animal Health Diagnostic Center, Cornell University to determine antibody levels of HAd5 upon completion of the Pre-ONRAB® sampling period and again after the Post-ONRAB® sampling period. The adenovirus virus neutralization (VN) assay for Ad-5 followed standard procedures for VN assays in microtiter plates. Two-fold serum dilutions (50 ul) in duplicate were mixed with 100-300 $TCID_{50}$ of Ad-5 (VR-5 ATCC) in a 50 ul volume. Mixtures were allowed to incubate for at least 1 hr at room temperature. A 100 ul volume of indicator cells (A549 - ATCC) was added to each well and the plates were placed in a CO_2 incubator at 37°C for 6 days. Wells were scored for the presence or absence of typical Ad-5 cytopathology. Positive serum controls were an equine anti Ad-5 (CDC) and a bovine anti Ad-5 (ARS-USDA).

Tetracycline Biomarker

First premolars extracted from each live captured and anesthetized raccoon or skunk were shipped in small, labeled Manila envelopes to Matson's Laboratory (Missoula, MT) for tetracycline and specific age determination based on cementum annuli counts. For the analysis in this report only juvenile and adult age classes could be evaluated until age specific age results become available.

ONRAB® Detection from Oral Swab Samples

Oral swab samples were shipped in universal viral transport tubes (Becton Dickinson, #220222) containing 3 ml of transport medium to the Animal Health Diagnostic Center, Cornell University for analysis. Complete descriptions of the swab sampling protocol and the quantitative real time polymerase chain reaction analysis (*q*PCR) can be found in Appendices B and C, respectively.

Histopathology

Field studies

These studies are being conducted under contract with MD Anderson Cancer Center, Houston, Texas. A complete description the protocol is present in Appendix B.

Captive studies

The study protocol for captive histopathology studies conducted at the WS, NWRC animal research facilities in Ft. Collins, Colorado is provided in Appendix D. The species evaluated included: Eastern Woodrat *(Neotoma floridana),* Cottontail Rabbit (*Sylvilagus floridanus),* Opossum (*Didelphis virginiana),* Fox Squirrel *(Sciurus niger)* and Eastern Wild Turkey (*Meleagris gallopavo silvestri).* Because animals in the wild could consume more than one bait, this study evaluated animals exposed to a 10x dose of ONRAB® and controls.

ONRAB® Bait Contact

Enhanced surveillance for human and domestic animal bait and vaccine contact was a critical component of the ONRAB® field trial to measure and address potential human and pet safety issues. Typically during ORV projects, reports of human or pet contact with ORV baits occurs when a person who has found a bait calls the toll-free number printed on the bait, or when a person directly contacts federal, state or local agencies after a bait is found (Roess 2012). Each ONRAB® bait distributed as part of this field trial contained a warning label ("Rabies vaccine, DO NOT TOUCH. Live adenovirus vector") (Figure3) and was marked with a toll-free number that put callers in direct contact with public health officials at the West Virginia Department of Health and Human Services (WVDOH). To assist the WVDOH in documenting and managing human bait contacts, CDC and WS developed a recommended SOP for ONRAB® bait contacts through a detailed algorithm (Figure 5).

To improve surveillance for bait and vaccine contacts, a communication campaign was cooperatively implemented by WS specifically for the ONRAB® field trial. This campaign focused on raising public awareness of the field trial, enhancing public understanding of ORV and the associated risk of contact with baits and vaccine, and to increase reporting of potential vaccine exposures.

At the foundation of these communication efforts was the preparation and publication of a WS environmental assessment (EA) in cooperation with the USDA Forest Service (USFS) entitled "Field Trial of an Experimental Rabies Vaccine, Human Adenovirus Type 5 Vector in West Virginia." The EA analyzed the potential environmental effects of a proposed field trial in West Virginia, including portions of USFS lands but excluding Wilderness areas, to determine the safety and immunogenicity of the HAd5-rabies glycoprotein recombinant vaccine in raccoons and striped skunks. A 30-day public comment period (August 8th through September 7th, 2012) resulted in a total of 13 comments received from private citizens and representatives from public health, agriculture, and natural resources agencies in the U.S. and Canada. The majority of comments were supportive. Three comments were addressed, including one comment that expressed concern about the potential for human exposures and methods for managing these events, and another comment that focused on concerns that WS had not properly informed the residents of WV about the pending field trial. The final comment was related to a National Park Service request for language changes in the EA.

WS also enhanced communication efforts through preparation and distribution of a national press release about the field trial. The press release was picked up by the Associated Press and widely distributed. In addition, at least four T.V. and newspaper interviews were conducted at a media event and at a Greenbrier County, WV Commission meeting, where a detailed presentation on the ONRAB® field trial was given to County Commissioners and the public. Finally, WS in collaboration with WVDOH, Ontario Ministry of Natural Resources, West Virginia Department of Agriculture, West Virginia University, West Virginia Division of Natural Resources and the USFS developed a list of talking points to assist in the coordination of key field trial messages provided to the public, facilitate data collection on potential bait and vaccine exposures, and ensure safety concerns were adequately addressed by all cooperating agencies.

Statistical Methods

Descriptive statistics were used to characterize the proportion of raccoons that seroconverted between pre- and post-baiting trapping operations. We used contingency tables and the Cochran-Mantel-Haenszel (CMH) (Agresti, 1996) test to determine whether the proportions of individuals sampled in pre- and post-ORV capture efforts differed among sampling cells (1-4). The CMH tests were used to determine if age, sex and seroconversion status were conditionally independent across sampling cells. Separate CMH analyses were conducted on pre- and post-ORV data sets. We used the McNemar test for dependent samples to determine whether the proportion of seroconverted individuals captured differed between the pre- and post-baiting sampling sessions. Finally, we conducted a factorial ANOVA to determine if recapture distance differed between sampling sites, and age and sex classes.

We used Cochran-Mantel-Haenszel tests to determine whether relationships existed between bio-marking, serology, age and sex. Among post-ORV samples, we explored detected differences by multiple logistic regression. Significance was set at $P<0.05$.

All statistical analyses were conducted in SAS 9.1 (SAS Institute, 2002)

RESULTS AND DISCUSSION

Target Species Serology

Raccoons sampled during the pre-baiting trapping period displayed a 9.6 percent sero-prevalence for RVNA. Geographic cell-to-cell proportion of seroconverted raccoons trapped during pre-ORV sampling did not differ relative to sex (CMH = 2.53, df = 1, p = 0.11) or age (CMH = 2.81, df = 1, p = 0.094) classes. The sampled cell-to-cell (Figure 4) proportion of sero-positive demographic classes ranged from 0-6%, and adults comprised a greater proportion of sampled individuals than did juveniles (Table 4).

By contrast, 49.4 percent of raccoons sampled during the post-ORV trapping period had detectable RVNA. The sampled cell-to-cell proportion of sero-positive demographic classes ranged from 0-37% (Table 4) differed both by sex (CMH = 3.98, df = 1, p = 0.046) and age (CMH = 6.46, df = 1, p = 0.011) classes. These differences can be attributed to a lower proportion of sero-positive females in cell 1 (χ^2 = 6.81, df = 1, p = 0.009), and a lower proportion of sero-positive juveniles in cell 4 (χ^2 = 5.82, df = 1, p = 0.016). The difference in the proportions of individuals that were categorized as sero-positive was significantly higher (S=102.25, df = 1, p < 0.001) in the post-ORV with ONRAB® (49.4%) sampling period than in the pre- ONRAB® (9.6%) period.

The distance between pre and post- ONRAB® recaptures differed by cell (F = 6.78, p = 0.002) and not by sex (F = 0.51 p = 0.48) or age class (F = 1.77, p = 0.19). The mean distance between recaptures was greatest for cell 2 (553 m), followed by cell 3 (260 m), cell 4 (212 m), and cell 1 (101 m).

Sero-positivity of 9.6 percent among raccoons (n=395) sampled prior to ONRAB® baiting (Table 5) in the ORV naïve raccoon rabies enzootic field trial area was higher than has been recorded for areas of similar status (mean=4.9%, SD=4.8, n=13) (Tables 6 and 7). Possible reasons for the higher baseline include naturally acquired immunity from sub-lethal exposures to raccoon rabies virus or movements of orally vaccinated raccoons into sampling cells from the adjacent Raboral V-RG® zone. However, only 3 of 31 sero-positive raccoons were also biomarker-positive (Table 8). In addition, the coated sachet is the primary bait type distributed in the adjacent Raboral V-RG®, which does not contain biomarker. Twenty-five of 310 sero-negative raccoons were biomarker-positive from the pre-ORV sampling period (Table 8), suggesting that the environment may be a source of tetracycline exposure. Raccoon rabies in this general area during the five years prior to the field trial (Figure 6) suggest the higher sero-positivity in the pre-ORV sample is more likely related to naturally acquired immunity. Previous studies have reported higher levels of RVNA in raccoon populations in areas naïve to the raccoon rabies virus variant where there have been no vaccination efforts (McLean, 1971;

Ramey et al., 2008). Lastly, there are no known wildlife rehabilitation facilities in the immediate area that may have released sufficient numbers of hand-inoculated raccoons by off-label use of commercial rabies vaccines to affect pre-ORV levels. Such activities are not legal in West Virginia.

The 49.4 percent post-ORV with ONRAB® (uncorrected for the 9.6%) sero-positivity represents the highest population RVNA level WS has observed after a first baiting of a naïve area at 75 baits/km^2 where antibody baselines had been measured prior to ORV. Nine percent (3/32) were tetracycline and sero-positive in the pre-ONRAB® sample (Table 8), compared to 40 percent (48/123) post-ORV with ONRAB® (Table 9). Among pre-ORV samples, no relationships were detected between bio-marking, serology, age, or sex (P=0.6336). However, among post-ORV samples, differences were noted relative to age (P=0.0007) predicted sero-positivity, with adults having >3 times higher odds of being sero-positive than juveniles (OR=3.133). Once specific age class data become available, biomarking will be evaluated further as younger raccoons have been reported to biomark with tetracycline more easily (PM1 and canine teeth only) than older animals (Algeo et al., *in revision*) due to active tooth growth (Linhart and Kennelly, 1967), Most important is that tetracycline biomarking was related to serological response (P=0.0001).

That biomarker-positives were less than RVNA-positives can be attributed in part to the use of the first premolar, a tissue known to be inferior to canines or mandibular bone to evaluate tetracycline deposition (Linhart and Kenelly, 1967; Algeo et al., *in revision*). The premolar was selected because it was more important to release raccoons and skunks to increase the probability of recaptures in the post-ORV sample than to euthanize animals to extract canine teeth for evaluation. In spite of this, only 27 raccoons caught during post-ORV trapping were recaptured from the pre-ORV period. One of 27 raccoons was RVNA-positive during pre-ORV and 8 of 27 had evidence of RVNA post-ORV. The single individual that had antibodies pre-ORV (I.U.=0.06) was also positive post-ORV with an apparent anamnestic response (I.U.=0.32). No skunks were recaptured in the post-ORV period.

While this field trial was not specifically designed to compare ONRAB® and Raboral V-RG®, a sero-positivity of 17.0 percent (n=11, SD 9.9%, uncorrected for RVNA of 4.9%, (n=13, SD 4.8%) was observed after a first baiting with Raboral V-RG® in naïve areas under essentially the same initial ORV baiting characteristics during 2001 to 2009 in Alabama, Florida, Georgia, Maine, North Carolina Pennsylvania, Tennessee and Virginia and similar raccoon density indices (U.S. Department of Agriculture, 2011c) as in this field trial except in Florida (Tables 6 and 7). These results are in line with those reported by Fehlner-Gardiner et al. (2012) who found essentially a two-fold higher sero-positivity at 73 percent for ONRAB® in New Brunswick, compared to 29 percent for Raboral V-RG® across the border in Maine.

Although comparative seroconversion samples were not collected in the adjacent Raboral V-RG® zone in West Virginia post-ORV during 2011, in the four years previous to the ONRAB®

field trial, RVNA population levels ranged from 18.5 percent (n=135; 2008) to 46.4 percent (n=84; 2007, Table 10). These percentages represent RVNA levels for areas that have been subject to annual ORV campaigns at 75 baits/km^2 since 2001.

The Raboral V-RG® zone in Virginia is the closest area for reference, at 166 km linear distance southwest from the ONRAB® field trial zone (Figure 7). During 2011, a 57.1 percent (n=177) sero-positivity was recorded in this area, which contains similar habitats but has been baited at 75 baits/km^{2}since 2002. This represents a rare high RVNA, which may be partially explained by the presence of raccoon rabies virus variant in the area (Figure 7). While the same effect may apply to the field trial area (figure 6), biomarker in the ULTALITE® bait provides context for a vaccine-induced RVNA response. Unfortunately, the coated sachet used to deliver Raboral V-RG® does not contain biomarker, which would have aided in this evaluation. Considerable variation in RVNA has been observed 2007 to 2010 in the general vicinity of the Virginia reference area, with levels ranging from18.4 (n=114; 2010) to 39.0 percent (n=210; 2007) (Table 10). As an index, 11 percent (5/45) of ORV events examined during a 3-year period (2006 to 2008) at 75 baits/km^2 from 12 eastern states exceeded the sero-positive rate observed in this field trial. The mean sero-positivity was 32.3±12.8 (SD) percent for these 45 ORV events. RVNA for all of these ORV events was in relation to areas that had been baited at least twice; most had been baited for several successive years. However, given this rare high level of 57.1 percent, we plan to evaluate the same ORV area in Virginia in 2012, with a similar sampling cell format as used in West Virginia. In addition, formal sampling cells are being planned for pre- and post-ORV within the Raboral V-RG® and the ONRAB® zone in West Virginia in 2012. This design improvement will facilitate determining if population immunity will increase significantly in response to a second successive year of ONRAB® baiting at 75 baits/km^2 and allow for a more formal comparison with Raboral V-RG®.

An inadequate sample of skunks was captured to establish a population baseline for RVNA and evaluate the response to ONRAB®. Only one of 20 skunks had detectable RVNA in the Pre-ORV sample; one of 27 had RVNA post-ORV with ONRAB® (Table 5). All other species in Table 5 are reflective of incidental captures of rabies reservoir species in North America (Blanton et al., 2011).

There was no evidence of HAd5 antibodies in 416 animals sampled during the pre-ORV period (Appendix E). Seven of 296 (2.4%) samples evaluated post-ORV demonstrated HAd5 VNA; 6 raccoons and 1 skunk (Appendix F). Low seroconversion may be expected for a human adapted virus. Monitoring for anamnestic responses will be incorporated into the evaluation of baiting with ONRAB® in after a second baiting as projected for September 2012.

Histopathology

Field study

Diverse fauna were sampled for histopathological analysis in the pre and post-ONRAB® periods. Small rodents such as *Peromycus sp.* and *Microtus sp.* constituted the largest group sampled that included other rodents, lagomorphs, cervids and birds (Table 11). The results are not available at this time, but will be provided as an addendum to this report after they are received from MD Anderson Cancer Center (Houston, Texas, USA). Our expectation is that these results will be available by the end of April.

Captive study

The species selected for captive studies represent common fauna to southeastern West Virginia and will serve to complement the spectrum of species that have already been evaluated for histopathologic effects relating to ONRAB® exposure in Canada (Knowles et al., 2009). Similar histopathologic results in vaccinates and control animals were observed for the four species in which the analysis is completed, suggesting no untoward effects from ONRAB® consumption at a 10x dose above exposure to single bait. The woodrat analysis is nearing completion. The results from this captive study in tandem with previous results from Canada (Fehlner-Gardiner et al., 2012) serve as the reference for histopathology from wildlife in southeastern West Virginia. The summary report from the captive histopathology study submitted by WS, NWRC is in Appendix G.

ONRAB® Detection from Oral Swab Sampling Day 1 – 6 Post-ORV

Swab samples were collected from 125 target and nontarget individuals live-trapped from day 1-6 post ORV baiting to determine ONRAB® presence in the oral cavity. Individual raccoons captured on days 2, 3 and 4 and an opossum on day 4 had *q*PCR values <35, which are considered strong positives (Knowles et al. 2009) (figure 8). An additional nine raccoons and four opossums with >35 *q*PCR values <40 were captured on days 1-4. No ONRAB® was detected on days 5-6. Two of 15 raccoons and two of 12 opossums had >35 *q*PCR values <40 on day 1. While these are not considered strong positives, they represent the only animals sampled that could have had vaccine contact during the first night after ONRAB® distribution. It is not possible to determine the specific day in which vaccine contact may have occurred for remainder of the animals deemed positive from day 2-4.

ONRAB® Bait Contacts

No human or domestic animal bait or vaccine contacts were reported to the WVDOH or WS associated with the ONRAB® field trial in 2011, despite notification of the field trial and media events that included bait descriptions and the integration of enhanced to document potential bait exposures and reduce potential safety issues.

Enhanced surveillance and tracking of bait and vaccine contacts are an integral component of ORV projects conducted by WS in the U.S. (Roess et al. 2012). About 120 million Raboral V-RG® baits have been distributed since the WS ORV program inception in 1995, yet only 1,464 people reported contacting or potentially contacting a bait (i.e., picking up bait, finding a bait in yard, or removing bait or sachet from pet's mouth, feces, or vomit - any type of contact with a bait is also defined throughout the document as an "exposure"). This equates to one human exposure per 68,521 baits distributed, or 0.0015% contact cases (U.S. Department of Agriculture, 2011b). In addition, exposure cases were generally insignificant, as most involved finding an intact bait. Very few cases involved touching a broken bait, sachet, or liquid vaccine. Furthermore, of the 0.0015% of contact cases reported since 1995, only two known adverse reactions have been reported (USDA 2010a; CDC 2009).

From 1995 - 2008, U.S. Department of Agriculture (2011b) documented only 1,327 instances where a pet or other domestic animal had contact with a bait. This equates to 1 domestic exposure per 75,596 baits disbursed or 0.001 % contact cases. In addition, U.S. Department of Agriculture (2011b) documented that 261 incidents were reported in which pets came into contact with a bait in 2008. However, there were no reports of pets or other domestic animals experiencing any type of adverse reaction, other than 8 dogs who experienced vomiting or diarrhea after ingesting a number of baits. The dogs involved in these adverse reactions have reportedly not experienced any substantive or long term adverse effects. Domestic animals that bite into and ingest a bait of either Raboral V-RG® or ONRAB® are most likely to be immunized against rabies virus or receive a boost from a previous vaccination.

As in the U.S., ONRAB® baits distributed in Canada are marked with a toll-free number that the public can call if they see or have contact with bait or vaccine. Between 2006 and 2011 over 3.9 million ONRAB® baits were distributed in Ontario. There were a total of 64 calls received in which a person reported finding or contacting a bait during this period. This equates to approximately 1 call for every 65,140 baits distributed or 15 calls for every 1 million baits distributed, essentially the same as the call rate for Raboral V-RG®. The total number of calls received involving possible contact (i.e. vaccine contact not officially confirmed) with vaccine as a result of handling a punctured bait or removing a bait from a dog's mouth or dog chewing a bait was 15. The rate of possible contact with vaccine equates to 1 contact for every 260,560 baits distributed. There were no reports of adverse events associated with humans from any of the reported contacts (Ontario Ministry of Natural Resources 2007, 2008, 2009, 2010, 2011). The province of Quebec has also been distributing ONRAB® vaccine baits since 2007. Between 2007 and 2009, despite the distribution of almost 2 million baits, there were only 16 reports of human exposures (Mainguy and Canac-Marquis unpublished). In 2010, just over 1 million ONRAB® baits were distributed with no human bait contacts reported (Ontario Ministry of Natural Resources, 2011)

The relatively low numbers of bait and vaccine contacts associated with ONRAB® in Canada and no reported bait or vaccine exposures documented during this field trial in the U.S. could be related to several factors. The ONRAB® ULTRALITE bait differs from Raboral V-RG® baits (coated sachets and fishmeal polymer baits) in color and attractants. The ONRAB® bait matrix is a khaki green color, which may aid in camouflaging in most habitats. Also, the ONRAB® baits contain a sweet scent and flavor rather than scent associated with fishmeal for Raboral V-RG®, which may decrease the likelihood of baits being found by cats and dogs. Maintaining a system for enhanced surveillance for human and domestic animal bait contact will be important as field trials expand to other areas that include more urban and suburban habitats with increased human and pet populations that often require higher baits densities to meet management objectives.

RECOMMENDATIONS

The following recommendations are the result of the encouraging outcome of this initial ONRAB® field trial.

1) The West Virginia trial should be replicated and include at least two similar sized, buffered sampling cells in the adjacent Raboral V-RG® ORV zone for comparative purposes. Pre and Post-ORV raccoon and skunk samples should be collected at sufficient sample size for RVNA serological analysis to address the question: does a successive second year of ONRAB® vaccination significantly increase raccoon population immunity above year one? These data will be required as a basis for year three ORV to determine if the population immune response becomes asymptotic at year two similar to the pattern generally observed for Raboral V-RG®, but at a significantly higher level. Leveling off of the population immune response should in turn be analyzed in the context of raccoon age structure, which is likely a correlate as a reflection of population dynamics.

2) Field trails should be expanded in 2012 to areas with an elevated risk of raccoon rabies spreading to naïve areas. The Ohio Contingency Action zone (east of Cleveland, Ohio) , which is interspersed with urban and suburban development, represents the highest priority site and is ideally suited for an evaluation of safety with respect to bait contacts and immunogenicity associated with hand baiting at 150 baits/km^2. This proposed expansion would introduce new complexities associated with unique bait distribution patterns that should be evaluated for broader use of ONRAB®. As a results of trap-vaccinate-release efforts in the Ohio Contingency Action zone, a history of raccoon populations and RVNA exists. The challenge will be to focus sampling effort on the naïve juvenile class as well as the overall population immunity for evaluating the post-ORV with ONRAB® treatment. In addition, measuring bait contacts under higher bait and human densities represents a key area of interest.

3) In 2006, raccoon rabies was first confirmed in Quebec, with Vermont as the likely source of the emergence of this rabies virus variant. Since 2006, Quebec has made extensive commitments to raccoon rabies control and surveillance (Rees et al., 2011). No cases of raccoon rabies have been detected in the Province since May 2009, with the use of ONRAB® central to their strategy for elimination. Quebec has expressed an interest in

creating a greater distance buffer to enhance protection against the emergence of raccoon rabies in Montreal. Therefore, a field trial in northern Vermont and New Hampshire and Northeastern New York should be elevated in priority to prepare for collaboration with Quebec and the likelihood of a commitment of Quebec resources to this effort in 2013. In the U.S., this area is largely rural, not unlike West Virginia, but the field trial would occur in an area that has been subject to ORV and trap-vaccinate-release (TVR). This field trail should be structured to allow for a comparison of naive areas in Vermont to similar areas in West Virginia, as well as first ONRAB® baiting in previously vaccinated (Vermont) and (unvaccinated) areas (West Virginia).

4) The Niagara Frontier, connecting New York and Ontario, represents another high risk corridor for raccoon rabies movement and therefore is a priority for a future field trial in 2012, or more likely 2013, depending on available resources and other factors. TVR had been used for several years between the Welland Canal and the Niagara River in Ontario to provide an immune barrier against spread and establishment of raccoon rabies from New York. In New York, ORV with Raboral V-RG® has been in place on the Niagara Frontier since 1996 (U.S. Department of Agriculture, 2007), with rabies perpetuating in raccoons and skunks in Erie and Niagara Counties, New York, which border Canada. The Niagara Frontier includes the City of Buffalo and Niagara Falls, and as such has some similarities with the proposed field trial area east of Cleveland, Ohio.

5) In 2013, the St. Lawrence River Valley, New York and northeastern Tennessee represent high risk areas that should be considered as field trial sites, if additional field data are required to support the licensing of ONRAB®.

TABLES

Table 1. List of county, state, federal and international entities from governments and non-government organizations that collaborated and participated in the West Virginia ONRAB® field trial, 2011.

Agency/Organization Name
United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center (USDA, APHIS, WS, NWRC)
USDA, APHIS, Center for Veterinary Biologics (CVB)
USDA, APHIS, Legislative and Public Affairs (LPA)
USDA, APHIS, Policy and Program Development (PPD)
Centers for Disease Control and Prevention (CDC)
United States Forest Service (USFS)
West Virginia Department of Agriculture
West Virginia Department of Health and Human Resources
West Virginia Division of Natural Resources
Greenbrier, Monroe and Summers County Commissioners
Animal Health Diagnostic Center, Cornell University College of Veterinary Medicine
Ontario Ministry of Natural Resources
Quebec Ministère des Ressources naturelles et de la Faune
Canadian Food Inspection Agency
Trent University
Artemis Technologies, Inc.
Dynamic Aviation Group, Inc.

Table 2. Candidate oral rabies vaccines considered for field testing in the U.S. over the past five years.

Vaccine	ONRAB®	SAG2	CAV2	SPN SASGAS
Recombinant?	yes	no, modified-live	yes	yes, reverse genetics
Licensed in U.S.?	no	no	no	no
Licensed outside U.S.?	pending	yes	no	no
Interest in U.S.?	yes	yes	yes	yes
Proprietor[1]	Artemis	Virbac	MTTI	IDT

[1] Artemis Technologies, Inc. (Ontario, Canada); Virbac (Carros, France); Molecular Targeting Technologies, Inc. (Pennsylvania, USA); IDT Biologika (Dessau, Germany).

Table 3. Pre- (baseline) and post-ONRAB® oral rabies vaccine sampling emphasis.

Activity	Pre-ONRAB®	Post-ONRAB®
Enhanced rabies surveillance	yes	yes
Rabies virus serology	yes	yes
Human adenovirus serology	yes	yes
Biomarker	yes	yes
Histopathology (target and non-target species)	yes	yes
Oral cavity swab samples	no	yes
Monitoring for abnormalities among animals	yes	yes
ONRAB® bait contacts	n/a	yes

Table 4. Percent of sero-converted raccoons and corresponding sample size for pre- and post-ONRAB® baiting.

		Adult Male		Adult Female		Juvenile Male		Juvenile Female	
Interval	Cell	%	n	%	n	%	n	%	n
Pre-bait	1	3.9	32	1.9	31	0.0	21	0.0	17
	2	4.1	41	4.1	27	2.0	18	0.0	10
	3	4.4	49	3.6	33	0.8	12	0.8	15
	4	5.9	24	1.1	25	0.0	12	3.6	20
Post-bait	1	21.6	27	8.4	27	7.2	16	4.8	13
	2	14.6	14	17.1	8	9.7	11	7.3	8
	3	37.3	28	25.4	25	5.1	4	1.7	2
	4	25.0	39	16.2	30	1.2	7	0.0	4

Table 5. Percent of sero-converted animals sampled for pre- and post-ONRAB® field trial in West Virginia, 2011.

	Pre-ONRAB®		Post-ONRAB®	
Species	%	n	%	n
Raccoons	9.6	395	49.4	263
Striped skunks	5.0	20	3.7	27
Gray foxes	0.0	1	100	2
Red foxes		0	66.7	3
Coyotes		0	100	1
Total	9.4	416	45.9	296

Table 6. Percent of sero-converted raccoons where Raboral V-RG® oral rabies vaccine was distributed for the first time following baseline sample collection in an ORV naive area and indexes to raccoon population density in eastern states (2001-2009).[1]

State	Year	Bait Density (per km^2)	%	n	Region[2]	Mean Raccoon Density	n Studies
Alabama	2002	naive	2.9	68	Georgia-Alabama-Tennessee (GAT)		
	2003	75	32.8	67	Georgia-Alabama-Tennessee (GAT)	6.5	2
	2002	naive	11.3	##	Selma		
	2004	75	13.1	99	Selma	8.9	2
	2005	naive	1.2	84	Birmingham		
	2005	75	2.7	##	Birmingham	n/a	0
Florida	2003	naive	9.1	99	Mainland (not Pinellas County)		
	2003	75	9.2	65	Mainland (not Pinellas County)	17	5
Georgia	2003	naive	0	19	Georgia-Alabama-Tennessee (GAT)		
	2004	75	30.2	##	Georgia-Alabama-Tennessee (GAT)	n/a	0
Maine	2002	naive	14.3	21	Hodgdon		
	2003	naive	0	11	Hodgdon		
	2003	70	19.5	82	Hodgdon	3.6	2
	2007	naive	1.4	74	Caribou		
	2009	75	26.4	##	Caribou	12.1	2
North Carolina	2005	naive	3.3	30	Appalachian Ridge		
	2005	75	7.5	##	Appalachian Ridge	n/a	0
Pennsylvania	2002	naive	9.9	##	Appalachian Ridge		
	2002	75	12.8	39	Appalachian Ridge	n/a	0
Tennessee	2002	naive	3.3	30	Appalachian Ridge		
	2002	75	10.8	74	Appalachian Ridge	9	1
Virginia	2001	naive	9.1	33	Appalachian Ridge		
	2002	naive	3.4	29	Appalachian Ridge		
	2002	75	22.1	68	Appalachian Ridge	4.7	2
Total		naive	mean=4.9±4.8	n=13			
Total		70/75	mean=17.0±9.9	n=11			

[1] Information subject to minor change based on revised data entry to Wildlife Services' Management Information System; accessed March 2012.

[2] Regions represent distinct ORV zones within a given state where sampling was conducted independent of other regions.

3 Means and standard deviations of naive vs. baited areas were generated from 13 and 11 events, respectively (n=13, n=11).

Table 7. Raccoon density indexes within or near ONRAB® field trial area in West Virginia, 2006-2010.

Year	Study Name	County	Density (raccoons/km^2)	Area Habitat Class
2006	WVGREENB06	Greenbrier	8.2	Rural (mixed forest)
2007	WVGREENB07	Greenbrier	12.2	Rural (mixed forest)
2010	WV00310	Greenbrier	12.0	Agriculture (pasture/hay)
2010	WV00410	Greenbrier	10.7	Rural (mixed forest)
2010	WV00510	Monroe	6.6	Rural (mixed forest)
2010	WV00610	Monroe	7.0	Rural (mixed forest)

Table 8. Seroconversion and biomarker results for 341 raccoons sampled prior to ONRAB® ORV distribution in West Virginia, 2011.

Pre-ORV		Biomarker Negative		Biomarker Positive	
		male	female	male	female
Sero-negative	adult	117	87	5	6
	juvenile	38	43	11	3
Sero-positive	adult	15	9	1	1
	juvenile	3	1	1	0

Table 9. Seroconversion and biomarker results for 250 raccoons sampled after ONRAB® ORV distribution in West Virginia, 2011.

Post-ORV		Biomarker Negative		Biomarker Positive	
		male	female	male	female
Sero-negative	adult	33	41	7	5
	juvenile	19	13	4	5
Sero-positive	adult	41	28	19	13
	juvenile	2	4	12	4

Table 10. Percent of sero-converted raccoons sampled post-ORV (Raboral V-RG® in West Virginia and Virginia, 2007-2011.

	West Virginia		Virginia	
Year	%	n	%	n
2007	46.4	84	39.0	210
2008	18.5	135	32.0	147
2009	24.0	179	18.8	319
2010	20.5	239	18.4	114
2011	no sampling conducted		57.1	177

Table 11. Number of histopathology samples collected pre- and post-ORV during the ONRAB® field trial in West Virginia, 2011.

Species	Pre-ONRAB®	Post-ONRAB®
Bear (*Ursus americanus*)	4	1
Coyote (*Canis latrans*)	3	3
Crow (*Corvus brachyrhynchos*)	9	9
Deer (*Odocoileus virginianus*)	4	2
Fox (*Urocyon cinereoargenteus* and *Vulpes vulpes*)	3	3
Opossum (*Didelphis virginiana*)	9	10
Rabbit (*Sylvilagus floridanus*)	8	9
Raccoon (*Procyon lotor*)	9	10
Rodents, small (*Peromyscus* and *Microtus spp.*)	201	221
Skunk (*Mephitis mephitis*)	7	4
Squirrel (*Sciurus* carolinensis and *Sciurus niger*)	13	10
Vulture (*Cathartes aura*)	10	8
Woodchuck (*Marmotamonax*)	10	10
Total	290	300

FIGURES

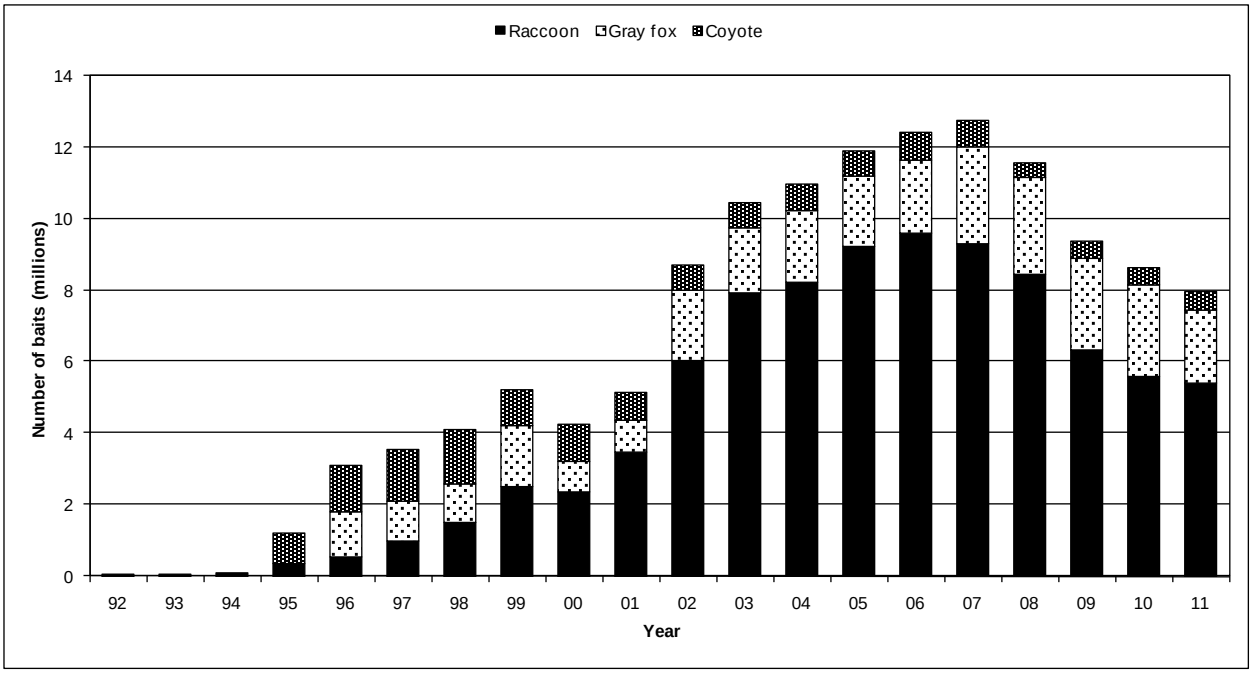

Figure 1. Raboral V-RG® oral rabies vaccine distribution targeting raccoons, gray foxes and coyotes in the United States, 1992-2011.

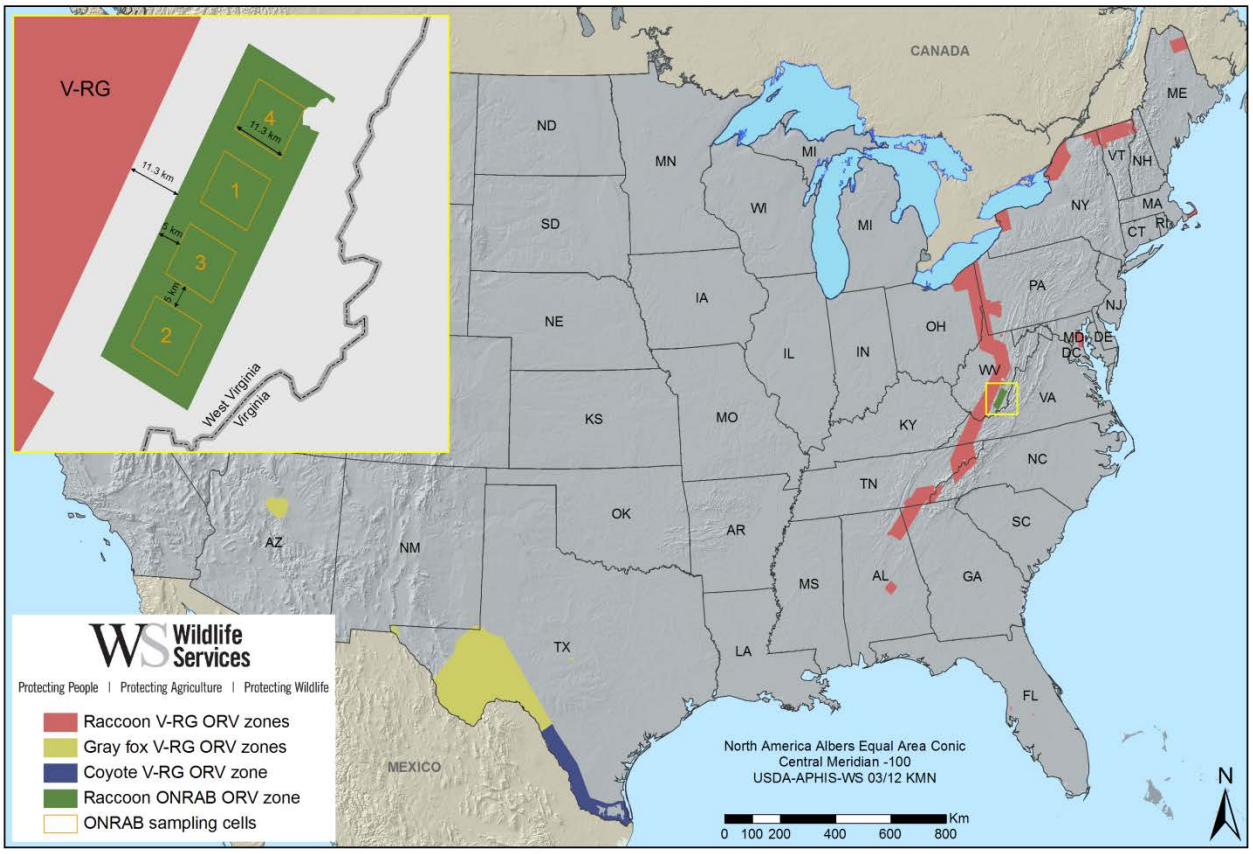

Figure 2. Oral rabies vaccine distribution zones targeting raccoons, gray foxes and coyotes in the United States, 2011. Inset includes ONRAB® ORV zone relative to Raboral V-RG® zone and ONRAB® sampling cell details.

Figure 3. ONRAB® baits utilized in oral rabies vaccine field trial in West Virginia, 2011. (Photo: http://www.mnr.gov.on.ca/en/Business/Rabies/2ColumnSubPage/STEL02_166285.html accessed March 2012.)

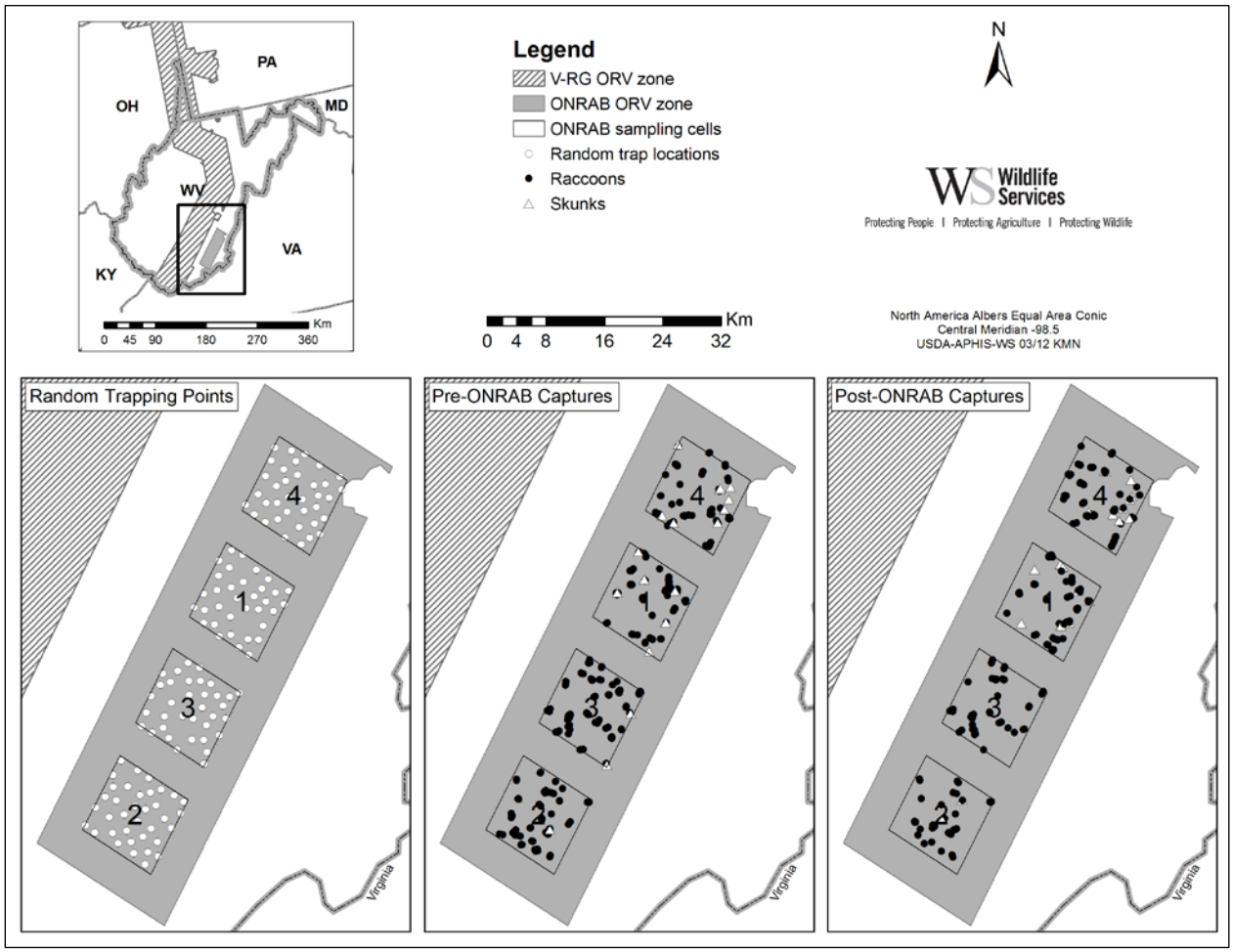

Figure 4. Preselected random trapping points and subsequent raccoons and skunks captured during pre- and post-ONRAB® trapping periods in West Virginia, 2011.

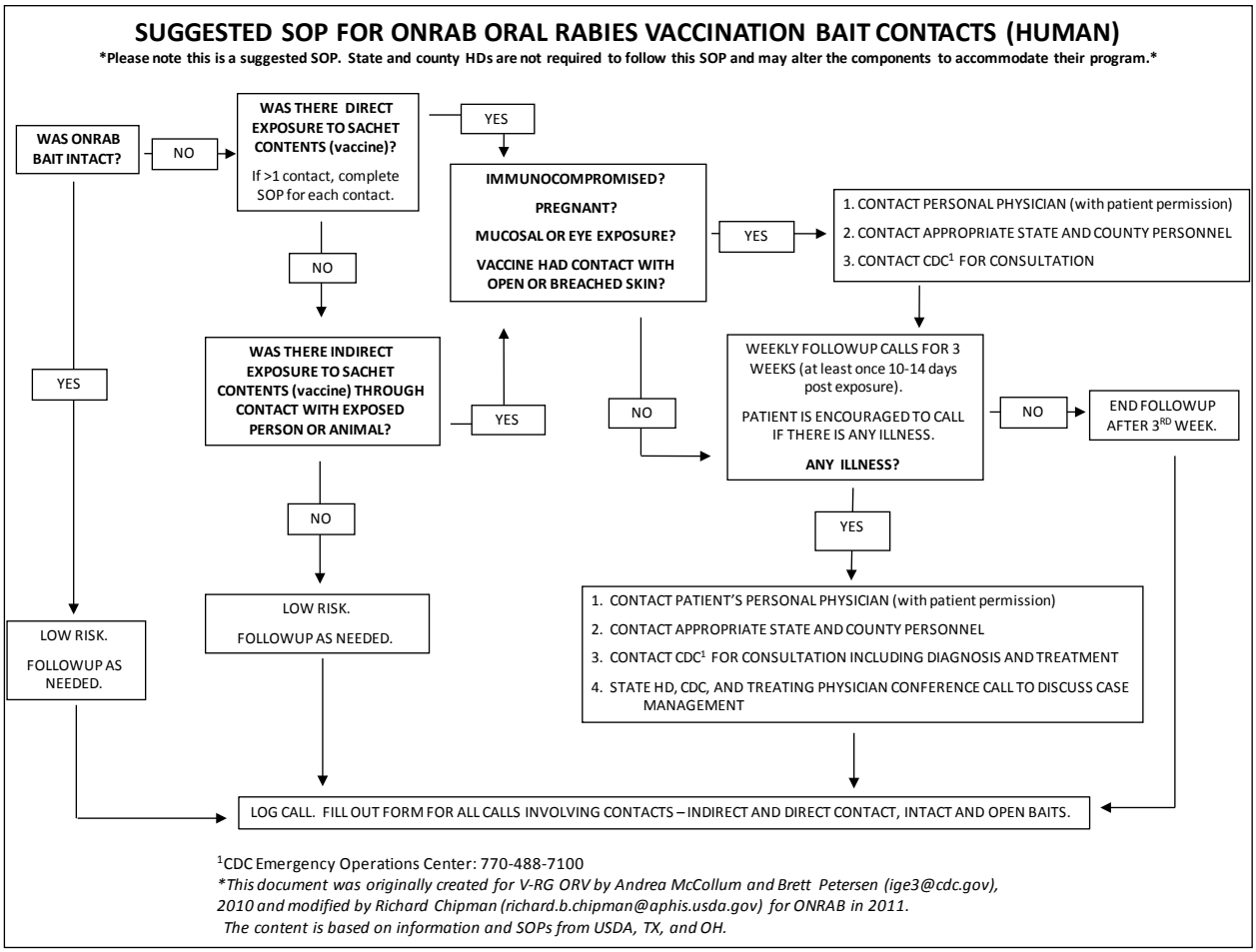

Figure 5. Suggested standard operating procedure (SOP) for documenting and managing human contacts with ONRAB® baits during field trial in West Virginia, 2011.

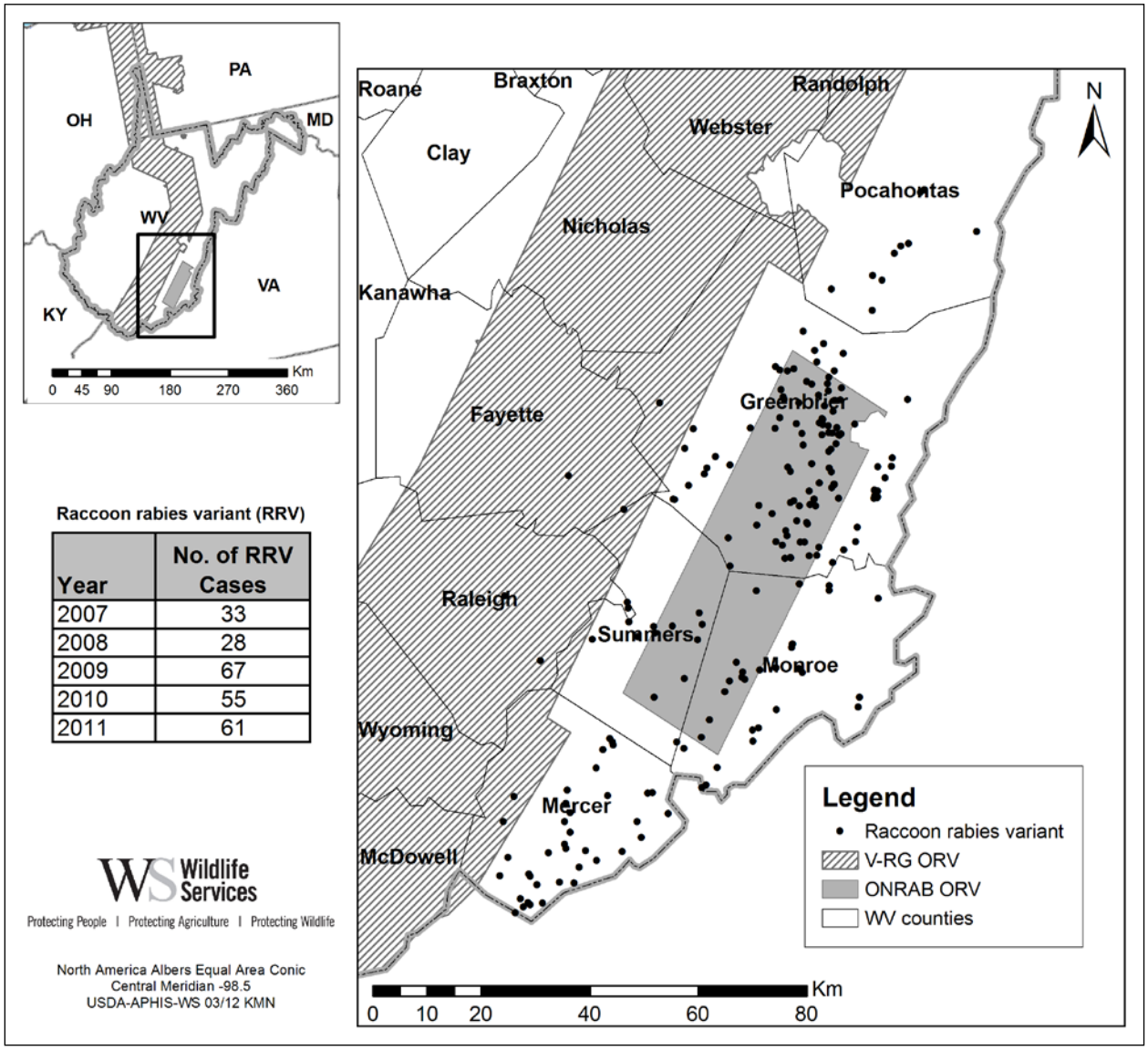

Figure 6. Raccoon rabies variant case history in West Virginia 2007-2011 within an 83 km radius of the center of the 2011 ONRAB® ORV zone.

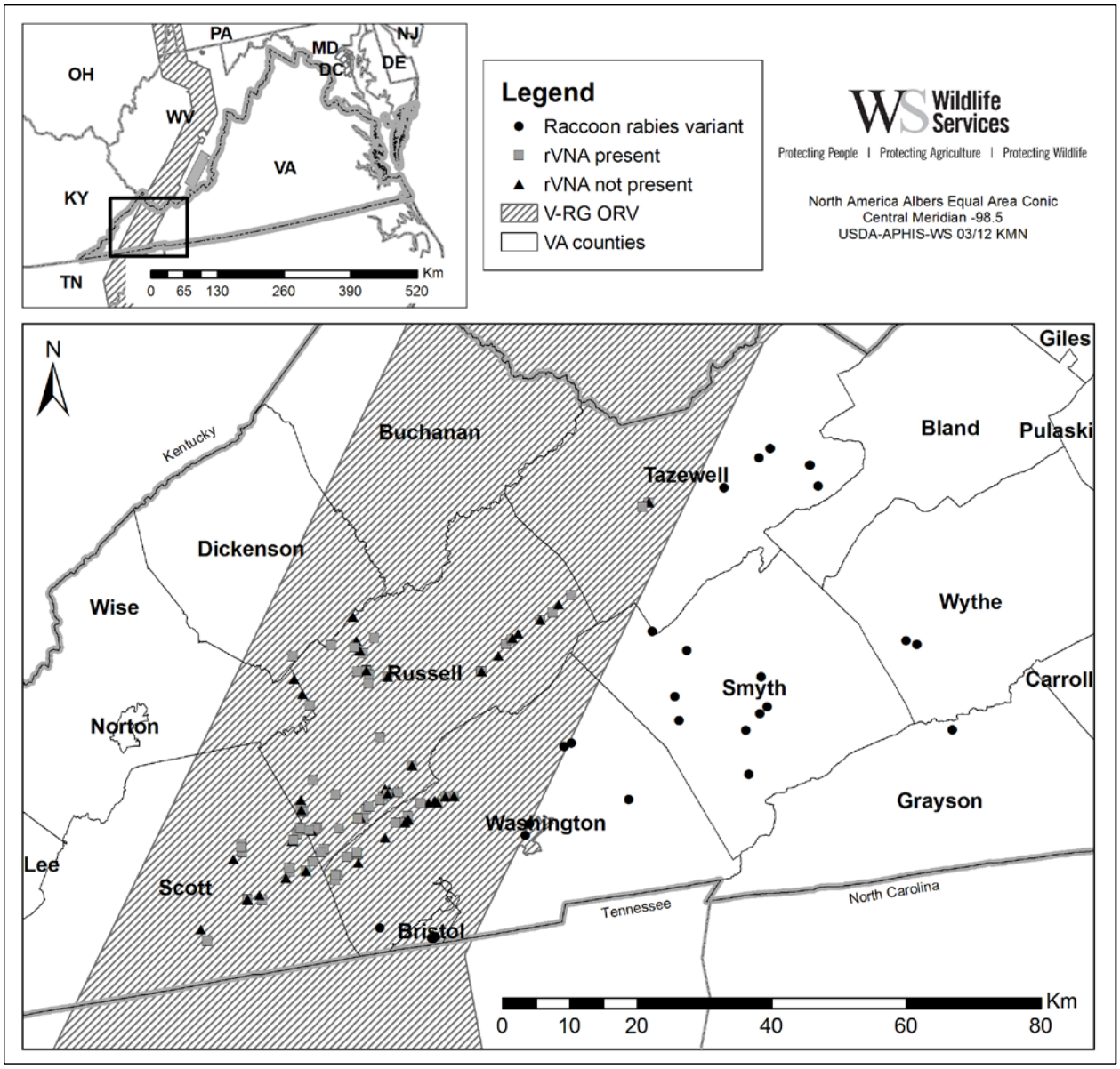

Figure 7. Raccoons sampled for rabies virus neutralizing antibodies (RVNA) post-ORV Raboral V-RG® distribution and cases of raccoon rabies virus variant within an 83 km radius of the center of the sampled raccoons, Virginia, 2011.

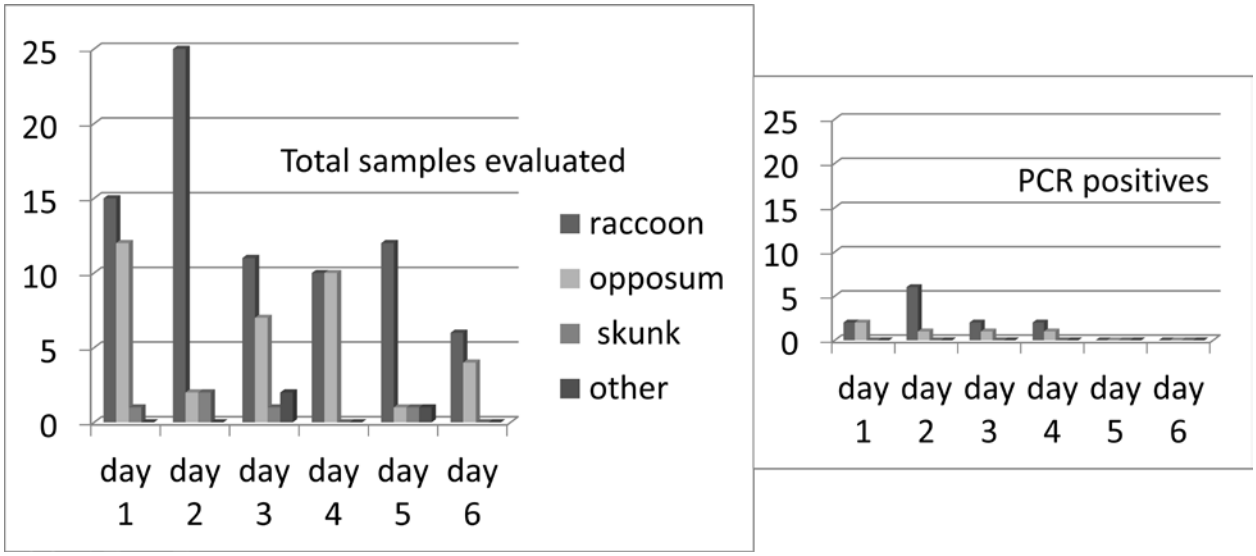

Figure 8. Total unique captures for oral swab sampling and PCR positives by species, day 1-6 post-ORV with ONARB® distribution September 16, 2011 (others included gray squirrel and cottontail rabbit).

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APPENDICES

Appendix A: Final Outline for Proposed ONRAB® Oral Rabies Vaccine Field Trial in West Virginia

1) Site location (Figure 1)

- State: Southeastern West Virginia
- Counties: portions of Greenbrier, Monroe, Summers
- Towns: Alderson, Fairlea, Falling Springs, Lewisburg, Ronceverte
- Field trial plot coordinates: 38.044, -80.421; 37.920, -80.241; 37.566, -80.932; 37.445, -80.753.

2) Rationale for field trial site selection

- Near the strategic center of the Appalachian Ridge ORV zone created with Raboral V-RG®
- Raccoons and skunks present
- Raccoon rabies perpetuates east of existing ORV zone
- Proposed zone in a single state
- Local support within state and county
- Low human population
- WS infrastructure in place
- Site allowed for a broader seasonal window for baiting than potential sites farther north

3) Field trial plot size

- Total area: 1,478.11 km2 [1,450.28 km2 (fixed wing baiting) and 27.83 km2 (ground baiting)]
- 4 distance buffered cells: 11.2 x 11.2 km post-ORV sampling cells: 126.84 km2

4) Baiting characteristics

- Total baits: 79,594 (78,016 fixed wing and 1,578 ground)
- Bait density: 75 baits/km2
- Flight line spacing: 750 meters
- Off-time: 28% for fixed wing and 24% for ground using NLCD to determine "baitable" habitat
- Projected baiting dates: September 15-16, 2011
- Baiting duration: 2 days, 1 plane and ground crews for hand-baiting

5) Bait-vaccine characteristics

- Each bait contains 1.8 ± 0.1ml of ONRAB® vaccine (titer of not < $10^{9.5}$ cell culture infectious dose 50% [$CCID_{50}$]/ml)
- Bait matrix is comprised of partially hydrogenated vegetable shortening (34%), Microbond® wax (30%), stearine (12.5%), Icing sugar (20%), vegetable oil (1%), artificial marshmallow flavor (1%), artificial sweet flavor (1%), and a fat-soluble food dye (0.5%)
- Bait matrix contains 100 mg of tetracycline hydrochloride as a biomarker
- Each vaccine-bait weighs approximately 4g
- The body of the blister pack is an elongated oval with dimensions of 1.81 x 0.55 x 0.39in (30x14x10mm)
- Each bait contains a conspicuous advisory label with a toll free number in the event of a bait contact and potential vaccine exposure

6) Pre-ORV sampling (baselines)

- Enhanced rabies surveillance has been in place for > than 1 year
- Early baseline raccoon and skunk samples collected in 2010 from 5 density index sites
- In late summer 2011, 150 raccoon-sized cage traps will be tended for 10 consecutive days within each of the 4 buffered sampling cells
- Traps will be deployed at random roadside trapping locations
- Focus is to characterize target and non-target community
- Expect capture rate at >100 raccoon/cell based on recent previous trapping efforts in area
- Maximize skunk captures by additional targeted trapping if needed
- Collect pertinent biological, physical and spatial-temporal data from live captured raccoons and skunks and sera for rabies and human adenovirus serological analysis
- Euthanize target species with unusual lesions or behaviors for pathological analysis
- Late spring/summer 2011 collect nontarget tissue and sera from 5-day trapping studies for serological and histopathological analysis
- Implement continuous opportunistic sampling for target and nontarget species (e.g., roadkills) for histopathological analysis

7) Post-ORV sampling (treatment effects)

- Continue enhanced rabies surveillance
- Continue opportunistic sampling for target and nontarget species (e.g., roadkills, hunter harvest) for histopathological analysis for up to 1 year post ORV
- Upon completion of ORV collect buccal and anal swab samples and other pertinent biological, physical and spatial-temporal data from target species for a 10-day period for virus detection/isolation
- Beginning 2 weeks post-ORV collect nontarget organ tissue and sera from 5-day trapping studies for serological and histopathological analysis

- 5 weeks post ORV sample ≥100 raccoons and as many skunks as practical within each of the 4 buffered cells using the pre-ORV target species trapping protocol
- Collect pertinent biological, physical and spatial-temporal data from raccoons and skunks as well as sera for rabies and human adenovirus analysis
- Monitor for abnormalities in captures/animals reported and collect samples as appropriate

8) Sample Analysis

- Rabies virus serological titers to be determine by CDC
- Human adenovirus titers potentially determined at Cornell
- Histopathological analysis sources to be finalized

9) Captive pen safety studies at NWRC (separate protocol for review)

- Species of concern: opossum, fox squirrel, wild turkey, cottontail and wood rats.

10) Report Findings

- Expect results from analysis of field data by March 1, 2012
- Draft report by May 1, 2012

Appendix B: Post-ORV Sampling to Detect Human Adenovirus Serotype-5 as a Part of the September 2011 ONRAB® Field Trial, Southeastern West Virginia

Trapping design and animal handling

Samples will be collected day 1 through day 6 to obtain buccal and anal swab samples from live-trapped target and non-target species for PCR and virus isolation. Fifty live traps should be set the day of ONRAB® (Artemis Technologies, Guelph, Ontario, Canada) distribution within the three intercellular spaces between the four sampling cells (Figure 1). This spatial trapping design should minimize trap "happiness" or "shyness" within the raccoon and skunk populations in the four sampling cells that will be trapped five weeks post-ORV. Traps should not be set in buffer areas to eliminate edge effects.

All captured animals will be handled according to WS' SOP used by the NRMP.

Swab sampling, processing and shipping

Swab samples will be collected from all target and non-target species captured during the first 6 days post-ORV using this design.

Once each live-trapped animal is anesthetized or in some cases euthanized (if warranted based on signs or other factors), duplicate buccal and duplicate anal swab samples should be obtained immediately using BD* Universal Viral Transport Kits (see vendor information under Supplies at the end of this Appendix).

Thoroughly swab the inside of the mouth ensuring the each swab makes contact within the mouth (e.g., tongue, roof, etc.). Place each swab into a properly labeled 1 mL viral media vial, snap off the swab, properly seal each vial and immediately place on dry ice or in a freezer (following vendor instructions). For anal swab samples, insert the swab into the anus the length of the swab tip and gently complete one rotation and place each swab into the 1 mL viral media as detail for buccal swabs. Place all swabs immediately on dry ice. Cooling quickly is a critical step.

Each day (or every other day) samples should be sent by overnight mailed on dry ice to the following address:

Dr. Edward Dubovi
Animal Health Diagnostic Lab
College of Veterinary Medicine
Upper Tower Road
Ithaca, NY 14853
607-253-3923

An email with courtesy copy to Kathleen Nelson (knelson@aphis.usda.gov) should be sent to Dr. Edward Dubovi(ejd5@cornell.edu) informing him that samples have been sent. Include an excel spreadsheet with data and the UPS tracking number. Do not ship samples on Fridays unless otherwise instructed to do so.

Recording data

If data collection is in MIS: Collect standard information for each capture, plus record time of day each sample was collected. Time of sample collection/capture can be obtained by using the time the animal was anesthetized from the PDA database file (dbf). The dbf file should be retained to recall time of day for each capture, as this information is not uploaded to the MIS.

If data collection is outside of MIS: Collect data for the following fields: date, time of day, latitude, longitude, species, sex, relative age, swab sample (yes or no), comments.

Supplies

BD* Universal Viral Transport Kits may be purchased at Fischer Scientific, catalog number: 22-031-15.
http://www.fishersci.com/ecomm/servlet/fsproductdetail?storeId=10652&productId=13751252&catalogId=29104&matchedCatNo=2203115&endecaSearchQuery=%23store%3DScientific%23N%3D0%23rpp%3D15&fromSearch=1&searchKey=031||22||15&highlightProductsItemsFlag=Y

Appendix C: Materials and Methods Used in qPCR Screening of Human Adenovirus Serotype-5 Rabies Virus Glycoprotein Recombinant Vaccine (ONRAB®)

Animal Health Diagnostic Center, Cornell University
January 19, 2012

Primers and probe for ONRAB® qPCR assay

The primers and probe sequences for the ONRAB® qPCR assay were from Knowles et al. (2009a, 2009b). Primers were synthesized by Invitrogen and were purified by desalting. The probe was sourced from Sigma Life Science and was HPLC purified.

AdRG-For	CAACTGTCCTAACCTTGGATTACATCA	HAd5 (upstream of rabies G)
AdRG-Rev	GAATTTCCCAAAACACAATGGAA	rabies G gene
AdRG-probe	FAM™-AGGCTCTCCTGTTTGTACCCCTTCTGGTT-BHQ®1	rabies G gene

Positive amplification control and assay verification

A positive amplification control (PAC) amplicon was generated using primers that flank the assay target region.

Ad5E3-F	GCGGACGGCTACGACTGAATGTTA
RVG-R	TTGTTTGGGCAGCTGAGGTGATGT

Vaccine was drawn directly from an ONRAB® bait and nucleic acid was extracted from a 200 µl volume (Qiagen #69504). The PAC fragment was amplified from the purified DNA using a commercially available PCR mix (Invitrogen, #11306-016) and a conventional thermocycler (Stratagene Robocycler).

Cycling conditions were 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, an annealing temperature for 30 sec, and 72°C for 1 min. The annealing temperature gradient included reactions at 55°C, 57°C, 59°C and 61°C. Gel electrophoresis confirmed single bands in reactions at all annealing temperatures. The reactions were pooled and purified (Qiagen #28106). The purified PAC was quantified spectrophotometrically (Nanodrop 1000) and the copy number was calculated.

An Applied Biosystems (ABI) Path-ID kit (#4388644) was used for PCR amplification. Forward and reverse primers were used at a concentration of 400 nM and the probe was used at a concentration of 120 nM. Four µl of the PAC dilution series, ranging from 1×10^7 copies /4 µl to 1×10^{-1}/4 µl, was used per reaction, in a total volume of 25 µl. Three initial test runs were

conducted on a SmartCycler 2 thermocycler (Table 1 and Figure 1). Cycling conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, 60°C for 1 min. A subsequent run of the PAC dilution series on an ABI 7500 thermocycler gave equivalent Ct values at each dilution and an overall amplification efficiency of 101.8% (not shown). The PAC dilution amplification series showed the assay to be consistently sensitive to a level of detection approximating 10 copies of viral DNA.

Table 1. Example summary of a run showing the Ct values of the PAC dilution series.

Run Summary	(Smart Cycler 2.0d)						
Run Name	OnRab Test 1 11-7-11						
Started At	11/7/2011 16:42						
Number o	10						
Results Table							
Site ID	Protocol	Sample ID	Sample T	Notes	Status	FAM Std/Res	FAM Ct
A1	PATH ID qPCR	ntc	UNKN		OK	ND	0
A2	PATH ID qPCR	10^-1	STD		OK	0.1	0
A3	PATH ID qPCR	10^0	STD		OK	1	0
A4	PATH ID qPCR	10^1	STD		OK	10	35.98
A5	PATH ID qPCR	10^2	STD		OK	100	31.33
A6	PATH ID qPCR	10^3	STD		OK	1000	27.86
A7	PATH ID qPCR	10^4	STD		OK	10000	24.47
A8	PATH ID qPCR	10^5	STD		OK	100000	21.15
A9	PATH ID qPCR	10^6	STD		OK	1000000	17.39
A10	PATH ID qPCR	10^7	STD		OK	10000000	14.44

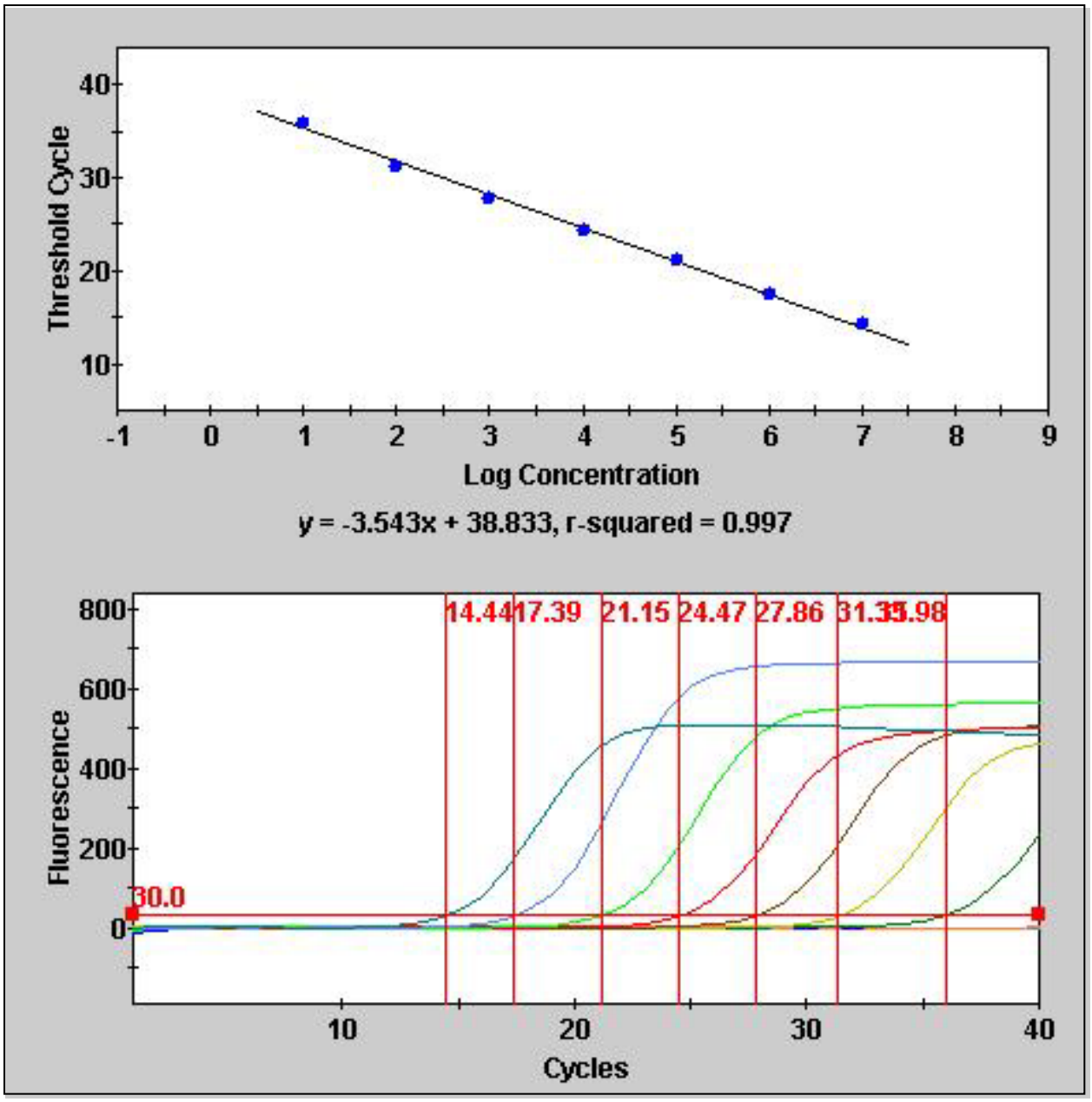

Figure 1.Upper panel shows the amplification plot of PAC dilution series. The lower panel shows the standard curve generated from the data in Table 1 (Efficiency = 91.5%).

Purification and qPCR of samples

Swabs were received in universal viral transport tubes (Becton Dickinson, #220222) containing 3 ml of transport medium. Nucleic acid (NA) was purified from 175 µl of the medium using a MagMax total Nucleic Acid Isolation Kit (ABI #1840). Purified NA was eluted in a final volume of 50 µl.

The ABI Path-ID mastermix (#4388644) was used for PCR amplification. Forward and reverse primers were used at a concentration of 400 nM and the probe was used at a concentration of 120 nM. Four µl of purified NA was used per reaction in a total volume of 25 µl. The qPCR reactions were run in 96-well plates on an ABI 7500 thermocycler. Cycling conditions were 95^{o}C for 10 min, followed by 40 cycles of 95^{o}C for 15 sec, 60^{o}C for 1 min.

In addition to the test samples, each set of reactions included at least two negative control wells (NTC), a positive extraction control (PEC) well with ONRAB® vaccine diluted at 1×10^{-5} in transport medium, a negative extraction control (NEC) consisting of transport medium only, and a PAC well with a calculated copy number of 100.

Number	Date	Time of Day	Latitude	Longitude	Species	Sex	Rel. Age	Oral #1	Oral #2	Anal #1	Anal #2	Extraction #	Accession #	ID	PCR DATA
14000 WV	9/17/2011	8:30	37.82704	80.42957	OPOSSUM	F	JUV	X	X	X	X	52	110198-1	14000	Undetermined
14001 WV	9/17/2011	9:55	37.83657	80.45701	OPOSSUM	M	AD	X	X	X	X	53	110198-2	14001	Undetermined
14002 WV	9/17/2011	10:21	37.83705	80.45468	RACCOON	F	AD	X	X	X	X	54	110198-3	14002	Undetermined
14003 WV	9/17/2011	10:40	37.83818	80.4557	RACCOON	M	AD	X	X	X	X	55	110198-4	14003	Undetermined
14004 WV	9/17/2011	11:16	37.83362	80.45618	RACCOON	M	AD	X	X	X	X	56	110198-5	14004	35.1223
14005 WV	9/17/2011	11:40	37.83209	80.45869	RACCOON	M	AD	X	X	X	X	57	110198-6	14005	Undetermined
14006 WV	9/17/2011	12:27	37.82688	80.46329	OPOSSUM	M	AD	X	X	X	X	58	110198-7	14006	Undetermined
14007 WV	9/17/2011	13:41	37.86965	80.47001	RACCOON	F	JUV	X	X	X	X	59	110198-8	14007	38.5782
14008 WV	9/17/2011	9:46	37.82629	80.43301	SKUNK	M	AD	X	X	X	X	60	110198-9	14008	Undetermined
14009 WV	9/17/2011	8:30	37.73788	80.60128	OPOSSUM	F	JUV	X	X	X	X	61	110198-10	14009	Undetermined
14010 WV	9/17/2011	9:05	37.745	80.60285	OPOSSUM	F	AD	X	X	X	X	62	110198-11	14010	37.1908
14011 WV	9/17/2011	9:50	37.75204	80.58514	OPOSSUM	F	AD	X	X	X	X	63	110198-12	14011	Undetermined
14012 WV	9/17/2011	10:45	37.74026	80.61771	RACCOON	F	JUV	X	X	X	X	64	110198-13	14012	Undetermined
14013 WV	9/17/2011	10:45	37.74026	80.61771	RACCOON	F	AD	X	X	X	X	65	110198-14	14013	Undetermined
14014 WV	9/17/2011	11:10	37.74082	80.61704	OPOSSUM	M	AD	X	X	X	X	66	110198-15	14014	37.1854
14015 WV	9/17/2011	12:40	37.75591	80.61449	RACCOON	M	JUV	X	X	X	X	67	110198-16	14015	Undetermined
14016 WV	9/17/2011	13:10	37.75463	80.6142	RACCOON	F	AD	X	X	X	X	68	110198-17	14016	Undetermined
14017 WV	9/17/2011	9:05	37.63006	80.71993	OPOSSUM	F	JUV	X	X	X	X	69	110198-18	14017	Undetermined
14018 WV	9/17/2011	9:20	37.63054	80.71915	RACCOON	M	AD	X	X	X	X	70	110198-19	14018	Undetermined
14019 WV	9/17/2011	9:34	37.62905	80.71915	OPOSSUM	F	AD	X	X	X	X	71	110198-20	14019	Undetermined
14020 WV	9/17/2011	10:10	37.62978	80.71467	RACCOON	M	AD	X	X	X	X	72	110198-21	14020	Undetermined
14021 WV	9/17/2011	12:20	37.64814	80.72614	OPOSSUM	F	AD	X	X	X	X	73	110198-22	14021	Undetermined
14022 WV	9/17/2011	12:50	37.64647	80.72885	OPOSSUM	F	JUV	X	X	X	X	74	110198-23	14022	Undetermined
14023 WV	9/17/2011	13:10	37.64444	80.73098	RACCOON	F	AD	X	X	X	X	75	110198-24	14023	Undetermined
14024 WV	9/17/2011	13:40	37.64565	80.72527	OPOSSUM	M	AD	X	X	X	X	76	110198-25	14024	Undetermined
14025 WV	9/17/2011	15:30	37.63354	80.68318	RACCOON	M	JUV	X	X	X	X	77	110198-26	14025	Undetermined
14026 WV	9/17/2011	15:48	37.6335	80.68127	RACCOON	F	JUV	X	X	X	X	78	110198-27	14026	Undetermined
14027 WV	9/17/2011	16:20	37.62096	80.672	RACCOON	M	JUV	X	X	X	X	79	110198-28	14027	Undetermined
14028 WV	9/17/2011	16:20	37.62103	80.67184	RACCOON	M	AD	X	X	X	X	80	110198-29	14028	Undetermined
14029 WV	9/18/2011	9:40	37.7662	80.58402	RACCOON	F	AD	X	X	X	X	81	110198-30	14029	35.1551
14030 WV	9/18/2011	10:00	37.6604	80.58505	RACCOON	F	JUV	X	X	X	X	82	110198-31	14030	Undetermined
14031 WV	9/18/2011	10:30	37.76266	80.61134	RACCOON	F	JUV	X	X	X	X	83	110198-32	14031	Undetermined
14032 WV	9/18/2011	11:30	37.76369	80.61176	RACCOON	F	JUV	X	X	X	X	84	110198-33	14032	Undetermined
14033 WV	9/18/2011	12:00	37.75416	80.61408	OPOSSUM	M	AD	X	X	X	X	85	110198-34	14033	Undetermined
14034 WV	9/18/2011	12:45	37.74026	80.61771	RACCOON	F	JUV	X	X	X	X	86	110198-35	14034	Undetermined
14035 WV	9/18/2011	13:30	37.73788	80.60128	RACCOON	M	JUV	X	X	X	X	87 11/18	110198-36	14035	38.4313
14036 WV	9/18/2011	14:05	37.74257	80.60308	RACCOON	F	AD	X	X	X	X	88	110198-37	14036	Undetermined
14037 WV	9/18/2011	15:00	37.76357	80.58318	RACCOON	F	AD	X	X	X	X	7 12/1	110198-38	14037	37.8832
14038 WV	9/18/2011	10:15	37.83006	80.43278	SQUIRREL	M	AD	X	X	X	X	8	110198-39	14038	Undetermined
14039 WV	9/18/2011	11:43	37.83705	80.45468	RACCOON	M	AD	X	X	X	X	9	110198-40	14039	Undetermined
14040 WV	9/18/2011	12:07	37.84025	80.45819	OPOSSUM	F	AD	X	X	X	X	10	110198-41	14040	39.5265
14041 WV	9/18/2011	13:13	37.82688	80.46329	RACCOON	M	AD	X	X	X	X	11	110198-42	14041	Undetermined
14042 WV	9/18/2011	13:39	37.83544	80.46277	RACCOON	M	AD	X	X	X	X	12	110198-43	14042	Undetermined
14043 WV	9/18/2011	14:00	37.83401	80.46442	RACCOON	M	AD	X	X	X	X	13	110198-44	14043	Undetermined
14044 WV	9/18/2011	15:19	37.86937	80.4691	RACCOON	F	AD	X	X	X	X	14	110198-45	14044	39.7765
14045 WV	9/18/2011	15:40	37.87078	80.46799	SKUNK	M	AD	X	X	X	X	15	110198-46	14045	Undetermined
14046 WV	9/18/2011	16:05	37.87462	80.46687	SKUNK	F	AD	X	X	X	X	16	110198-47	14046	Undetermined
14047 WV	9/18/2011	10:15	37.63006	80.71993	RACCOON	M	AD	X	X	X	X	17	110198-48	14047	Undetermined
14048 WV	9/18/2011	10:20	37.63054	80.71915	RACCOON	F	AD	X	X	X	X	18	110198-49	14048	Undetermined
14049 WV	9/18/2011	10:58	37.62905	80.71915	RACCOON	F	AD	X	X	X	X	19	110198-50	14049	Undetermined
14050 WV	9/18/2011	11:20	37.62978	80.71467	RACCOON	F	AD	X	X	X	X	20	110198-51	14050	Undetermined
14051 WV	9/18/2011	12:18	37.64451	80.72998	RACCOON	F	AD	X	X	X	X	21	110198-52	14051	Undetermined
14052 WV	9/18/2011	12:57	37.64659	80.72134	RACCOON	F	AD	X	X	X	X	22	110198-53	14052	Undetermined
14053 WV	9/18/2011	13:29	37.64506	80.21504	RACCOON	F	AD	X	X	X	X	23	110198-54	14053	Undetermined
14054 WV	9/18/2011	14:30	37.6335	80.68122	RACCOON	F	AD	X	X	X	X	24	110198-55	14054	Undetermined
14055 WV	9/18/2011	15:05	37.62096	80.672	RACCOON	F	JUV	X	X	X	X	25	110198-56	14055	29.9788
14056 WV	9/18/2011	15:28	37.61995	80.67099	RACCOON	M	AD	X	X	X	X	26	110198-57	14056	38.3745
14057 WV	9/18/2011	16:19	37.61642	80.6678	RACCOON	F	AD	X	X	X	X	27	110198-58	14057	Undetermined
14058 WV	9/18/2011	16:23	37.61682	80.66807	RACCOON	M	AD	X	X	X	X	28	110198-59	14058	Undetermined
14059 WV	9/19/2011	8:19	37.63006	80.71993	RACCOON	F	JUV	X	X	X	X	29	110198-60	14059	Undetermined
14060 WV	9/19/2011	8:24	37.63054	80.71915	RACCOON	M	AD	X	X	X	X	30	110198-61	14060	Undetermined
14061 WV	9/19/2011	8:59	37.62905	80.71915	RACCOON	M	JUV	X	X	X	X	31	110198-62	14061	Undetermined
14062 WV	9/19/2011	10:06	37.64631	80.72919	OPOSSUM	F	JUV	X	X	X	X	32	110198-63	14062	Undetermined
14063 WV	9/19/2011	10:36	37.64806	80.7246	OPOSSUM	F	JUV	X	X	X	X	33	110198-64	14063	Undetermined
14064 WV	9/19/2011	11:51	37.62096	80.672	RACCOON	M	JUV	X	X	X	X	34	110198-65	14064	38.3116
14065 WV	9/19/2011	8:20	37.76711	80.61655	OPOSSUM	M	AD	X	X	X	X	35	110198-66	14065	Undetermined
14066 WV	9/19/2011	8:50	37.7649	80.61355	RACCOON	F	AD	X	X	X	X	36	110198-67	14066	Undetermined
14067 WV	9/19/2011	11:00	37.73752	80.60233	OPOSSUM	M	JUV	X	X	X	X	37	110198-68	14067	Undetermined
14068 WV	9/19/2011	11:50	37.75024	80.58485	RACCOON	M	AD	X	X	X	X	38	110198-69	14068	Undetermined
14069 WV	9/19/2011	12:40	37.76378	80.58132	RACCOON	F	AD	X	X	X	X	39	110198-70	14069	Undetermined
14070 WV	9/19/2011	12:50	37.76402	80.58195	GRAY SQUIRREL	F	AD	X	X	X	X	40	110198-71	14070	Undetermined
14071 WV	9/19/2011	13:20	37.76357	80.58318	OPOSSUM	M	AD	X	X	X	X	41	110198-72	14071	39.8211
14072 WV	9/19/2011	10:25	37.86937	80.4691	SKUNK	F	AD	X	X	X	X	42	110198-73	14072	Undetermined
14073 WV	9/19/2011	11:23	37.83705	80.45468	RACCOON	F	AD	X	X	X	X	43	110198-74	14073	34.5375

9/19/2011	11:49	37.83818	80.4557	OPOSSUM	M	JUV	X	X	X	X	44	110198-75	14074	Undetermined
9/19/2011	12:11	37.84025	80.45819	RACCOON	M	AD	X	X	X	X	45	110198-76	14075	Undetermined
9/19/2011	12:32	37.83743	80.46046	OPOSSUM	M	AD	X	X	X	X	46	110198-77	14076	Undetermined
9/19/2011	13:15	37.82646	80.45865	RACCOON	F	AD	X	X	X	X	47	110198-78	14077	Undetermined
9/19/2011	13:50	37.82688	80.46329	RACCOON	F	AD	X	X	X	X	48	110198-79	14078	Undetermined
9/19/2011	15:04	37.82713	80.43448	RABBIT	F	AD	X	X	X	X	49	110198-80	14079	Undetermined
9/20/2011	8:15	37.76627	80.58346	OPOSSUM	F	JUV	X	X	X	X	32	110780-1	14080	Undetermined
9/20/2011	8:45	37.76542	80.58667	OPOSSUM	M	AD	X	X	X	X	33	110780-2	14081	Undetermined
9/20/2011	9:30	37.76711	80.61655	RACCOON	M	JUV	X	X	X	X	34	110780-3	14082	Undetermined
9/20/2011	10:00	37.76738	80.61276	RACCOON	M	AD	X	X	X	X	35	110780-4	14083	Undetermined
9/20/2011	10:20	37.76369	80.61176	OPOSSUM	M	AD	X	X	X	X	36	110780-5	14084	34.2905
9/20/2011	11:45	37.74591	80.60189	RACCOON	F	JUV	X	X	X	X	37	110780-6	14085	Undetermined
9/20/2011	8:04	37.63045	80.71915	OPOSSUM	M	AD	X	X	X	X	38	110780-7	14086	Undetermined
9/20/2011	8:58	37.6294	80.71185	RACCOON	M	AD	X	X	X	X	39	110780-8	14087	Undetermined
9/20/2011	10:00	37.64346	80.72662	RACCOON	M	AD	X	X	X	X	40	110780-9	14088	Undetermined
9/20/2011	10:21	37.64444	80.73098	OPOSSUM	F	AD	X	X	X	X	41 11/18	110780-10	14089	Undetermined
9/20/2011	11:10	37.64506	80.71504	OPOSSUM	M	AD	X	X	X	X	42	110780-11	14090	Undetermined
9/20/2011	11:50	37.63867	80.68528	RACCOON	M	AD	X	X	X	X	43	110780-12	14091	Undetermined
9/20/2011	12:30	37.62096	80.672	RACCOON	F	JUV	X	X	X	X	44	110780-13	14092	34.1932
9/20/2011	10:03	37.83647	80.45802	RACCOON	F	AD	X	X	X	X	45	110780-14	14093	Undetermined
9/20/2011	10:24	37.83551	80.45918	OPOSSUM	M	AD	X	X	X	X	46	110780-15	14094	Undetermined
9/20/2011	11:07	37.83209	80.45869	RACCOON	F	AD	X	X	X	X	47	110780-16	14095	Undetermined
9/20/2011	11:33	37.82978	80.4574	RACCOON	M	AD	X	X	X	X	48	110780-17	14096	39.6448
9/20/2011	11:58	37.82919	80.45629	OPOSSUM	M	AD	X	X	X	X	49	110780-18	14097	Undetermined
9/20/2011	13:57	37.83285	80.42165	OPOSSUM	M	AD	X	X	X	X	50	110780-19	14098	Undetermined
9/20/2011	14:19	37.82788	80.42899	OPOSSUM	F	JUV	X	X	X	X	51	110780-20	14099	Undetermined
9/21/2011	8:20	37.76607	80.58416	RACCOON	F	JUV	X	X	X	X	7 11/18	111406-1	14100	Undetermined
9/21/2011	9:30	37.76542	80.58667	RACCOON	M	JUV	X	X	X	X	8	111406-2	14101	Undetermined
9/21/2011	10:20	37.7649	80.61355	RACCOON	M	AD	X	X	X	X	9	111406-3	14102	Undetermined
9/21/2011	11:20	37.75463	80.6142	RACCOON	M	AD	X	X	X	X	10	111406-4	14103	Undetermined
9/21/2011	12:50	37.75199	80.58295	RACCOON	F	JUV	X	X	X	X	11	111406-5	14104	Undetermined
9/21/2011	13:30	37.76378	80.58132	RACCOON	F	JUV	X	X	X	X	12	111406-6	14105	Undetermined
9/21/2011	8:30	37.63239	80.718	RACCOON	M	JUV	X	X	X	X	13	111406-7	14106	Undetermined
9/21/2011	10:05	37.64444	80.73098	RACCOON	M	JUV	X	X	X	X	14	111406-8	14107	Undetermined
9/21/2011	11:40	37.62096	80.672	OPOSSUM	F	JUV	X	X	X	X	15	111406-9	14108	Undetermined
9/21/2011	12:33	37.61682	80.66807	RACCOON	F	AD	X	X	X	X	16	111406-10	14109	Undetermined
9/21/2011	7:35	37.87078	80.46799	RACCOON	F	AD	X	X	X	X	17	111406-11	14110	Undetermined
9/21/2011	7:58	37.87462	80.46687	SKUNK	M	AD	X	X	X	X	18	111406-12	14111	Undetermined
9/21/2011	9:22	37.83705	80.45468	RACCOON	M	AD	X	X	X	X	19	111406-13	14112	Undetermined
9/21/2011	10:00	37.83505	80.45889	RABBIT	F	AD	X	X	X	X	20	111406-14	14113	Undetermined
9/21/2011	11:37	37.82667	80.43024	RACCOON	M	AD	X	X	X	X	21	111406-15	14114	Undetermined
9/22/2011	10:35	37.75416	80.61408	RACCOON	M	JUV	X	X	X	X	22	111406-16	14115	Undetermined
9/22/2011	11:30	37.76782	80.61362	RACCOON	M	AD	X	X	X	X	23	111406-17	14116	Undetermined
9/22/2011	11:35	37.76723	80.61722	RACCOON	F	AD	X	X	X	X	24	111406-18	14117	Undetermined
9/22/2011	10:41	37.82646	80.45865	RACCOON	M	AD	X	X	X	X	25	111406-19	14118	Undetermined
9/22/2011	11:02	37.83218	80.4613	OPOSSUM	M	AD	X	X	X	X	26	111406-20	14119	Undetermined
9/22/2011	11:17	37.83505	80.45889	OPOSSUM	M	AD	X	X	X	X	27	111406-21	14120	Undetermined
9/22/2011	9:47	37.64642	80.71317	RACCOON	F	AD	X	X	X	X	28	111406-22	14121	Undetermined
9/22/2011	9:54	37.64343	80.72237	RACCOON	M	JUV	X	X	X	X	29	111406-23	14122	Undetermined
9/22/2011	12:00	37.62096	80.672	OPOSSUM	M	JUV	X	X	X	X	30	111406-24	14123	Undetermined
9/22/2011	12:07	37.63054	80.71915	OPOSSUM	F	AD	X	X	X	X	31	111406-25	14124	Undetermined

Appendix D: Safety of ONRAB® in Select Captive Non-target Species

United States Department of Agriculture
Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	**Safety of ONRAB® in select non-target species.**
NWRC Study Director:	Tricia Fry
Approved NWRC Project:	Rabies Project - Methods and Strategies For Controlling

PROTOCOL CLASSIFICATION

1 ☐	NWRC staff are not involved in study design, data collection, experiments, or animal studies, **and** there is generally no commitment of NWRC resources other than personnel time, **and** activities are not regulated research activities. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 3 (Description of Activities)	Examples: • Writing or collaborating on review papers and synthesis reports • Student committee participation • Analyzing or writing up data collected under operational or other contexts
2 ☐	NWRC staff are not involved in study design, data collection or experiments, **but** the activity involves regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 3 (Description of Activities) ☐ Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable. ☐ Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]	Examples: • Training programs requiring the use of animals • Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) • Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 ☐	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **but** the NWRC portion of the study does not include regulated research activities*. Complete & Submit: ☐ Cover Page ☐ Part 1 (Signature Page) ☐ Part 4 (full NWRC Study Protocol) ☐ Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.	Examples: • Collaborating on study design, data analysis, or economic analysis. • Minor participation on a regulated study at the collaborating host institution • A study that does not include animal use, etc.
4 ☒	NWRC staff actively participate in all or some aspects of the research, **and** the study involves NWRC facilities and staff, **and** the study includes regulated research activities*. Complete & Submit: ☒ Cover Page ☒ Part 1 (Signature Page) ☒ Part 2 (Regulatory Considerations) ☒ Part 4 (full NWRC Study Protocol) ☒ Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.	Examples: • A typical NWRC led study • Major NWRC staff participation in regulated activity • Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director: Tricia Fry Date: _____

Position (check one):

☒ Biologist/Chemist/Technician
Supervisor signature required:
_____ Date_____ ☒ Res. Scientist ☒ Proj. Leader

☐ Research Scientist

☐ Project Leader

☐ Visiting Scientist: NWRC Representative/Contact:_____

☐ Student: NWRC Representative/Contact:_____

Concur:
NWRC Research Project Leader _____ Date _____

Review and Processing:
QAU:_____ Date _____

Concur:
NWRC Assistant Director _____ Date _____

Approved:
NWRC Director _____ Date _____

Note: Additional approvals are located in the attached appendices.

PART TWO: REGULATORY CONSIDERATIONS

NO	YES	Item
Animal Use		
☐	☒	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals. ☒ NWRC is responsible for all or part of live animal phase; attach **NWRC Animal Use Appendix** ☐ Collaborating institution is responsible for all or part of live animal phase; attach **IACUC protocol & approval** ☐ Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are not involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
☐	☒	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach **Microbiological/Biohazardous Materials Use Appendix**.
Permits		
☐	☒	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. – We are working on securing these permits. All permits will be in place prior to starting research. Colorado Special Collecting License - CODOW Biologic Permit for Research and Evaluation - CVB State of Colorado Importation Permit Permit(s) description Number Date
-		
☐	☒	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the **NEPA & ESA Appendix.**
☒	☐	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the **NEPA & ESA Appendix**. Contact QA/NEPA staff for ESA or eagle incidental take requirements.
☒	☐	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
☒	☐	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
☒	☐	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: _____
☒	☐	Will this study be conducted under any regulatory standard? If yes please check: ☐ *CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA)* ☐ Other:_____
☒	☐	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		
☒	☐	Will this study include the testing of any article, material or device? If yes, attach the **Test, Control and Reference Material/Devices Formulation and Use Appendix**. Please indicate if otherwise described in the protocol.
Historical Resources		
☒	☐	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
☐	☒	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate **Material Transfer Agreement**.
Analytical Chemistry		
☒	☐	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach **Analytical Chemistry Appendix**.

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
Tricia Fry	NWRC	PI
Other Investigators, Collaborators, Cooperators, and Consultants		
Kaci VanDalen	NWRC	Co-investigator
Kurt Vercauteren	NWRC	Co-investigator
Colleen Duncan	CSU	Co-investigator
Susan Shriner	NWRC	Consultant
J. Jeff Root	NWRC	Consultant

2. Testing Facilities

Name	Address	Role in Study
NWRC	Fort Collins, CO	Animal research, virology
CDC	Atlanta, GA	Diagnostic Testing
Colorado State University Veterinary Diagnostic lab	Fort Collins, CO	Necropsy, diagnostic testing

3. Sponsor

Name	Address	Contract No.
Artemis Technologies, Inc.	51 Watson Rd., Guelph, Ontario, Canada	pending

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: August 15, 2012
Proposed Study Completion/Archive Date: March 15. 2013

5. Background and Justification

The National Rabies Management Program (NRMP), administered by USDA-APHIS Wildlife Services (WS), is attempting to control and eventually eliminate terrestrial rabies in the United States. Oral rabies vaccines are distributed by hand and with the help of both fixed-wing aircraft and helicopters in most states east of the Appalachian Mountains as well as portions of Ohio, Texas, New Mexico, and Arizona. It is estimated that at least 50% of raccoons in these areas require vaccination for the oral rabies vaccination (ORV) program to be successful (Hethcote 1978). At present, it is estimated that approximately 30% of raccoons are vaccinated (Slate et al. 2009) by the WS ORV program.

Raboral V-RG® (Merial Ltd., Athens, Georgia), the vaccine presently used in the program, is a recombinant vaccine comprised of a live vaccinia virus with the rabies glycoprotein gene inserted into its genome. Presently, raccoons, gray

foxes, coyotes, and possibly other terrestrial mammals can be vaccinated by ingesting this liquid vaccine, which is contained in a sachet. Ideally, when the animal bites into the sachet the liquid vaccine coats the mucosal lining of the mouth and stimulates an immune response. However, the efficacy, as measured by immunogenicity, of this vaccine is limited. Brown et al. (2010) reported that of 19 wild raccoons vaccinated by Raboral V-RG, 14 succumbed to a rabies challenge. Additionally, cross-border evaluations conducted in the US by WS NRMP operations suggested that approximately 30% of raccoons vaccinated in the US by Raboral V-RG demonstrated detectable (0.05 IU/ml) rabies virus neutralizing antibodies (rVNA), while nearly 70% of raccoons administered ONRAB on the Canadian side of the border showed rVNA tites ≥ 0.5 IU/mL (L. Kempf, ME WS, pers. comm.). The lack of congruity in these two vaccines leads to concern at many levels, thus the WS NRMP is preparing to field test the ONRAB vaccine manufactured by Artermis Technologies (Guelph, Ontario, Canada).

ONRAB, like Raboral V-RG is a recombinant vaccine. However, the viral vector in ONRAB is human adenovirus type 5 (Had5). Had5 is a relatively safe and well-studied virus, which is used in many vaccine formulations. Wild-type Had5 often causes mild respiratory or gastrointestinal manifestations although immunocompromised individuals may develop a more serious disease (Textbook of Pediatric infectious diseases, Vol 2: Ralph Feigin). Extensive studies on the efficacy and safety of the ONRAB vaccine in animals have been completed in Canada (Yarosh et al. 1996, Lutz-Wallace et al. 1995, Randrianarison-Jewthoukoff and Perricaudet 1995, Rosette et al. 2009, Knowles et al. 2009), and the encouraging findings of these studies initiated ONRAB's distribution via aerial bait drop in 2006 targeting raccoons and skunks. Rabies VNA seroconversion rates in areas baited with ONRAB averaged 80% in the first two years (Rosatte et al. 2009), well above the seroconversion rates when the vaccine Raboral V-RG is used (Slate et al. 2009, Brown et al. 2010).

Canadian researchers have conducted vaccine efficacy and safety studies on 18 species: striped skunk (*Mephitis mephitis*), red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), meadow vole (*Microtus pennsylvanicus*), deer mouse (*Peromyscus leucopus*), grey squirrel (*Sciurus carolinensis*), laboratory rabbit (*Oryctologu cuniculus*), ground squirrel (*Marmota monax*), cow (*Bos taurus*), horse (*Equus ferus*), pig (*Sus domesticus*), chicken (*Gallus domesticus*), sheep (*Ovis aries*), dog (*Canis familiaris*), cat (*Felis domesticus*), cotton rat (*Sigmodon hispidus*), SCID mouse (*Mus musculus*), and nude mouse (*Mus musculus*, Knowles et al. 2009). For the non-target safety studies, researchers administered an ONRAB dose 10 times the dose needed to vaccinate target species. Animals were monitored daily and all tolerated exposure to the vaccine. Analyses of tissues resulted in viral RNA detected in some lung and kidney samples. Following histopathological analysis, the most marked findings were in the lungs with pulmonary congestion and some possible vaccine aspiration effects. Oral swabs and/or fecal samples were collected from skunks, raccoons, fox, cotton rats, cats and dogs. Viral shedding (if any) was generally complete by 4 days post-inoculation (dpi) except for one skunk, which continued to shed virus in feces through 14 dpi (Knowles et al. 2009).

Our research will expand on the species evaluated by Knowles et al. and investigate the vaccine as it relates to its safety in wildlife species likely to come in contact with the ONRAB vaccine as part of WS NRMP ORV operations, as well as, identify whether Had5 shed from certain wildlife species may increase of the chance of humans contacting the Had5. We do not expect, based on previous research and the use of ONRAB in field operations, for the vaccine to negatively affect the health and well-being of animals used in this study. Knowles et al. did not note any clinical signs in the animals tested as part of her study and only a fraction of individuals shed the human adenovirus. This research is being conducted and is essential in order to confirm these results and to protect wildlife and domestic animals in the United States. The conclusions of our research are required by the Center for Veterinary Biologics to assist in determining if additional research is necessary to license ONRAB in the United States.

6. Related Protocols

none

7. Assurance of Non-Duplication of Studies

An extensive literature review using the databases Scopus and Biological Abstracts was conducted as well as personal communication with researchers in Ontario, Canada. While some safety studies on the effects of ONRAB in non-target species have been completed in Canada, it is the first time the effects of ONRAB® will be tested in the species included in this protocol.

8. Objective/Hypotheses

The objective of this study is to assess the safety of ONRAB® in non-target wildlife species found in the eastern U.S. and evaluate the possible shedding of Had5 viral DNA.

9. Methods/Procedures

While initial safety studies conducted in Canada provide a wealth of information the Veterinary Services Center for Veterinary Biologics (CVB) believes there is reason to continue to explore the potential effects of ONRAB in additional species, specifically wildlife that are likely to come in contact with the vaccine as part of ORV campaigns. We propose to investigate the effects of high doses of the ONRAB vaccine in wood rats (*Neotoma cinerea*), eastern cottontail rabbits (*Sylvilagus floridanus*), opossums (*Didelphis virginiana*), Eastern wild turkey (*Meleagris gallopavo silvestri*), and fox squirrel (*Sciurus niger*), species not considered by Canadian researchers, but whose habitat overlaps with ORV target species in the U.S. To evaluate the effects of ONRAB, we will attempt to isolate Had5 viral DNA through the collection of oral, rectal/clocoal, and/or fecal swabs, and via post mortem examination. Shedding will allow us to determine if environmental contamination and potential transfer between and among species is a risk. In addition, we will look at the immunogenicity of the vaccine and determine, at necropsy, any gross or histological pathology that may be linked to the vaccine.

Trapping and Accessioning Study Animals

All animals will be accessioned into the NWRC ARB population and placed in quarantine for a minimum of 14 days (SOP AC/CO 016.00). Prior to vaccination we will restrain and/or anesthetize all animals with isoflurane and collect baseline blood samples and evaluate serum for rabies VNA using the RFFIT test. Rabies VNA will be determined prior to the start of the study, animals that have rabies titers > 0.5 IU/mL will not be included in the study. We will also examine the animal for health and dust the animal for ectoparasites as appropriate. Species specific methods will be detailed below. Two to four individuals of each species will be held as controls. Controls will be held as substitute animals and to confirm the absence of Had5in tissues and products of non-treated animals. Each treatment group will have a minimum of 16 vaccinated animals at the start of the study. With a minimum of 16 animals per treatment group we will be able evaluate shedding and the distribution of the virus in tissue at two time intervals, 4 and 28 days post vaccination durations consistent with the research conducted in Canada by Knowles et al. (2009).

Eastern Wild Turkey – Twenty-four Eastern wild turkeys will be purchased from a commercial turkey breeder (e.g. McMurry Hatchery, Webster City, Iowa). ONRAB will not be administered to turkeys that are less than 90 days old. Blood from turkeys will be collected via jugular, leg vein, or brachial vein, no more than 1-3cc of blood will be collected once the turkeys are over 30 days old.

Fox Squirrels – Fox squirrels will be trapped from within Larimer County, Colorado, (n=16-24) using appropriate sized

Tomahawk and/or Sherman traps and baits such as peanuts, fruit, and oats, traps will be checked daily. Cottontails will be transported to NWRC in the bed of a pickup truck covered either with a cap or a tarp. Fox squirrels will be held with the Animal Research Building or Invasive Species Research Building at the NWRC. Squirrels will be housed individually in appropriate sized cages, a den box and enrichment objects will be provided. When squirrels are accessioned into NWRC facilities, blood will be collected via the lateral femoral vein or the saphenous vein and no more than 3 cc of blood will be collected.

Wood rats – Wood rats will be trapped from within Larimer County, Colorado, (n=16-24) using appropriate sized Tomahawk and/or Sherman traps baited with peanut butter and oats, traps will be checked daily. Wood rats will be transported to NWRC in the bed of a pickup truck covered either with a cap or a tarp. Wood rats will be held in the Animal Research Building or Invasive Species Research Building at the NWRC. Wood rats will be housed individually in appropriate sized cages, a den box and enrichment objects will be provided. When woodrats are accessioned into NWRC facilities, blood will be collected via the jugular, tail, or lateral femoral vein or via the saphenous vein and no more than 0.7 to 2 cc of blood will be collected.

Opossums – Opossums will be collected with the help of the Nebraska Wildlife Service's Operations program. Prior to being transported opossums will be held in tomahawk traps or dog crates in shaded areas. They will be provided food (an omnivore kibble and fresh fruits or vegetables) and water daily. Animals will be held a maximum of 10 days before being transported to the NWRC. Opposums will be transported in a covered trailer or bed of a pick-up truck (with a cap) to NWRC. . A waterer such as the 16oz. Lixit Pet Fount will be fastened in all cages to provide water or ice chips will be provided every 4-6 hours,. Food will be provided every 24 hours during transport.

Opossums will be housed individually in the Outdoor Animal Research facility either in 10'x10'x8 pens that include den boxes and enrichment structures or in building 11, dependent on ambient temperatures, final housing arrangements will be made by the Attending Veterinarian. When opossums are accessioned into NWRC facilities, blood will be collected via an appropriate vessel such as the jugular or femoral vein and no more than 5 cc of blood will be collected.

Eastern Cottontail Rabbits – Sixteen to twenty-four eastern cottontail rabbits will be trapped from within Larimer and Weld Counties, Colorado using appropriate sized Tomahawk traps using vegetable baits, traps will be checked daily. Cottontails will be transported to NWRC in the bed of a pickup truck covered either with a cap or a tarp. Rabbits will be housed in the Animal Research Building or Invasive Species Research Building at the NWRC in appropriately size cages and husbandry practices will follow SOP AC/CO 015.02 Rabbit Maintenance ARB. When rabbits are accessioned into the NWRC population they will be dusted for ectoparasites and blood will be collected from the medial or lateral ear vein, the lateral femoral vein, or other appropriate vessel. No more than 3cc of blood will be collected per individual. If rabbits cannot be physically retrained they will be anesthetized with isoflurane.

Vaccination

In order to determine the titration of the ONRAB virus and determine the most appropriate doses we will perform a $TCID_{50}$ on the vaccine prior to administering to animals. We will follow the methods for conducting a $TCID_{50}$ outlined by Knowles et al. (2009b). The volume of ONRAB given to each species will be determined after the $TCID_{50}$ is confirmed with the goal of obtaining a $TCID_{50}$ up to 10 times greater than typical ORV dose with a $TCID_{50}$ of $10^{9.5}$. We will administer the ONRAB orally to physically restrained, but not anesthetized animals using a needleless syringe.

Post Vaccination monitoring

We will evaluate the safety of the ONRAB vaccine via numerous tests and observations. On a daily basis we will monitor animals for changes in eating habits, behavior or changes in health. We will look for shedding of Had5 DNA via

oral and nasal swabs in the case of mammals, oropharyngeal swabs for turkeys, and in feces for all species tested. Prior to administering the vaccine we will collect pre-treatment samples and then repeatedly sample individuals on days 4, 7, 14, 21, 28 (post-treatment) for oral and nasal swabs. Fecal swabs will be collected on days 0-7, 14, 21, 28. On sampling day s 4, 7, 14, 21, 28 , we also will collect blood samples (in the volumes indicated above for each species upon accession into our facility) to monitor blood sera for rabies virus neutralizing antibodies. Rabies virus neutralizing antibodies will be determined via RFFIT to an endpoint titer at the CDC, Atlanta.

One-half of the study animals for each species will be euthanized at day 4 post vaccination, when viral replication should be at or near its peak, with the other half being euthanized at the conclusion of the study (approximately day 28) following the protocol described by Knowles et al. 2009. After being humanely euthanized, each animal will be necropsied by a board certified veterinary pathologist animal. All gross findings will be reported as will findings related to histology, all histology will be completed at the Colorado State University Veterinary Diagnostic Laboratory,. Select tissues will also be examined for the presence of Had5 viral DNA. In mammal species, tissues examined for Had5 DNA and histology will include lung, nasal turbinate, small intestine, large intestine, liver and kidney. Tissues examined for Had5 DNA and histology from wild turkeys will include lung, small intestine, large intestine, liver and kidney. We will use quantitative real-time PCR analysis to evaluate tissues and swabs for Had5 DNA following procedures described by Knowles et al. (2009b).

10. Experimental Design and Statistical Analyses

The number of animals used for each species will be approximately N= 16 - 22 inoculated individuals plus 2 controls. The number of individuals for each species is consistent with the published vaccine safety study from Canada (Knowles 2009) and other published, experimental wildlife vaccine work (Grosenbaugh 2007, Henderson 2009). These sample sizes will be sufficient for publication and for licensure consideration by the VS-Center for Veterinary Biologics (CVB).

We will calculate summary statistics such as percentage of animals that shed for each species/sample type; the mean concentration of viral RNA found in swabs, feces, and tissues; mean duration of viral RNA shedding; percentage of individuals with lesions; and percentage of individuals that show an antibody response.

If animals do shed virus, the time period of shedding and the mean concentration of virus found in swabs and tissues will be characterized for each day post inoculation.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
AC 004.00	Handling and restraint of medium-sized mammals
HS 004.00	Personal Protective Equipment
BT 013.01	Inventory and storage procedures for BL-2 agents and diagnostic samples
FP 013.00	Medium sized mammal capture, handling, and transport to NWRC
AC/CO 027.00	Raccoon maintenance for OARF
ACCO 16.00	Animal quarantine procedures at Fort Collins.
AC/CO 105.02	Rabbit Maintenance ARB should

12. List of Records to be Maintained

A. Protocol and Amendments
B. Correspondence, telephone logs and related records
C. Data records including:
    a. Diagnostic testing results

b. Data sheets
c. Laboratory notebooks
d. Animal records

D. Trapping Permit
E. Final Report

13. Cost Estimate for Each Fiscal Year

	FY-11	FY-12		
A. Salary and Benefits	$45,000	$15,000		
B. Facilities (in addition to existing facility or space costs)	$0	$0		
C. Equipment	$0	$0		
D. Supplies	$30,000	$3,000		
E. Animal Care Costs	neg.	$0		
F. Operating Costs (travel, misc. services, etc)	$5,000	$5,000		
TOTAL	$80,000	$23,000		

14. Human Health and Safety

Employees will be informed of necessary safety precautions and procedures in SOPs and by the study director. This includes safety procedures when handling ONRAB® in both the laboratory and pens, as well as chemical immobilization, and blood collection of study animals.

15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. All SOPs and study specific training logs will be completed and documented in study or personnel records prior to participation in that aspect of the study. Individuals working independently or leading a crew will be limited to Kaci VanDalen, or Tricia Fry.

16. Archiving

All raw data, documentation, records, protocols, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

Brown, L. J., R. C. Rosatte,C. Fehlner-Gardiner, M. K. Knowles, P. Bachmann, J. C. Davies, A. Wandeler, K. Sobey, and D. Donovan. 2011. Immunogenicity and efficacy of two rabies vaccines in wild-caught, captive raccoons. Journal of Wildlife Diseases, 47(1): 182–194.

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Grosenbaugh, DA, Maki, JL, Ruppercht CE, Wall DK. Rabies challenge of captive stiped skunks (*Mephitis mephitis*) following oral administration of a live vaccinia-vectored rabies vaccine. Journal of Wildlife Diseases 2007; 42(1) 124-128.

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Slate, D., Algeo, TP, Nelson, KM, et al. Oral rabies vaccination in north america: opportunities, complexities, and challenges. PLOS 2009; 3(13)1-9, e529.

Yarosh OK, Wandeler AI, Graham FL, Campbell JB, Prevec L. 1996. ;Human adenovirus type 5 vectors expressing rabies glycoprotein. Vaccine 14:1257–64.

19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☐ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☒ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☐ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title)_____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Animal Use Appendix

An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

A. Animal Description

1) Animals: Eastern Woodrat
- Species, subspecies (if applicable): *Neotoma floridana*
- Breed, strain and substrain (if applicable): not applicable
- Total Number and Sex: 16 to 24, mixed, we will have a minimum of 6 individuals of each sex
- Body weight range: 0.5 to 1.5 lb
- Age: all ages

2) Animals: Cottontail Rabbit
- Species, subspecies (if applicable): *Sylvilagus floridanus*
- Breed, strain and substrain (if applicable): not applicable
- Total Number and Sex: 16 to 24, mixed, we will have a minimum of 6 individuals of each sex
- Body weight range: 1.5 to 5 lb
- Age: all ages

2) Animals: Opossum
- Species, subspecies (if applicable): *Didelphis virginiana*
- Breed, strain and substrain (if applicable): not applicable
- Total Number and Sex: 16 to 24, mixed, we will have a minimum of 6 individuals of each sex
- Body weight range: 4 -14 lb.
- Age: >1 year old

2) Animals: Fox squirrel
- Species, subspecies (if applicable): *Sciurus niger*
- Breed, strain and substrain (if applicable): not applicable
- Total Number and Sex: 16 to 24, mixed, we will have a minimum of 6 individuals of each sex
- Body weight range: 1 -2 lb
- Age: all ages

2) Animals: Eastern Wild Turkey
- Species, subspecies (if applicable): *Meleagris gallopavo silvestri*
- Breed, strain and substrain (if applicable): not applicable
- Total Number and Sex: 16 to 24, mixed, we will have a minimum of 6 individuals of each sex
- Body weight range: approximately 100 g when shipped, will not be vaccinated for at least 90 days after arrival
- Age: 1-2 days at arrival

B. Rationale for involving animals, for appropriateness of species, and for numbers. Provide justification why this study requires the use of animals, and for the numbers to be used.

1) Rationale for involving animals: The only method to evaluate the safety of ONRAB® is to test the vaccine in non-target species, we will evaluate safety by various means including determining if non-target species shed the human adenovirus, the vaccines immunogenicity, and identify if systemic effects of the vaccine are noted. These questions can only be obtained through live animal studies, no surrogate is possible.

2) Rationale for appropriateness of the species to be used: The species selected for this safety study compliments the list of non-target species evaluated in Canada (Knowles et al. 2009). The species we selected are typical residents of the ecosystems where ORV campaign are held. These species are likely to encounter, investigate, and possibly ingest the vaccine when encountered. Knowing the effects on these species is integral for the safe delivery of the vaccine.

3) Rational for numbers of animals to be used (include description of any animals to be obtained as extra if appropriate):
Our research aguments Knowles et al. (2009) findings and our sample size of 16-22 individuals sampled to day 4 is appropriate based on what is known about the humanadeno virus. Shedding beyond day 4, when our sample size decrease by 50% is not likely to occur. We will have multiple samples from each individual over the course of the study (eg. Feces – n= 60 samples/species by day 4) thus increasing the likelihood that we will be able to detect virus shedding. With this design we will be able to detect virus shedding when only 5% of the population is involved. This resolution is consistent with other vaccine safety studies.

C. Source

Woodrats, fox squirrels, and cottontail rabbits will be trapped from Larimer and/or Weld Counties, Colorado. Under permit TR1232 issued by the Colorado Division of Wildlife.

Opossums will be trapped with the help of Wildlife Services Operations in Oklahoma or Nebraska and transported to NWRC. Appropriate trapping permits and import permits be obtained, prior to trapping.

Wild Turkeys will be purchased commercially.

D. Method of identification of animals

Animals accessioned into the OARF will be individually identified using leg bands (Turkeys) and ear tags (mammals). Cage cards will also identify individuals.

E. Trapping/Collecting

All mammals will be trapped in appropriate size Tomahawk live trap and traps will be checked once to twice daily.
Woodrats and squirrels - 19 x 6 x 6 (checked a minimum of once daily)
Rabbits – 26x9x9 (checked a minimum of once daily)
Opossums - 26x9x9 or 32x10x12 (checked a minimum of once daily)
Our intent is to trap opossums in Nebraska or Oklahoma and transport them to the NWRC. Prior to being transported opossums will be held in tomahawk traps or dog crates in shaded areas. They will be provided food and water daily. Food offered will an omnivore diet kibble, such as dog food and fresh fruits and vegetables. Animals will be held a maximum of 10 days before being transported to the NWRC.

Turkeys will be shipped from a commercial dealer.

F. Transport

For animals trapped locally, during extreme weather (>90 degrees Fahrenheit) , trucks with covered beds or tarps will be used to protect the animals.

Opossums will be transported in the Tomahawk traps (26x9x9 or 32x10x12) in the bed of a pickup truck (covered with a cap or canvas tarp) or covered trailer. If ambient temperatures exceed 90 degrees Fahrenheit we will transport opossum

during cooler hours of the day. Animals will be held in shaded areas. A waterer such as the 16oz. Lixit Pet Fount will be fastened in all cages to provide water or ice chips will be provided every 4-6 hours,. Food will be provided every 24 hours during transport.

We will not trap or transport animals if temperatures are below 32F.

G. Handling/restraint

For Opossums we will follow the SOP - AC 004.00-Handling and restraint of medium sized mammals.

Handling of squirrels and woodrats will be faciliated by using a conical cloth bag placed over the entrance of traps that allows squirrels to move from the trap directly into the restraint. This will minimize the risk of escape and injury, reduce the amount of handling time, and allow for normal respiration. Additional and appropriate handling techniques maybe employeed including the use of plastic restaint bags called DecapiCones (www.braintreesci.com).

Rabbits will be restrained in a cloth bag containing a zipper that only allows the head to be outside the bag.

If needed all mammals will be retrained using an inhalant anestheia such as isoflurane.

Turkeys will be physically retrained.

H. Quarantine

All animals will be quarantined for a minimum of 14 days following SOP AC/CO 016.00 Animal quarantine procedures at Fort Collins

I. Housing/maintenance

Fox Squirrels and woodrats will be maintained in stainless steel rabbit racks following SOP AC/CO 036.00.
Rabbits will be maintained in plastic cages on steel racks specifically designed for rabbits in the ARB (SOP AC/CO 015.00).
Opossum will be housed in the OARF in outdoor animal pens such as those typically used for raccoons. Determination of exact pen configureation will be made by the Attending Veterinarian and dependent on space. Opossums maybe housed in either elevated coyote cages (eg, building 17) or raccoon cages (eg, building 13).

Upon arrival turkeys will be housed in stock tanks with appropriate food, bedding, water and heat. As they grow, their housing will be adjusted appropriately as per the Attending Veterinarian. Housing will depend on size of the birds and whether housing them individually is preferable to group housing.

J. Dietary contaminant exposure

none

K. Disposition of animals

At the completion of this study, all mammals will be anesthetized and euthanized either via an overdose of barbiturates via intracardiac or intravenous injection or with the use of isoflurane and $C0_2$. Turkeys will be euthanized via an IV injection of barbiturates in the leg vein. Alternative methods for euthanasia as listed in the 2007 AVMA Guidelines for Euthanasia may be used if deemed necessary these will be approved by the attending veterinarian. All animal carcasses will be double-bagged and digested or incinerated through the Colorado State University Diagnostic Lab.

L. Animal pain or distress

1) Consultation with Attending Veterinarian:
Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Note: Consult separately, and with appropriate advance notice, the Animal Facilities Supervisory Personnel for space allocation in designated Animal Facilities.

Name of Attending Veterinarian; Gordon Gathright

Date of Consultation: Monday, May 9, 2011

2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian ?

☒ No

☐Yes If yes, continue with the following items.

a) Alternative procedures:
not applicable

b) Sedatives, analgesics, or anesthetics or Column E Explanation:
not applicable

c) Surgery:
No surgical procedures are necessary for this research.

M. Euthanasia

All mammals will be anesthetized (with gas inhalant or ketamine/xylazine) and euthanized either via an overdose of barbiturates via intracardiac or intravenous injection or with the use of isoflurance and $C0_2$. Turkeys will be euthanized via an IV injection of barbiturates in the leg vein. Alternative methods for euthanasia as listed in the 2007 AVMA Guidelines for Euthanasia may be used if deemed necessary these will be approved by the attending veterinarian.

N. IACUC Approval

Date of IACUC Approval Letter:_____

O. Staff Qualifications

Tricia Fry, Wildlife Biologist, has been working on rabies research for nearly 5 years and has extensive experience in handling raccoons, small mammal and birds, I and E drugs, and veterinary biologics.

Kaci VanDalen, Biologist, as BSL-3 laboratory manager, is proficient in all laboratory procedures that will be conducted as part of this study. She is trained in the use of I and E drugs and has been handling and cooperating with the study director on a number of studies for the last 3 years. Kaci has handled a variety of small mammals and birds over the course of her research.

Shylo Johnson, Biologist, has been working with wildlife and rabies research for the last 5 years. She has led research projects that include the handling of raccoons and the use of I and E drugs.

Cody Minor, Wildlife Science Technician, Cody has been working at a Technician for two years. He is trained in WS I and E drug use and has trapped raccoons as part of the Rabies Project. He has exceptional animal handling skills and will be involved with the trapping and transporting animals for the purpose of this study.

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NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☒ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☐ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

- ☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity
- ☐ B) not cause contaminants to enter water bodies
- ☐ C) not adversely affect any federally protected species or critical habitat
- ☐ D) not cause bioaccumulation

☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

When trapping animals we will trap at multiple locations as to not remove a large number of animals from any one property. In no way will our trapping efforts effect the populations of the species we are targeting.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

Any non-target species trapped in our Sherman/Tomhawk live traps will be immediately released.

Effects on T&E species and eagles:
Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? ☒ No ☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact: ☐ Other:
Consultations:
Did you consult with a state or federal agency specifically on this action. ☒ No ☐ Yes If yes, describe the date/mode/contact person and outcome of this consultation:
Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner. ☐ No, permission not needed because: ☐ Yes ☒ Other: Permission will be obtained to trap prior to entering private property.

Microbiological/Biohazardous Materials Use Appendix

NWRC proposed research or testing activities which involve the use of microbiological organisms or biohazardous agents at or above a Biosafety Level 2 or Risk Level 2, or use recombinant DNA *in vivo*, require this appendix to be completed and submitted to the NWRC IBC for review and approval.

Reference the Centers for Disease Control's (CDC) "Biosafety in Microbiological and Biomedical Laboratories (BMBL)," current (BMBL) edition at www.cdc.gov/od/ohs/biosfty/biosfty.htm for the definitions and lists of BioSafety Level 2 organisms and above.

Reference the American Biological Safety Association's (ABSA) "Risk Group Classification for Infectious Agents" at http://www.absa.org/resriskgroup.html for the definitions and lists of Risk Level 2 agents and above.

Reference the National Institute of Health's (NIH) Guidelines for Recombinant DNA and Gene Transfer at www4.od.nih.gov/oba/rac/documents1.htm for specific practices for constructing and handling recombinant DNA and organisms/viruses containing recombinant DNA molecules. Definition of recombinant DNA; 1) Molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or 2) Molecules that result from the replication of those in 1 above.

A. Identify the organism(s)/agent to be used (e.g., species, strain, type, etc.):
AdRG1.3 – ONRAB®. A replication-competent human adenovirus rabies glycoprotein recombinant vaccine.

B. Is this a Select Agent (see www.selectagents.gov/agentToxinList.htm)?
No

C. Does the organism contain recombinant DNA, or will recombinant DNA be constructed *in vivo* as a biologically active polynucleotide or polypeptide product? If yes, then address each of the following (if no, then N/A):

1. The source(s) of the DNA.
   Human adenovirus 5 (HAd5) and rabies G gene of the ERA strain
2. The nature of the inserted DNA sequences.
   Rabies glycoprotein responsible for rabies virus binding and cell entry
3. The host(s) and vector(s) to be used.
   HAd5 is the vector with the rabies G gene inserted into the genome.
4. Will an attempt be made to obtain expression of a foreign gene? If so, indicate the protein that will be produced.
   Rabies G will be expressed as a result of in vivo trials in wildlife species specified in this protocol and in vitro vaccine titration in tissue culture.
5. The containment conditions that will be implemented.
   Laboratory studies and most animal studies will be conducted under BSL-2 conditions. Previous safety trials in Canada (Knowles, 2009) were conducted in BSL-2 containment facilities. However, similar vaccine studies (RABORAL V-RG) have been previously conducted at the NWRC in outdoor animal pens with enhanced biosafety precautions. Therefore, if indoor space is limited, outdoor animal pens at NWRC may also be used with enhanced biosafety precautions such as waste autoclaving and PPE.

D. Source of the organism(s)/agent (e.g., location or name and address of lab/vendor):
Artemis Technologies, Inc.
Guelph, Ontario, Canada

E. Procedures for shipping and transportation (e.g., from facility to facility, and from room to room):

ONRAB vaccine will be shipped from Artemis Technologies via standard shipping requirements for biologics and dry ice. Throughout the NWRC (Ft Collins), vaccine will be transported on ice in sealed, leak-proof, shatter-proof bio-transport containers.

F. Location(s) where the materials are to be used and stored (include all buildings and room number and laboratories):
All storage and usage will be at NWRC, 4101 LaPorte Ave, Fort Collins, CO.

ONRAB will be stored at -80°C in Room 222 of the WSB.

ONRAB may be used in WSB 222, WSB 160, ARB 14N, ARB animal rooms, OARF 11, and/or OARF Bldg 13, 14, or equivalent

G. Permit information:
Application for a VS Permit for Biologic Research and Evaluation has been submitted.

H. Inventory and tracking procedures (e.g., chain of custody procedures):
Inventory and tracking of ONRAB will be kept in the QA laboratory notebook, as well as electronically.

I. Quality control measures (e.g., procedures to prevent contamination of stocks): Manipulation of ONRAB in BSL-2 laboratories will take place in certified HEPA filtered biosafety cabinets by trained personnel.

Agent Hazards:

J. What particular hazards to humans, animals, and the environment are associated with these organisms/agents? (e.g., infective dose, severity of disease, mode of transmission, susceptibility to humans, stability in the environment, etc.)
Had5 (the backbone of ONRAB) is a relatively safe and well-studied virus, which is used in many vaccine formulations. Wild-type Had5 often causes mild respiratory or gastrointestinal manifestations although immunocompromised individuals may develop a more serious disease (Textbook of Pediatric infectious diseases, Vol 2: Ralph Feigin). Accidental needle-sticks or other suspected human exposures will be reported to the NWRC Safety Officer. No vaccinations or post exposure care (other than washing any potential entry sites) should be necessary. Any signs or symptoms more severe than mild-, cold-like or GI symptoms should be reported to a physician immediately.

Extensive studies on the efficacy and safety of the ONRAB vaccine in animals common to Canada have been completed (Yarosh et al. 1996, Lutz-Wallace et al. 1995, Randrianarison-Jewthoukoff and Perricaudet 1995, Rosette et al. 2009, Knowles et al. 2009). According to Knowles et al. (2009) of the individuals that shed the virus in feces the vast majority stopped shedding 3-4 days after the vaccine was administered. In a single case, one skunk did continue to shed virus in feces for 14 days.

The stability of the ONRAB recombinant vaccine in natural environments is unknown (or unpublished). However, adenoviruses, in general, are relatively stable (>80 days) in surface and ground water at cool temperatures (10°C and 19°C; Rigotto 2011). In the laboratory, adenoviruses typically survive for <10 days for porous surfaces but up to 12 weeks on non-porous surfaces (Valtierra, 2008).

Laboratory Procedure Hazards:

K. Estimated volume, amount or concentration of agents or solutions:
Approximately 300 mL of ONRAB vaccine will be used at an approximate concentration of $10^{10.3}$ $TCID_{50}$/mL or lower.

L. Identify known or potential sources of contamination or exposure (e.g., infected live animals, tissues, fluids, byproducts, waste, sharps, etc.)
Study participants may be inadvertently exposed to ONRAB through recently vaccinated animals and contaminated sharps. Proper PPE (gloves, protective eye wear, lab coats, overalls, etc) will be worn and sharps safety handling will be followed to minimize potential exposures.

M. Identify any procedures and equipment which could produce aerosols (e.g., pipetting, blenders, centrifuges, sonication and vortexing), and describe how the creation of aerosols and/or exposures to those aerosols will be minimized.
Aerosols may be produced during vortexing of ONRAB stock. All vortexing and other manipulations of the stock vaccine (except for actual vaccination of study animals) will be performed in a biosafety cabinet.

Biosafety, Security and Additional Precautions:

N. Biosafety Level / Risk Level (from the CDC or ABSA reference above): BSL-2

O. Biosecurity Plan: (the Biosecurity Plan is a description of a number of different aspects which together define the mechanisms by which biohazardous agents will be safely and securely used)

1. **Physical Security:**
   ONRAB® stock will be stored in a locked -80°C freezer within the locked WSB 222 Rabies virology laboratory.

2. **Biosecurity:**
   ONRAB vaccine stock will be stored in a locked -80°C freezer within the locked WSB 222 Rabies virology laboratory. Vaccine stock may be titrated on 293 cells in ARB 14N (tissue culture), which has restricted badge-entry access. BSL-2 practices will be followed for all vaccine manipulations in WSB 222 and/or in ARB 14N (tissue culture laboratory). Only personnel trained in BSL-2 practices and biosafety cabinet use will be allowed to manipulate the vaccine. All laboratory waste (disposable pipet tips, plates, etc) will be autoclaved. If disposable waste must be transported outside of the laboratory, it will be double-bagged with the first bag sprayed with disinfectant prior to placing into the second bag. Bagged waste must then be transported within the facility in a labeled, hard-sided, closed container for final disposition (incineration or autoclaving). All potentially contaminated non-disposable items will be surface decontaminated with 70% ethanol (or equivalent). When transported between the laboratory and animal pens, ONRAB will be carried in a leak-proof, shock-proof biosafety transport carrier.

   During vaccination of animals, a minimum number of trained individuals will orally deliver (via needleless syringe or pipet) the vaccine. Most adult individuals have had natural adenoviral infection early in childhood, which results in immunity to subsequent adenoviral infections so risk of HAd5 infection is low. Rabies vaccination will also be offered and documented for all personnel. Appropriate clothing including Tyvek/coveralls, gloves, boot covers, eye protection, and N-95 masks (95% filtration) will be worn when inoculating animals with ONRAB. Measures will be taken to avoid vaccine spillage and animals will be placed on absorbent liners when vaccinated. In the event of a spill the area will be disinfected with 1% Virkon (or equivalent). All disposable waste (eg. Gloves, absorbent towels, contaminated syringes) will be double-bagged with the first bag sprayed with disinfectant prior to placing into the second bag. Bagged waste must then be transported within the facility in a labeled, hard-sided, closed container for final disposition (incineration or autoclaving). Any potentially contaminated work surfaces will be surface decontaminated with 70% ethanol or equivalent.

   At the conclusion of the study, all animal carcasses will be double-bagged with the first bag sprayed with disinfectant prior to placing into the second bag. Bagged carcasses will then be transported in a labeled, hard-

sided, closed container to the CSU Veterinary Hospital for necropsy. Carcasses will be sent to chemical digesters as per their disposal protocol.

P. Specialized Risk Control Measures:

All laboratory staff will follow BSL-2 standards as stated in the BMBL 5th edition. Lab coats and gloves will be worn at all times. When working in indoor animal rooms, ABSL-2 standards as stated in the BMBL 5th edition will be followed. Staff will wear tyveks or lab coats, and foot coverings dedicated to each room. Gloves and eye protection will also be worn. N-95 masks will be worn during inoculation and for at least 96 hours post-inoculation. If outdoor animal space is used, staff will follow the same PPE procedures as in ABSL-2 facilities.

Because previous studies (Knowles, 2009) have shown that animals may shed the ONRAB recombinant virus for 3 – 4 days, all items in contact with inoculated animals will be considered contaminated for at least 96 hours post-inoculation. All disposable waste will be double-bagged with the first bag sprayed with disinfectant prior to placing into the second bag. Bagged waste must then be transported within the facility in a labeled, hard-sided, closed container for final disposition (incineration or autoclaving). Durable, solid-surface items such as cages, feed bowls, etc. will be cleaned and disinfected before removal from ABSL-2 rooms or outdoor animal pens. No live animals will be removed from the area during the first 96-hour period. Any carcasses will be double-bagged with the first bag sprayed with disinfectant prior to placing into the second bag. Bagged carcasses will then be transported in a labeled, hard-sided, closed container to the CSU Veterinary Hospital for necropsy. Carcasses will be sent to chemical digesters as per their disposal protocol.

In addition to the ONRAB recombinant virus, care must be taken when manipulating any wildlife as they have the potential to harbor a number of pathogens. Because we are unable to test for all possible pathogens (viral, bacterial, parasitic) the following biosafety measures are in place to reduce these unknown potential exposures, as well.

T. Provide an assurance statement that all practices and procedures are in accordance with the appropriate guidelines for that biosafety/risk level of organism/materials:

All practices will be in accordance with the US Dept. of Health and Human Services' Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition.

U. NWRC Institutional Biosafety Committee (IBC):

Date of IBC approval letter:_____

Knowles, M. K., S.A. Nadin-Davis, M.Sheen, R.Rosatte, R. Mueller and A. Beresford. 2009. Safety studies on an adenovirus recombinant vaccine for rabies (AdRG1.3-ONRAB®) in target and non-target species. *Vaccine*. 27:6619-6626.

Rigotto C., K. Hanley, P.A. Rochelle, R. De Leon, C.R.M. Barardi, and M.V. Yates. 2011. Survival of Adenovirus Types 2 and 41 in Surface and Ground Waters Measured by a Plaque Assay. *Environ. Sci. Technol. 45*:4145–4150.

Valtierra, H.N. 2008. Stability of Viral Pathogens in the Laboratory Environment. *Applied Biosafety*. 13:21-26.

Appendix E: Human Adenovirus Serotype-5 Titers Pre-ONRAB®

Cornell University
Animal Health Diagnostic Center
240 Farrier Road, Cornell University, Ithaca, NY 14853
Ph: 607-253-3900 Fax: 607-253-3943
http://diagcenter.vet.cornell.edu

Finalized Report

Owner: PRE BAIT SERUM SAMPLES

Accession Number: **102289-11**

Usda-Aphis-Ws-Serum Study - (16515)
Attn Nancy Bennett
474-8315
Ithaca, NY 14853
(607) 253-3923

Sampled: Not Given
Received: 08/30/2011
Finalized: 09/19/2011
Case Coordinator: Edward Dubovi
Reference Number: PRE BAIT SERUM SAMPLES

VIROLOGY
607-253-3900

Human Adenovirus 5 SN (VN)

Item		Result
1	1001WV - Procyonidae Raccoon Female	<4
2	1002WV - Procyonidae Raccoon Female	<4
3	1003WV - Procyonidae Raccoon Male	<4
4	1004WV - Procyonidae Raccoon Female	<4
5	1005WV - Procyonidae Raccoon Male	<4
6	1006WV - Mustelidae Skunk, Nos Female	<4
7	1007WV - Procyonidae Raccoon Female	<4
8	1008WV - Procyonidae Raccoon Male	<4
9	1009WV - Procyonidae Raccoon Female	<4
10	1010WV - Procyonidae Raccoon Female	<4
11	1011WV - Procyonidae Raccoon Female	<4
12	1012WV - Procyonidae Raccoon Female	<4
13	1013WV - Procyonidae Raccoon Female	<4
14	1014WV - Procyonidae Raccoon Female	<4
15	1015WV - Procyonidae Raccoon Female	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
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Human Adenovirus 5 SN (VN)

Item		Result
16	1016WV - Procyonidae Raccoon Female	<4
17	1017WV - Procyonidae Raccoon Male	<4
18	1018WV - Procyonidae Raccoon Male	<4
19	1019WV - Procyonidae Raccoon Male	<4
20	1020WV - Procyonidae Raccoon Female	<4
21	1021WV - Procyonidae Raccoon Male	<4
22	1022WV - Procyonidae Raccoon Male	<4
23	1023WV - Procyonidae Raccoon Female	<4
24	1024WV - Procyonidae Raccoon Male	<4
25	1025WV - Procyonidae Raccoon Male	<4
26	1026WV - Procyonidae Raccoon Male	<4
27	1027WV - Procyonidae Raccoon Female	<4
28	1028WV - Procyonidae Raccoon Female	<4
29	1029WV - Procyonidae Raccoon Male	<4
30	1030WV - Procyonidae Raccoon Male	<4
31	1031WV - Procyonidae Raccoon Female	<4
32	1032WV - Procyonidae Raccoon Female	<4
33	1033WV - Procyonidae Raccoon Female	<4
34	1034WV - Procyonidae Raccoon Male	<4
35	1035WV - Procyonidae Raccoon Male	<4
36	1036WV - Procyonidae Raccoon Male	<4
37	1037WV - Procyonidae Raccoon Female	<4
38	1038WV - Procyonidae Raccoon Female	<4
39	1039WV - Procyonidae Raccoon Female	<4
40	1040WV - Procyonidae Raccoon Female	<4
41	1041WV - Procyonidae Raccoon Female	<4

Report Date: 9/19/2011 3:07:25PM

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Animal Health Diagnostic Center

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Human Adenovirus 5 SN (VN)

Item		Result
42	1042WV - Procyonidae Raccoon Male	<4
43	1043WV - Procyonidae Raccoon Male	<4
44	1044WV - Procyonidae Raccoon Male	<8
45	1045WV - Procyonidae Raccoon Male	<4
46	1046WV - Procyonidae Raccoon Male	<4
47	1047WV - Procyonidae Raccoon Male	<4
48	1048WV - Procyonidae Raccoon Male	<4
49	1049WV - Mustelidae Skunk, Nos Male	<4
50	1050WV - Procyonidae Raccoon Female	<4
51	1051WV - Procyonidae Raccoon Female	<4
52	1052WV - Procyonidae Raccoon Male	<4
53	1053WV - Procyonidae Raccoon Female	<4
54	1054WV - Procyonidae Raccoon Male	<4
55	1055WV - Procyonidae Raccoon Male	<4
56	1056WV - Procyonidae Raccoon Male	<4
57	1057WV - Procyonidae Raccoon Male	<4
58	1058WV - Procyonidae Raccoon Female	<4
59	1059WV - Procyonidae Raccoon Female	<4
60	1060WV - Procyonidae Raccoon Female	<4
61	1061WV - Procyonidae Raccoon Female	<4
62	1062WV - Procyonidae Raccoon Female	<4
63	1063WV - Procyonidae Raccoon Female	<4
64	1064WV - Procyonidae Raccoon Female	<4
65	1065WV - Procyonidae Raccoon Female	<4
66	1066WV - Procyonidae Raccoon Male	<4
67	1067WV - Procyonidae Raccoon Male	<4

Report Date: 9/19/2011 3:07:25PM

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Animal Health Diagnostic Center

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PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
68	1068WV - Mustelidae Skunk, Nos Female	<4
69	1069WV - Procyonidae Raccoon Male	<4
70	1070WV - Procyonidae Raccoon Female	<4
71	1071WV - Procyonidae Raccoon Female	<4
72	1072WV - Procyonidae Raccoon Female	<4
73	1073WV - Procyonidae Raccoon Female	<4
74	1074WV - Procyonidae Raccoon Male	<4
75	1075WV - Procyonidae Raccoon Female	<4
76	1076WV - Mustelidae Skunk, Nos Female	<4
77	1077WV - Procyonidae Raccoon Male	<4
78	1078WV - Procyonidae Raccoon Female	<4
79	1079WV - Procyonidae Raccoon Male	<4
80	1080WV - Procyonidae Raccoon Female	<4
81	1081WV - Procyonidae Raccoon Female	<4
82	1082WV - Procyonidae Raccoon Female	<4
83	1083WV - Procyonidae Raccoon Male	<8
84	1084WV - Procyonidae Raccoon Female	<4
85	1085WV - Procyonidae Raccoon Female	<4
86	1086WV - Procyonidae Raccoon Female	<4
87	1087WV - Procyonidae Raccoon Male	<4
88	1088WV - Procyonidae Raccoon Female	<4
89	1089WV - Procyonidae Raccoon Female	<4
90	1090WV - Procyonidae Raccoon Female	<4
91	1091WV - Procyonidae Raccoon Male	<4
92	1092WV - Procyonidae Raccoon Male	<4
93	1093WV - Procyonidae Raccoon Female	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

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PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
94	1094WV - Procyonidae Raccoon Female	<4
95	1095WV - Procyonidae Raccoon Male	<4
96	1096WV - Procyonidae Raccoon Female	<4
97	1097WV - Procyonidae Raccoon Male	<4
98	1098WV - Procyonidae Raccoon Male	<4
99	1099WV - Procyonidae Raccoon Male	<4
100	1100WV - Procyonidae Raccoon Male	<4
101	1101WV - Procyonidae Raccoon Female	<4
102	1102WV - Mustelidae Skunk, Nos Female	<4
103	1103WV - Procyonidae Raccoon Female	<4
104	1104WV - Procyonidae Raccoon Female	<4
105	1105WV - Procyonidae Raccoon Female	<4
106	1106WV - Procyonidae Raccoon Female	<4
107	1107WV - Procyonidae Raccoon Male	<4
108	1108WV - Procyonidae Raccoon Female	<4
109	1109WV - Mustelidae Skunk, Nos Female	<4
110	1110WV - Procyonidae Raccoon Male	<4
111	1111WV - Procyonidae Raccoon Male	<4
112	1112WV - Procyonidae Raccoon Female	<4
113	1113WV - Procyonidae Raccoon Female	<4
114	1114WV - Procyonidae Raccoon Female	<4
115	1115WV - Procyonidae Raccoon Female	<4
116	1116WV - Mustelidae Skunk, Nos Female	<4
117	1117WV - Procyonidae Raccoon Female	<4
118	1118WV - Procyonidae Raccoon Female	<4
119	1119WV - Mustelidae Skunk, Nos Male	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
120	1120WV - Procyonidae Raccoon Female	<4
121	1121WV - Mustelidae Skunk, Nos Female	<4
122	1122WV - Procyonidae Raccoon Male	<4
123	1123WV - Procyonidae Raccoon Female	<4
124	1124WV - Procyonidae Raccoon Female	<4
125	1125WV - Procyonidae Raccoon Female	<4
126	1126WV - Procyonidae Raccoon Male	<4
127	1127WV - Procyonidae Raccoon Male	<4
128	1128WV - Procyonidae Raccoon Female	<4
129	1129WV - Procyonidae Raccoon Male	<4
130	1130WV - Procyonidae Raccoon Male	<4
131	1131WV - Procyonidae Raccoon Female	<4
132	1132WV - Procyonidae Raccoon Female	<4
133	1133WV - Procyonidae Raccoon Male	<4
134	1134WV - Procyonidae Raccoon Male	<4
135	1135WV - Procyonidae Raccoon Male	<4
136	1136WV - Procyonidae Raccoon Female	<4
137	1137WV - Mustelidae Skunk, Nos Male	<4
138	1138WV - Procyonidae Raccoon Male	<4
139	1139WV - Procyonidae Raccoon Male	<4
140	1140WV - Procyonidae Raccoon Male	<4
141	1141WV - Procyonidae Raccoon Male	<4
142	1142WV - Procyonidae Raccoon Female	<4
143	1143WV - Procyonidae Raccoon Male	<4
144	1144WV - Procyonidae Raccoon Male	<4
145	1145WV - Procyonidae Raccoon Male	<4

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Animal Health Diagnostic Center

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PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
146	1446WV - Procyonidae Raccoon Female	<4
147	1447WV - Procyonidae Raccoon Male	<4
148	1148WV - Procyonidae Raccoon Male	<4
149	1149WV - Procyonidae Raccoon Female	<4
150	1150WV - Procyonidae Raccoon Male	<4
151	1151WV - Procyonidae Raccoon Male	<4
152	1152WV - Procyonidae Raccoon Male	<4
153	1153WV - Procyonidae Raccoon Male	<4
154	1154WV - Procyonidae Raccoon Male	<4
155	1555WV - Procyonidae Raccoon Male	<4
156	1156WV - Procyonidae Raccoon Male	<4
157	1157WV - Procyonidae Raccoon Female	<4
158	1158WV - Procyonidae Raccoon Female	<4
159	1159WV - Procyonidae Raccoon Female	<4
160	1160WV - Procyonidae Raccoon Female	<4
161	1161WV - Procyonidae Raccoon Female	<4
162	1162WV - Procyonidae Raccoon Male	<4
163	1163WV - Procyonidae Raccoon Male	<4
164	1164WV - Procyonidae Raccoon Female	<4
165	1165WV - Procyonidae Raccoon Male	<4
166	1166WV - Procyonidae Raccoon Female	<4
167	1167WV - Procyonidae Raccoon Female	<4
168	1168WV - Mustelidae Skunk, Nos Female	<4
169	1169WV - Procyonidae Raccoon Female	<4
170	1170WV - Procyonidae Raccoon Female	<4
171	1171WV - Procyonidae Raccoon Female	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
172	1172WV - Procyonidae Raccoon Male	<4
173	1173WV - Procyonidae Raccoon Male	<4
174	1174WV - Procyonidae Raccoon Female	<4
175	1175WV - Procyonidae Raccoon Female	<4
176	1176WV - Procyonidae Raccoon Male	<4
177	1177WV - Procyonidae Raccoon Male	<4
178	1178WV - Procyonidae Raccoon Female	<4
179	1179WV - Procyonidae Raccoon Female	<4
180	1180WV - Procyonidae Raccoon Female	<4
181	1181WV - Mustelidae Skunk, Nos Female	<4
182	1182WV - Procyonidae Raccoon Male	<4
183	1183WV - Mustelidae Skunk, Nos Female	<4
184	1184WV - Procyonidae Raccoon Male	<4
185	1185WV - Procyonidae Raccoon Male	<4
186	1201WV - Procyonidae Raccoon Male	<4
187	1202WV - Procyonidae Raccoon Male	<4
188	1203WV - Procyonidae Raccoon Female	<4
189	1205WV - Procyonidae Raccoon Male	<4
190	1206WV - Procyonidae Raccoon Male	<4
191	1207WV - Procyonidae Raccoon Male	<4
192	1208WV - Procyonidae Raccoon Female	<4
193	1209WV - Procyonidae Raccoon Male	<4
194	1210WV - Procyonidae Raccoon Male	<4
195	1211WV - Procyonidae Raccoon	<4
196	1212WV - Procyonidae Raccoon	<4
197	1213WV - Procyonidae Raccoon	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
198	1214WV - Procyonidae Raccoon Male	<4
199	1215WV - Procyonidae Raccoon Female	<4
200	1216WV - Procyonidae Raccoon Female	<4
201	1217WV - Procyonidae Raccoon Female	<4
202	1218WV - Mustelidae Skunk, Nos Female	<4
203	1219WV - Procyonidae Raccoon Male	<4
204	1220WV - Mustelidae Skunk, Nos Female	<4
205	1221WV - Procyonidae Raccoon Female	<4
206	1222WV - Procyonidae Raccoon Male	<8
207	1223WV - Procyonidae Raccoon Male	<8
208	1224WV - Procyonidae Raccoon Male	<4
209	1225WV - Procyonidae Raccoon Male	<8
210	1226WV - Mustelidae Skunk, Nos Female	<4
211	1227WV - Procyonidae Raccoon Female	<4
212	1228WV - Procyonidae Raccoon Male	<4
213	1229WV - Procyonidae Raccoon Male	<4
214	1230WV - Mustelidae Skunk, Nos Female	<4
215	1231WV - Procyonidae Raccoon Male	<4
216	1232WV - Procyonidae Raccoon Female	<4
217	1233WV - Procyonidae Raccoon Female	<4
218	1234WV - Procyonidae Raccoon Male	<4
219	12365WV - Procyonidae Raccoon Male	<4
220	1236WV - Procyonidae Raccoon Female	<4
221	1237WV - Procyonidae Raccoon Male	<4
222	1238WV - Procyonidae Raccoon Male	<4
223	1239WV - Procyonidae Raccoon Male	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
224	1240WV - Procyonidae Raccoon Female	<4
225	1241WV - Procyonidae Raccoon Male	<4
226	1242WV - Procyonidae Raccoon Female	<4
227	1243WV - Procyonidae Raccoon Female	<4
228	1244WV - Procyonidae Raccoon Female	<4
229	1245WV - Procyonidae Raccoon Female	<4
230	1246WV - Procyonidae Raccoon Female	<4
231	1247WV - Procyonidae Raccoon Male	<4
232	1248WV - Procyonidae Raccoon Female	<4
233	1249WV - Procyonidae Raccoon Male	<4
234	1250WV - Procyonidae Raccoon Female	<4
235	1251WV - Procyonidae Raccoon Female	<4
236	1252 - Procyonidae Raccoon Female	<4
237	1253WV - Procyonidae Raccoon Female	<4
238	1254WV - Procyonidae Raccoon Male	<4
239	1255WV - Procyonidae Raccoon Male	<4
240	1256WV - Procyonidae Raccoon Female	<4
241	1257WV - Procyonidae Raccoon Male	<4
242	1258WV - Procyonidae Raccoon Male	<4
243	1259WV - Procyonidae Raccoon Male	<4
244	1260WV - Procyonidae Raccoon Female	<4
245	1261WV - Mustelidae Skunk, Nos Female	<4
246	1262WV - Procyonidae Raccoon	<4
247	1263WV - Procyonidae Raccoon Male	<4
248	1264WV - Procyonidae Raccoon Male	<4
249	1265WV - Procyonidae Raccoon Male	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
250	1266WV - Procyonidae Raccoon Male	<4
251	1267WV - Procyonidae Raccoon Female	<4
252	1268WV - Procyonidae Raccoon Male	<4
253	1269WV - Procyonidae Raccoon Male	<4
254	1270WV - Procyonidae Raccoon Male	<4
255	1271WV - Procyonidae Raccoon Female	<4
256	1272WV - Procyonidae Raccoon Male	<4
257	1273WV - Procyonidae Raccoon Female	<4
258	1274WV - Procyonidae Raccoon Male	<4
259	1275WV - Procyonidae Raccoon Male	<4
260	1276WV - Procyonidae Raccoon Female	<4
261	1277WV - Procyonidae Raccoon Male	<4
262	1278WV - Mustelidae Skunk, Nos Male	<4
263	1279WV - Procyonidae Raccoon Male	<4
264	1280WV - Procyonidae Raccoon Male	<4
265	1281WV - Procyonidae Raccoon Male	<4
266	1282WV - Procyonidae Raccoon Male	<4
267	1283WV - Procyonidae Raccoon Male	<4
268	1284WV - Procyonidae Raccoon Male	<4
269	1285WV - Procyonidae Raccoon Male	<4
270	1286WV - Procyonidae Raccoon Female	<4
271	1287WV - Procyonidae Raccoon Female	<4
272	1288WV - Procyonidae Raccoon Female	<4
273	1289WV - Procyonidae Raccoon Male	<4
274	1290WV - Procyonidae Raccoon Female	<4
275	1291WV - Procyonidae Raccoon Female	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
276	1292WV - Mustelidae Skunk, Nos Male	<4
277	1293WV - Procyonidae Raccoon Male	<4
278	1294WV - Procyonidae Raccoon Male	<4
279	1295WV - Procyonidae Raccoon Male	<8
280	1296WV - Procyonidae Raccoon Female	<4
281	1297WV - Procyonidae Raccoon Female	<4
282	1298WV - Procyonidae Raccoon Male	<4
283	1299WV - Procyonidae Raccoon Female	<4
284	1300WV - Procyonidae Raccoon Male	<4
285	1301WV - Procyonidae Raccoon Female	<4
286	1302WV - Procyonidae Raccoon Female	<4
287	1303WV - Procyonidae Raccoon Male	<4
288	1304WV - Procyonidae Raccoon Male	<4
289	1305WV - Procyonidae Raccoon Female	<4
290	1306WV - Procyonidae Raccoon Female	<4
291	1307WV - Procyonidae Raccoon Female	<4
292	1308WV - Procyonidae Raccoon Female	<4
293	1309WV - Procyonidae Raccoon Female	<4
294	1310WV - Procyonidae Raccoon Male	<4
295	1311WV - Procyonidae Raccoon Male	<4
296	1312WV - Procyonidae Raccoon Male	<4
297	1313WV - Procyonidae Raccoon Female	<4
298	1314WV - Procyonidae Raccoon Male	<4
299	1315WV - Procyonidae Raccoon Male	<4
300	1316WV - Procyonidae Raccoon Male	<4
301	1317WV - Procyonidae Raccoon Male	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
302	1318WV - Procyonidae Raccoon Female	<4
303	1319WV - Procyonidae Raccoon Male	<4
304	1320WV - Procyonidae Raccoon Male	<4
305	1321WV - Procyonidae Raccoon Male	<4
306	1322WV - Procyonidae Raccoon Female	<4
307	1323WV - Procyonidae Raccoon Male	<4
308	1324WV - Procyonidae Raccoon	<4
309	1325WV - Procyonidae Raccoon	<4
310	1326WV - Procyonidae Raccoon	<4
311	1327WV - Procyonidae Raccoon	<4
312	1328WV - Procyonidae Raccoon	<4
313	1329WV - Procyonidae Raccoon	<4
314	1330WV - Procyonidae Raccoon	<4
315	1331WV - Procyonidae Raccoon	<4
316	1332WV - Procyonidae Raccoon	<4
317	1333WV - Procyonidae Raccoon	<4
318	1334WV - Procyonidae Raccoon	<4
319	1335WV - Procyonidae Raccoon	<4
320	1336WV - Procyonidae Raccoon	<4
321	1337WV - Procyonidae Raccoon	<8
322	1338WV - Procyonidae Raccoon	<4
323	1339WV - Procyonidae Raccoon	<8
324	1351WV - Procyonidae Raccoon	<4
325	1352WV - Procyonidae Raccoon	<4
326	1353WV - Procyonidae Raccoon	<4
327	1354WV - Procyonidae Raccoon	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
328	1355WV - Procyonidae Raccoon	<4
329	1356WV - Procyonidae Raccoon	<4
330	1357WV - Procyonidae Raccoon	<4
331	1358WV - Procyonidae Raccoon	<4
332	1359WV - Procyonidae Raccoon	<4
333	1360WV - Procyonidae Raccoon	<4
334	1361WV - Procyonidae Raccoon	<4
335	1362WV - Procyonidae Raccoon	<4
336	1363WV - Procyonidae Raccoon	<4
337	1364WV - Procyonidae Raccoon	<4
338	1365WV - Procyonidae Raccoon	<4
339	1366WV - Procyonidae Raccoon	<4
340	1367WV - Procyonidae Raccoon	<4
341	1368WV - Procyonidae Raccoon	<4
342	1369WV - Procyonidae Raccoon	<4
343	1370WV - Procyonidae Raccoon	<4
344	1371WV - Procyonidae Raccoon	<4
345	1372WV - Procyonidae Raccoon	<4
346	1373WV - Procyonidae Raccoon	<4
347	1374WV - Procyonidae Raccoon	<4
348	1375WV - Procyonidae Raccoon	<4
349	1401WV - Procyonidae Raccoon	<4
350	1402WV - Procyonidae Raccoon	<4
351	1403WV - Procyonidae Raccoon	<4
352	1404WV - Procyonidae Raccoon	<4
353	1405WV - Procyonidae Raccoon	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
354	1406WV - Procyonidae Raccoon	<4
355	1407WV - Procyonidae Raccoon	<4
356	1408WV - Procyonidae Raccoon	<4
357	1409WV - Procyonidae Raccoon	<4
358	1410WV - Procyonidae Raccoon	<4
359	1411WV - Procyonidae Raccoon	<4
360	1412WV - Procyonidae Raccoon	<4
361	1413WV - Procyonidae Raccoon	<4
362	1414WV - Procyonidae Raccoon	<4
363	1415WV - Procyonidae Raccoon	<4
364	1416WV - Procyonidae Raccoon	<4
365	1417WV - Procyonidae Raccoon	<4
366	1418WV - Procyonidae Raccoon	<4
367	1419WV - Procyonidae Raccoon	<4
368	1420WV - Procyonidae Raccoon	<4
369	1421WV - Procyonidae Raccoon	<4
370	1422WV - Procyonidae Raccoon	<4
371	1423WV - Procyonidae Raccoon	<4
372	1424WV - Procyonidae Raccoon	<4
373	1425WV - Procyonidae Raccoon	<4
374	1426WV - Procyonidae Raccoon	<4
375	1427WV - Procyonidae Raccoon	<4
376	1428WV - Procyonidae Raccoon	<4
377	1451WV - Procyonidae Raccoon	<4
378	1452WV - Procyonidae Raccoon	<4
379	1453WV - Procyonidae Raccoon	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
380	1454WV - Procyonidae Raccoon	<4
381	1455WV - Procyonidae Raccoon	<4
382	1456WV - Procyonidae Raccoon	<4
383	1457WV - Procyonidae Raccoon	<4
384	1458WV - Procyonidae Raccoon	<4
385	1459WV - Procyonidae Raccoon	<4
386	1460WV - Procyonidae Raccoon	<4
387	1461WV - Procyonidae Raccoon	<4
388	1462WV - Canidae Gray Fox	<4
389	1463WV - Procyonidae Raccoon	<4
390	1464WV - Procyonidae Raccoon	<4
391	1465WV - Procyonidae Raccoon	<4
392	1466WV - Procyonidae Raccoon	<4
393	1467WV - Procyonidae Raccoon	<4
394	1468WV - Procyonidae Raccoon	<4
395	1469WV - Procyonidae Raccoon	<4
396	1470WV - Procyonidae Raccoon	<4
397	1471WV - Procyonidae Raccoon	<4
398	1472WV - Procyonidae Raccoon	<4
399	1473WV - Procyonidae Raccoon	<4
400	1474WV - Procyonidae Raccoon	<4
401	1475WV - Procyonidae Raccoon	<4
402	1476WV - Procyonidae Raccoon	<4
403	1477WV - Procyonidae Raccoon	<4
404	1478WV - Procyonidae Raccoon	<4
405	1479WV - Procyonidae Raccoon	<4

Report Date: 9/19/2011 3:07:25PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **102289-11**
PRE BAIT SERUM SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
406	1480WV - Procyonidae Raccoon	<4
407	1481WV - Procyonidae Raccoon	<4
408	1501WV - Procyonidae Raccoon	<4
409	1551WV - Procyonidae Raccoon	<4
410	1552WV - Procyonidae Raccoon	<4
411	1553WV - Procyonidae Raccoon	<4
412	1554WV - Procyonidae Raccoon	<4
413	1555WV - Procyonidae Raccoon	<4
414	1556WV - Procyonidae Raccoon	<4
415	1557WV - Procyonidae Raccoon	<4
416	1558WV - Procyonidae Raccoon	<4

Human Adenovirus 5 SN (VN)
The virus neutralization antibody titer is the reciprocal of the highest dilution of serum that neutralizes the infectivity of the virus (endpoint dilution of 1:32 = antibody titer of 32). Titers are in units of antibody and as such all values reported without modifiers contain that specified amount of antibody in the sample. Values with a < (less than symbol) indicate no detectable antibody at the minimum readable dilution (<8 = no detectable antibody at a 1:8 dilution). An antibody titer can result from vaccination, infection, or passive maternal transfer.
Most SN tests start at the minimum serum dilution of 1:4 (final dilution of 1:8) unless regulatory requirements dictate otherwise.

Report Date: 9/19/2011 3:07:25PM

Appendix F: Human Adenovirus Serotype-5 Titers Post-ONRAB®

Cornell University
Animal Health Diagnostic Center
240 Farrier Road, Cornell University, Ithaca, NY 14853
Ph: 607-253-3900 Fax: 607-253-3943
http://diagcenter.vet.cornell.edu

Finalized Report

Owner: ONRAB POST SAMPLES

Accession Number: **132402-11**

Usda-Aphis-Ws-Serum Study - (16515)
Attn Nancy Bennett
474-8315
Ithaca, NY 14853
(607) 253-3923

Sampled: Not Given
Received: 11/15/2011
Finalized: 12/05/2011
Case Coordinator: Edward Dubovi
Reference Number: ONRAB POST SAMPLES

VIROLOGY

607-253-3900

Human Adenovirus 5 SN (VN)

Item		Result
1	1002WV - Procyonidae Raccoon	<4
2	1028WV - Procyonidae Raccoon	<4
3	1041WV - Procyonidae Raccoon	<4
4	1042WV - Procyonidae Raccoon	<4
5	1043WV - Procyonidae Raccoon	<4
6	1062WV - Procyonidae Raccoon	<4
7	1078WV - Procyonidae Raccoon	<4
8	1080WV - Procyonidae Raccoon	<4
9	1086WV - Procyonidae Raccoon	<4
10	1094WV - Procyonidae Raccoon	<4
11	1099WV - Procyonidae Raccoon	<4
12	1107WV - Procyonidae Raccoon	<4
13	1113WV - Procyonidae Raccoon	<4
14	1143WV - Procyonidae Raccoon	<4
15	1179WV - Procyonidae Raccoon	<4

Report Date: 12/5/2011 12:54:51PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **132402-11**
ONRAB POST SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
16	1186WV - Procyonidae Raccoon	<4
17	1187WV - Procyonidae Raccoon	<4
18	1188WV - Procyonidae Raccoon	<4
19	1189WV - Procyonidae Raccoon	<4
20	1190WV - Procyonidae Raccoon	<4
21	1191WV - Procyonidae Raccoon	<4
22	1192WV - Procyonidae Raccoon	<4
23	1193WV - Procyonidae Raccoon	<4
24	1194WV - Mustelidae Skunk, Nos	<4
25	1195WV - Procyonidae Raccoon	<4
26	1196WV - Procyonidae Raccoon	<4
27	1197WV - Procyonidae Raccoon	<4
28	1198WV - Procyonidae Raccoon	<4
29	1199WV - Procyonidae Raccoon	<4
30	1200WV - Procyonidae Raccoon	<4
31	1202WV - Procyonidae Raccoon	<4
32	1215WV - Procyonidae Raccoon	<4
33	1219WV - Procyonidae Raccoon	<4
34	1221WV - Procyonidae Raccoon	<4
35	1251WV - Procyonidae Raccoon	<4
36	1260WV - Procyonidae Raccoon	<4
37	1264WV - Procyonidae Raccoon	<4
38	1290WV - Procyonidae Raccoon	4
39	1291WV - Procyonidae Raccoon	<4
40	1295WV - Procyonidae Raccoon	<4
41	1376WV - Procyonidae Raccoon	<4

Report Date: 12/5/2011 12:54:51PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **132402-11**
ONRAB POST SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
42	1377WV - Procyonidae Raccoon	<4
43	1378WV - Procyonidae Raccoon	<4
44	1379WV - Mustelidae Skunk, Nos	<4
45	1380WV - Mustelidae Skunk, Nos	<4
46	1381WV - Procyonidae Raccoon	<4
47	1382WV - Mustelidae Skunk, Nos	<4
48	1383WV - Procyonidae Raccoon	<4
49	1384WV - Procyonidae Raccoon	<4
50	1385WV - Procyonidae Raccoon	<4
51	1386WV - Procyonidae Raccoon	<4
52	1387WV - Procyonidae Raccoon	<4
53	1388WV - Procyonidae Raccoon	<4
54	1389WV - Procyonidae Raccoon	<4
55	1390WV - Procyonidae Raccoon	<4
56	1391WV - Procyonidae Raccoon	<4
57	1421WV - Procyonidae Raccoon	<4
58	1429WV - Procyonidae Raccoon	<4
59	1430WV - Procyonidae Raccoon	<4
60	1431WV - Procyonidae Raccoon	<4
61	1432WV - Procyonidae Raccoon	<4
62	1433WV - Procyonidae Raccoon	<4
63	1434WV - Procyonidae Raccoon	<4
64	1435WV - Procyonidae Raccoon	<4
65	1436WV - Procyonidae Raccoon	<4
66	1437WV - Procyonidae Raccoon	<4
67	1438WV - Procyonidae Raccoon	<4

Report Date: 12/5/2011 12:54:51PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **132402-11**
ONRAB POST SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
68	1439WV - Procyonidae Raccoon	<4
69	1440WV - Procyonidae Raccoon	<4
70	1441WV - Procyonidae Raccoon	<4
71	1442WV - Procyonidae Raccoon	<4
72	1443WV - Procyonidae Raccoon	<4
73	1444WV - Procyonidae Raccoon	<4
74	1445WV - Procyonidae Raccoon	<4
75	1446WV - Procyonidae Raccoon	<4
76	1447WV - Procyonidae Raccoon	<4
77	1448WV - Procyonidae Raccoon	<4
78	1449WV - Procyonidae Raccoon	<4
79	1450WV - Mustelidae Skunk, Nos	<4
80	1482WV - Mustelidae Skunk, Nos	<4
81	1483WV - Procyonidae Raccoon	<4
82	1484WV - Procyonidae Raccoon	<4
83	1485WV - Procyonidae Raccoon	4
84	1486WV - Procyonidae Raccoon	<4
85	1487WV - Procyonidae Raccoon	<4
86	1488WV - Procyonidae Raccoon	<4
87	1489WV - Procyonidae Raccoon	<4
88	1490WV - Procyonidae Raccoon	<4
89	1491WV - Procyonidae Raccoon	<4
90	1492WV - Procyonidae Raccoon	<4
91	1493WV - Procyonidae Raccoon	<4
92	1494WV - Mustelidae Skunk, Nos	<4
93	1495WV - Procyonidae Raccoon	<4

Report Date: 12/5/2011 12:54:51PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **132402-11**
ONRAB POST SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
94	1496WV - Procyonidae Raccoon	<4
95	1497WV - Mustelidae Skunk, Nos	<4
96	1498WV - Mustelidae Skunk, Nos	<4
97	1499WV - Procyonidae Raccoon	<4
98	1500WV - Procyonidae Raccoon	<4
99	1502WV - Procyonidae Raccoon	<4
100	1503WV - Procyonidae Raccoon	<4
101	1504WV - Mustelidae Skunk, Nos	<4
102	1505WV - Procyonidae Raccoon	<4
103	1506WV - Procyonidae Raccoon	<4
104	1507WV - Procyonidae Raccoon	<4
105	1508WV - Procyonidae Raccoon	<4
106	1509WV - Mustelidae Skunk, Nos	<4
107	1510WV - Procyonidae Raccoon	<4
108	1511WV - Procyonidae Raccoon	<4
109	1512WV - Procyonidae Raccoon	<4
110	1513WV - Procyonidae Raccoon	<4
111	1514WV - Procyonidae Raccoon	<4
112	1515WV - Procyonidae Raccoon	<4
113	1516WV - Procyonidae Raccoon	<4
114	1517WV - Procyonidae Raccoon	<4
115	1518WV - Procyonidae Raccoon	<4
116	1519WV - Procyonidae Raccoon	<4
117	1520WV - Mustelidae Skunk, Nos	<4
118	1521WV - Procyonidae Raccoon	<4
119	1522WV - Mustelidae Skunk, Nos	<4

Report Date: 12/5/2011 12:54:51PM

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Acession Number: **132402-11**
ONRAB POST SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
120	1523WV - Mustelidae Skunk, Nos	<4
121	1524WV - Procyonidae Raccoon	<4
122	1525WV - Procyonidae Raccoon	<4
123	1526WV - Procyonidae Raccoon	<4
124	1527WV - Procyonidae Raccoon	<4
125	1528WV - Procyonidae Raccoon	<4
126	1529WV - Procyonidae Raccoon	<4
127	1530WV - Procyonidae Raccoon	<4
128	1531WV - Procyonidae Raccoon	<4
129	1532WV - Mustelidae Skunk, Nos	<4
130	1533WV - Procyonidae Raccoon	<4
131	1534WV - Procyonidae Raccoon	<4
132	1535WV - Mustelidae Skunk, Nos	<4
133	1536WV - Procyonidae Raccoon	6
134	1537WV - Procyonidae Raccoon	<4
135	1538WV - Procyonidae Raccoon	<4
136	1539WV - Procyonidae Raccoon	<4
137	1540WV - Canidae Gray Fox	<4
138	1541WV - Procyonidae Raccoon	<4
139	1542WV - Procyonidae Raccoon	<4
140	1543WV - Procyonidae Raccoon	<4
141	1544WV - Procyonidae Raccoon	<4
142	1545WV - Procyonidae Raccoon	<4
143	1546WV - Procyonidae Raccoon	<4
144	1547WV - Mustelidae Skunk, Nos	4
145	1548WV - Procyonidae Raccoon	<4

Report Date: 12/5/2011 12:54:51PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **132402-11**
ONRAB POST SAMPLES

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Item		Result
146	1551WV - Procyonidae Raccoon	<4
147	1559WV - Procyonidae Raccoon	<4
148	1560WV - Procyonidae Raccoon	<4
149	1561WV - Procyonidae Raccoon	<4
150	1562WV - Procyonidae Raccoon	<4
151	1563WV - Procyonidae Raccoon	<4
152	1564WV - Procyonidae Raccoon	64
153	1565WV - Procyonidae Raccoon	<4
154	1566WV - Procyonidae Raccoon	<4
155	1567WV - Procyonidae Raccoon	<4
156	1568WV - Procyonidae Raccoon	<4
157	1569WV - Procyonidae Raccoon	<4
158	1570WV - Procyonidae Raccoon	<4
159	1571WV - Procyonidae Raccoon	<4
160	1572WV - Procyonidae Raccoon	<4
161	1573WV - Procyonidae Raccoon	<4
162	1574WV - Procyonidae Raccoon	<4
163	1575WV - Procyonidae Raccoon	<4
164	1576WV - Procyonidae Raccoon	<4
165	1577WV - Procyonidae Raccoon	<4
166	1578WV - Procyonidae Raccoon	<4
167	1579WV - Mustelidae Skunk, Nos	<4
168	1580WV - Procyonidae Raccoon	<4
169	1581WV - Mustelidae Skunk, Nos	<4
170	1582WV - Procyonidae Raccoon	<4
171	1583WV - Procyonidae Raccoon	<4

Report Date: 12/5/2011 12:54:51PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **132402-11**
ONRAB POST SAMPLES

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Item		Result
172	1584WV - Procyonidae Raccoon	<4
173	1585WV - Canidae Coyote	<4
174	1586WV - Canidae Coyote	<32
175	1587WV - Procyonidae Raccoon	<4
176	1588WV - Procyonidae Raccoon	<4
177	1589WV - Procyonidae Raccoon	<4
178	1590WV - Procyonidae Raccoon	<4
179	1591WV - Procyonidae Raccoon	<4
180	1592WV - Procyonidae Raccoon	<4
181	1593WV - Procyonidae Raccoon	<4
182	1594WV - Mustelidae Skunk, Nos	<4
183	1595WV - Procyonidae Raccoon	<4
184	1596WV - Procyonidae Raccoon	<4
185	1597WV - Procyonidae Raccoon	<4
186	1598WV - Procyonidae Raccoon	<4
187	1599WV - Procyonidae Raccoon	<4
188	1600WV - Procyonidae Raccoon	<4
189	1626WV - Procyonidae Raccoon	<4
190	1627WV - Procyonidae Raccoon	<4
191	1628WV - Procyonidae Raccoon	<4
192	1629WV - Procyonidae Raccoon	<4
193	1630WV - Procyonidae Raccoon	<4
194	1631WV - Procyonidae Raccoon	<4
195	1632WV - Procyonidae Raccoon	<4
196	1633WV - Procyonidae Raccoon	<4
197	1634WV - Procyonidae Raccoon	<4

Report Date: 12/5/2011 12:54:51PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **132402-11**
ONRAB POST SAMPLES

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Item		Result
198	1635WV - Canidae Gray Fox	<4
199	1636WV - Procyonidae Raccoon	<4
200	1637WV - Procyonidae Raccoon	<4
201	1638WV - Procyonidae Raccoon	<4
202	1639WV - Procyonidae Raccoon	<4
203	1640WV - Procyonidae Raccoon	<4
204	1651WV - Procyonidae Raccoon	<4
205	1652WV - Procyonidae Raccoon	<4
206	1653WV - Procyonidae Raccoon	<4
207	1654WV - Procyonidae Raccoon	<4
208	1655WV - Procyonidae Raccoon	<4
209	1656WV - Procyonidae Raccoon	<4
210	1657WV - Procyonidae Raccoon	<4
211	1658WV - Procyonidae Raccoon	<4
212	1659WV - Procyonidae Raccoon	<4
213	1660WV - Procyonidae Raccoon	<4
214	1751WV - Procyonidae Raccoon	<4
215	1752WV - Procyonidae Raccoon	<4
216	1753WV - Procyonidae Raccoon	24
217	1754WV - Procyonidae Raccoon	<4
218	1755WV - Procyonidae Raccoon	<4
219	1756WV - Procyonidae Raccoon	<4
220	1757WV - Procyonidae Raccoon	<4
221	1758WV - Procyonidae Raccoon	<4
222	1759WV - Mustelidae Skunk, Nos	<4
223	1760WV - Procyonidae Raccoon	<4

Report Date: 12/5/2011 12:54:51PM

Human Adenovirus 5 SN (VN)

Item		Result
224	1761WV - Procyonidae Raccoon	<4
225	1762WV - Procyonidae Raccoon	<4
226	1763WV - Procyonidae Raccoon	<4
227	1764WV - Mustelidae Skunk, Nos	<4
228	1765WV - Procyonidae Raccoon	<4
229	1766WV - Procyonidae Raccoon	<4
230	1767WV - Procyonidae Raccoon	<4
231	1768WV - Procyonidae Raccoon	<4
232	1769WV - Procyonidae Raccoon	<4
233	1770WV - Procyonidae Raccoon	<4
234	1771WV - Procyonidae Raccoon	<4
235	1772WV - Procyonidae Raccoon	<4
236	1773WV - Procyonidae Raccoon	<4
237	1801WV - Procyonidae Raccoon	<4
238	1826WV - Procyonidae Raccoon	<4
239	1827WV - Procyonidae Raccoon	<4
240	1828WV - Procyonidae Raccoon	<4
241	1829WV - Procyonidae Raccoon	<4
242	1830WV - Procyonidae Raccoon	<4
243	1831WV - Procyonidae Raccoon	<4
244	1832WV - Procyonidae Raccoon	<4
245	1833WV - Procyonidae Raccoon	<4
246	1834WV - Procyonidae Raccoon	<4
247	1835WV - Procyonidae Raccoon	<4
248	1876WV - Procyonidae Raccoon	<4
249	1877WV - Procyonidae Raccoon	<4

Cornell University
Animal Health Diagnostic Center

Acession Number: **132402-11**
ONRAB POST SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
250	1901WV - Procyonidae Raccoon	<4
251	1902WV - Procyonidae Raccoon	<4
252	1903WV - Procyonidae Raccoon	<4
253	1904WV - Procyonidae Raccoon	<4
254	1905WV - Procyonidae Raccoon	<4
255	1906WV - Procyonidae Raccoon	<4
256	1907WV - Procyonidae Raccoon	<4
257	1908WV - Procyonidae Raccoon	<4
258	1909WV - Procyonidae Raccoon	<4
259	1910WV - Procyonidae Raccoon	<4
260	1911WV - Procyonidae Raccoon	<4
261	1912WV - Procyonidae Raccoon	<4
262	1913WV - Procyonidae Raccoon	<4
263	1914WV - Procyonidae Raccoon	<4
264	1915WV - Procyonidae Raccoon	<4
265	1916WV - Procyonidae Raccoon	<4
266	1917WV - Procyonidae Raccoon	<4
267	1918WV - Procyonidae Raccoon	<4
268	1919WV - Procyonidae Raccoon	<4
269	1920WV - Procyonidae Raccoon	32
270	1921WV - Procyonidae Raccoon	<4
271	1922WV - Procyonidae Raccoon	<4
272	1923WV - Procyonidae Raccoon	<4
273	1924WV - Procyonidae Raccoon	<4
274	1925WV - Procyonidae Raccoon	<4
275	1926WV - Procyonidae Raccoon	<4

Report Date: 12/5/2011 12:54:51PM

Cornell University
Animal Health Diagnostic Center

Acession Number: **132402-11**
ONRAB POST SAMPLES

Human Adenovirus 5 SN (VN)

Item		Result
276	1927WV - Procyonidae Raccoon	<4
277	1951WV - Procyonidae Raccoon	<4
278	1952WV - Mustelidae Skunk, Nos	<4
279	1953WV - Procyonidae Raccoon	<4
280	194WV - Mustelidae Skunk, Nos	<8
281	1955WV - Mustelidae Skunk, Nos	<4
282	1956WV - Mustelidae Skunk, Nos	<4
283	1957WV - Procyonidae Raccoon	<4
284	1958WV - Procyonidae Raccoon	<4
285	1959WV - Procyonidae Raccoon	<4
286	1960WV - Procyonidae Raccoon	<4
287	1961WV - Procyonidae Raccoon	<4
288	1962WV - Mustelidae Skunk, Nos	<4
289	1963WV - Procyonidae Raccoon	<4
290	1964WV - Procyonidae Raccoon	<4
291	1966WV - Procyonidae Raccoon	<4
292	1967WV - Procyonidae Raccoon	<4
293	1968WV - Procyonidae Raccoon	<4
294	1969WV - Procyonidae Raccoon	<4
295	1970WV - Canidae Coyote	<4
296	1971WV - Procyonidae Raccoon	<4

Human Adenovirus 5 SN (VN)
The virus neutralization antibody titer is the reciprocal of the highest dilution of serum that neutralizes the infectivity of the virus (endpoint dilution of 1:32 = antibody titer of 32). Titers are in units of antibody and as such all values reported without modifiers contain that specified amount of antibody in the sample. Values with a < (less than symbol) indicate no detectable antibody at the minimum readable dilution (<8 = no detectable antibody at a 1:8 dilution).
An antibody titer can result from vaccination, infection, or passive maternal transfer.
Most SN tests start at the minimum serum dilution of 1:4 (final dilution of 1:8) unless regulatory requirements dictate otherwise.

Report Date: 12/5/2011 12:54:51PM

Appendix G: Histopathology Results from Captive Studies at Wildlife Services, NWRC

Dr. Kurt VerCauteren, Rabies Research Project Leader

ONRAB®, like Raboral V-RG®, is a recombinant vaccine. However, the viral vector in ONRAB® is human adenovirus type 5 (Had5). Had5 is a relatively safe and well-studied virus, which is used in many vaccine formulations. Wild-type Had5 often causes mild respiratory or gastrointestinal manifestations in humans, although immunocompromised individuals may develop a more serious disease (Textbook of Pediatric infectious diseases, Vol 2: Ralph Feigin). Extensive studies on the efficacy and safety of the ONRAB® vaccine in animals have been completed in Canada (Yarosh et al. 1996, Lutz-Wallace et al. 1995, Randrianarison-Jewthoukoff and Perricaudet 1995, Rosette et al. 2009, Knowles et al. 2009), and the encouraging findings of these studies initiated ONRAB's distribution via aerial bait drop in 2006 targeting raccoons and skunks. Rabies VNA seroconversion rates in areas baited with ONRAB® averaged 80% in the first two years (Rosatte et al. 2009), well above the seroconversion rates when the vaccine Raboral V-RG® is used (Slate et al. 2009, Brown et al. 2010).

For the non-target safety studies in the literature, researchers administered an ONRAB® dose 10 times the dose needed to vaccinate target species. Animals were monitored daily and all tolerated exposure to the vaccine. Analyses of tissues resulted in viral RNA detected in some lung and kidney samples. Following histopathological analysis, the most marked findings were in the lungs with pulmonary congestion and some possible vaccine aspiration effects. Oral swabs and/or fecal samples were collected from skunks, raccoons, fox, cotton rats, cats and dogs. Viral shedding (if any) was generally complete by 4 days post-inoculation (dpi) except for one skunk, which continued to shed virus in feces through 14 dpi (Knowles et al. 2009).

Our research expands on the species evaluated by Knowles et al. (2009) and investigates the vaccine as it relates to its safety in wildlife species likely to come in contact with the ONRAB® vaccine as part of WS NRMP ORV operations, as well as, identify whether Had5 shed from certain wildlife species may increase the chance of humans contacting the Had5. As expected, histological changes described in the non-target species vaccinated with ONRAB® (eastern cottontails, fox squirrels, opossums and wild turkeys) are best attributed to common pathologic changes in free-ranging wildlife. None of the histological findings in tissues of the study animals appear to be related to the ONRAB® vaccine (Table 1).

Table 1. A summary of histological findings unrelated to vaccination by ONRAB® are summarized by species.

Species	n	Vaccinates	Non-vaccinates
Cottontail Rabbit (*Sylvilagus floridanus)*	16	14	2
Eastern Wild Turkey (*Meleagris gallopavo silvestri)*	23	21	2
Virginia Opossum (*Didelphis virginiana)*	19	17	2
Fox Squirrel (*Sciurus niger)*	23	21	2

Cottontail rabbits: The most common finding in the cottontails (n=16), both vaccinates and non-vaccinates, was the presence of tapeworms in the gastrointestinal tract. Parasites were observed on both gross and histologic examination in 12/16 animals; histologically there was often a very mild increase in the number of lymphocytes and plasma cells in the lamina propria of infected animals. In the liver of two vaccinated animals there were areas of inflammation, regionally extensive and histiocytic with multinucleated giant cells in one animal and a focal granuloma with fibrosis in another animal that was presumed to be secondary to parasite migration. A degenerate parasite was identified within the center of the granuloma. Very mild, patchy interstitial nephritis was observed in two cottontails and lymphoplasmacytic aggregates in portal areas of the liver were seen in 5 cottontails, both vaccinates and non-vaccinates. One cottontail had a small focus of inflammation in the lung while another had some mild bronchiole associated lymphoid tissue (BALT) hyperplasia. Findings in the large intestine or spleen of all cottontails were unremarkable. Histological variation described for cottontail rabbits are presumably unrelated to vaccination with the ONRAB® vaccine.

Fox Squirrels: The most significant changes in fox squirrels (n=23) were observed in the kidneys of both the vaccinated and non-vaccinated animals. Sixteen fox squirrels, both vaccinates and non-vaccinates, had some degree of inflammation in the interstitum of the kidney that ranged from mild and patchy to very severe and regionally extensive. In five animals (4 vaccinates, 1 non-vaccinate) suppurativetubulitis was also observed, possibly a result of naturally acquired leptospirosis infection. In the liver of fourteen fox squirrels, including both vaccinates and non-vaccinates, there were small aggregates of lymphocytes and plasma cells in portal areas. Mild bronchial associated lymphoid tissue hyperplasia was seen in the lung of 4/23 animals with no apparent cause (1 non-vaccinate, 3 vaccinates). A single fox squirrel had a small focus of acute inflammation in the lung. Mild, patchy increases in lymphocytes and plasma cells were seen in the small or large intestine of three fox squirrels (1 non-vaccinate, 2 vaccinates). Histological variation described for fox squirrels are presumably unrelated to vaccination with the ONRAB® vaccine.

Opossums: On gross examination of opossums (n=19) multiple metazoan parasites were seen throughout the gastrointestinal tracts of nearly all opossums. Histologically there were numerous eosinophils as well as lymphocytes and plasma cells in the small intestine of all opossums (vaccinates and non-vaccinates), but fewer inflammatory cells were present in the colon. On

gross examination, the livers of all opossums were mildly enlarged and fatty on palpation. Histologically fatty change was seen most commonly in centrilobular areas, but panlobular in many animals. Inflammation similar to that seen in the gastrointestinal tract (eosinophillic to mixed infiltrate) was observed in portal areas of the liver of 14 opossums (2 non-vaccinates, 12 vaccinate) and ranged from very mild to moderate. Mild interstitial, lymphoplasmacytic inflammation was seen in the kidney of 11 opossums (2 non-vaccinates, 9 vaccinates) and mineralization was identified in the collecting tubules of 2 (both vaccinates) opossums. A single animal had a moderate amount of interstitial inflammation that also included eosinophils and there was some mild, secondary tubular distortion. Foci of mineralization were identified in the lung of 8 animals (2 non-vaccinates, 6 vaccinates). Mineral was usually devoid of associated reaction however foci of inflammation were identified in 10 animals (2 non-vaccinate, 8 vaccinates). Inflammatory change ranged from small foci of foamy macrophages and lymphocytes, to a single opossum with a mineralized granuloma and a single opossum with a large protazoal cyst surrounded with mixed inflammation (non-vaccinate). Airway associated lymphoid tissue was often hyperplastic and contained amyloid. Histological variation described for opossums are presumably unrelated to vaccination with the ONRAB® vaccine.

Wild Turkeys: The most significant histologic change observed in the wild turkeys (n=23) was a variable amount of extramedullaryhematopoesis in multiple tissues. Change was most notable in the liver (2 non-vaccinates, 13 vaccinates) but also present in the intestinal tract and the lung (five turkeys, all vaccinates). Dust granulomas were seen in the lungs of six birds. Two vaccinated turkeys had small foci of heterophillic inflammation in the kidneys and three had notable extra medulla hematokinesis. Histologic changes were uncommon in the gastrointestinal tract; extra medulla hematokinesis was variable and a single turkey had a focal, small crypt abscess in the gastrointestinal tract. Histological variation described for wild turkeys are presumably unrelated to vaccination with the ONRAB® vaccine.

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Yarosh OK, Wandeler AI, Graham FL, Campbell JB, Prevec L. 1996. Human adenovirus type 5 vectors expressing rabies glycoprotein. Vaccine 14:1257–64.