Raccoon (*Procyon lotor*) Rabies in Alabama: Insights from Historical Trends, Oral Rabies Vaccine Bait Fate, and Spatial Epizootiology

by

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camera trapping; oral rabies vaccine; *Procyon lotor*; rabies virus; mesocarnivore, zoonosis

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Abstract

Rabies epizootiology and management in Alabama have evolved significantly over the past two centuries, yet the influence of long-term ecological and historical factors on the effectiveness of oral rabies vaccine (ORV) programs remains poorly understood. This study synthesizes historical trends in rabies occurrence and management, emphasizing Alabama's role as the westernmost extent of the raccoon rabies virus enzootic zone. Additionally, we investigated the effects of region, habitat, bait type, and climate on ORV bait uptake by raccoons (*Procyon lotor*). Our findings highlight substantial competition for baits from non-target species, particularly Virginia opossums (*Didelphis virginiana*), and a rapid decline in bait uptake over time as key factors limiting the success of current management efforts. These results underscore the need to tailor ORV strategies to Alabama's unique historical context and diverse ecosystems to enhance efficiency, minimize redundancy, and reduce the economic burden associated with zoonotic disease control.

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List of Abbreviations

ADPH	Alabama Department of Public Health
AIC	Akaike Information Criterion
AICc	Corrected Akaike Information Criterion
BIC	Bayesian Information Criterion
CDC	Centers for Disease Control and Prevention
CS	Coated Sachet
DFA	Direct Fluorescent Antibody
dRIT	Direct Rapid Immunohistochemical Test
EMM	Estimated Marginal Means
ERS	Enhanced Rabies Surveillance
FMP	Fishmeal Polymer
GAT	Georgia-Alabama-Tennessee
GLMM	Generalized Linear Mixed Model
HR	Hazard Ratio
KM	Kaplan-Meier
M1	Model 1
M2	Model 2
NRMP	National Rabies Management Program
ONRAB	Ontario Rabies Vaccine
OR	Odds Ratio
ORV	Oral Rabies Vaccine
PEP	Post-Exposure Prophylaxis

PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
R^2	Coefficient of Determination
rVNA	Rabies Virus Neutralizing Antibody
RV	Rabies Virus
SyS	Syndromic Surveillance
USDA	United States Department of Agriculture
V-RG	Recombinant Live Vaccinia Virus
WS	Wildlife Services
WHO	World Health Organization

Chapter 1: A Historical Review of Rabies Epidemiology and Management in Alabama

Abstract

The history of wildlife pathogens in time and space impacts current understanding, detection, and management of infectious diseases, which may best be appreciated dynamically at a local level. Over the past two centuries, the epizootiology, prevention, and control of rabies in Alabama changed significantly. Canine rabies was reported in the state during the US civil war and predominated until after World War II. The later appreciation of wildlife rabies grew only after the control of rabies in dogs. During the 1950s, rabies in gray foxes (*Urocyon cinereoargenteus*) spread throughout the southeastern US but declined unexpectedly in the ensuing decades. By the 21st century, besides multiple variants of rabies virus in bats, the only extant mesocarnivore reservoir was the raccoon (*Procyon lotor*). Currently, Alabama represents the western-most extent of raccoon rabies virus variant in the US. Historically, the evolution of rabies management, from passive surveillance dependent upon clinical signs toward the enhanced, laboratory-based surveillance and the progress from host population reduction toward oral rabies vaccination of wildlife in combination with comprehensive vaccination of companion animals and postexposure prophylaxis in humans, is exemplified in Alabama via a One Health context.

Introduction

Rabies is an ancient yet neglected zoonosis that threatens agriculture, public health, and wildlife globally (Tarantola 2017). In the US, wildlife species in the orders *Carnivora* and *Chiroptera* maintain unique rabies virus (RV) variants or genetic lineages (Ma et al. 2024). The distribution of oral rabies vaccine (ORV) baits by the US Department of Agriculture (USDA), Animal and Plant Health Inspection Service, Wildlife Services (WS), National Rabies Management Program (NRMP) is the primary strategy used to control rabies in mesocarnivores (Slate and Chipman 2020, R. B. Chipman et al. 2023). Following the elimination of canine rabies, raccoon (*Procyon lotor*) rabies caused by a specific raccoon rabies virus (RRV) variant, has been the primary focus of management along the eastern US (Guerra et al. 2003, Biek et al. 2007).

Alabama represents the westernmost state where RRV is present and the westernmost ORV distribution zone in the US (R. Chipman et al. 2023, USDA 2023, Ma et al. 2024). While management efforts since 2001 have prevented the spread of RRV beyond Alabama, local elimination has not occurred. Previous studies in Alabama examined RRV over short periods (Sheeler 2002, Arjo et al. 2005, 2008, Johnson et al. 2009) but no comprehensive review of disease occurrence, distribution, management, and surveillance exists (Biek et al., 2007; Chipman et al., 2023; Duke et al., 2014; Elmore et al., 2017; Slate et al., 2020). Planning, implementation, and refinement of management strategies requires an understanding of how accumulated actions and events have shaped rabies occurrence within Alabama's multifaceted ecosystem (Durant and Durant 2010). Moreover, reevaluating disease dynamics across space and time is essential to avoid redundancy, maximize use of limited resources, and reduce economic burden (Giles et al. 1978, Reynolds et al. 2015, Hirsch et al. 2016). The objective of this study

was to synthesize the historical qualitative epidemiology of rabies in Alabama, delineate the significance of prior contributions made by the state, and discuss recent disease prevention and control as an example of practical wildlife management in the southeastern US.

Methods

We followed the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines (Tricco et al. 2018) to systematically search four databases (HauthiTrust, PubMed, Scopus, and Web of Science) for literature pertaining to the history of rabies in Alabama from 1800 to 2023 (Fig. 1.1). Using a consistent structure of terms and Boolean operators, we conducted searches in each database. We used terms "Alabama" AND "rabies" OR "hydrophobia" OR "rabies virus" as keywords for the subject (HauthiTrust), topic (Web of Science and PubMed), and article title, abstract and keyworks (Scopus). We excluded self-referenced records and removed duplicate records. Eligible records included peer-reviewed articles, books, and gray literature. Gray literature was limited to theses and dissertations, reports, newspaper articles, and conference papers from legitimate government and academic sources. We screened the remaining reports and excluded those that did not refer to the location of Alabama, did not explicitly include rabies or RV as a topic, and did not have a vertebrate subject. Additionally, we excluded reports specifically focused on humans and bats to provide a more thorough examination of rabies and its management for mesocarnivores. We manually reviewed the references for the remaining reports to identify additional relevant records. To ensure completeness, we also searched the USDA WS, Centers for Disease Control and Prevention (CDC), Alabama Department of Public Health (ADPH), and Library of Congress websites.

We requested additional recent rabies occurrence data from the ADPH and USDA WS. The ADPH provided rabies laboratory records that included the number of positive and negative results by species, year and county from 2018 to 2022. The USDA WS provided enhanced rabies surveillance (ERS) records from 2001 to 2023 that included the latitude and longitude, county, date, species, collection method, rabies testing and results, sex, lactation status, relative age, weight, and additional notes. Suspect animals collected through ERS were tested by the direct rapid immunohistochemical test (dRIT), as described (Patrick et al. 2019) while suspect animals submitted to the ADPH were tested by the standard direct fluorescent antibody (DFA) test (Meslin et al. 1996, Ronald et al. 2003).

To support the contention that raccoon rabies is the only mesocarnivore RV variant in Alabama, we retrieved completed and partial nucleoprotein gene sequences of RV from the southeastern US available in GenBank (Appendix 1.1). To obtain these, we performed a search using keywords “rabies”, “nucleoprotein”, and territorial modifiers corresponding to the states of the southeastern US, either in their full names or abbreviations. We aligned the nucleotide sequences and performed a neighbor-joining phylogenetic analysis based on p-distances with pairwise gap deletion for 1000 bootstrap replicates in MEGA v.7 (Kumar et al. 2016).

Qualitative Epidemiology

Reports of a disease clinically consistent with rabies in folklore and anecdotal evidence suggest that rabies was present in the US prior to European colonization (John W. Krebs et al. 1995, Vos et al. 2011, Tarantola 2017, Streicker and Biek 2020), but an increase was recognized following the introduction of Old-World domestic dogs and livestock (Smith et al. 1995, Fisher et al. 2018, Barrett et al. 2019, Schell et al. 2021). Alabama was admitted as the 22nd state of the US in December of 1819 but a substantial portion of what is now within the state boundaries was

designated Cherokee, Chickasaw, Choctaw, or Creek. The earliest report retrieved that suggested rabies in Alabama involved an aggressive dog shortly before the Civil War (Fig. 1.2), although the disease may have been present longer (Wilkinson 1894). Physicians reported sporadic cases of mortality in dogs and livestock following the bite of a rabid animal (Salmon 1901) until 1890 when apparent wildlife rabies was first documented in red (*Vulpes vulpes*) and gray foxes (*Urocyon cinereoargenteus*) in the state (Hull 1963). By the end of the 19th century, only ten counties in Alabama maintained an apparent 'rabies free' status (Wilkinson 1894).

Alabama had a surge in animal rabies cases (Kerr and Stimson 1909, Mohler 1911, Denison et al. 1937, Webster 1942, Baker et al. 2022) but a decline in human mortalities early in the 20th century after introduction of the Pasteur vaccine (Pasteur 1880, 1885) for postexposure prophylaxis (PEP), which became available in Montgomery through the State Board of Health in 1908 (Kerr and Stimson 1909, Hull 1941, 1947, 1955). Birmingham experienced rapid population growth in the first three decades of the 20th century (Brownell 1972). By the 1940s, Birmingham was the epicenter of rabies in the United States (Bolman 2022). Domestic animals and foxes primarily accounted for rabies cases (CDC 1961) until Alabama public health programs mandated vaccination of dogs and cats in the 1960s (Fig. 1.3A) (Lederle Laboratories Division 1948, ADPH et al. 1975, Smith et al. 1995, ADPH 2023). While dogs and cats comprised 70% of the 358 positive rabies positive tests in 1948, reported rabies cases in wildlife surpassed those in domestic animals in the 34 positive rabies tests by 1963 (Sikes and Tierkel 1961, ADPH n.d.) and represented 78% of the 57 positive rabies tests by 1969 (McLean 1970, 1975, Riddle et al. 1987, Blanton et al. 2010). Alabama reported an increase in rabies in foxes after an epizootic was recognized in the southeastern US in the late 1960s (CDC 1965*a*, *b*, 1971*a*, 1977*a*, Plotkin and Clark 1971). By 1970, the ADPH reported an increase in rabid foxes

that was concentrated in southern state localities (Johnson 1969, CDC 1970*a*, *b*, *a*, *c*, 1971*b*, 1973*a*, *b*). The ADPH reported rabies cases in foxes, raccoons, and skunks (*Mephitis mephitis* and *Spilogale putorius*) until 1976 (CDC 1973*a*, 1974*a*, 1975, 1976*a*, 1977*b*). Although skunks accounted for the greatest number of ADPH reported cases during 1974 (CDC 1974*a*, *b*), raccoons surpassed skunks as the mesocarnivore species most frequently diagnosed with rabies by 1976 (CDC 1976*a*, *b*, *c*, ADPH n.d.). While rabies in dogs had declined, animal bites injuries remained a substantial public health concern through the late 1970s in areas with greater human populations such as Jefferson County (Maetz 1979).

Development of monoclonal antibody typing methods in the late 1970s allowed for identification and differentiation of RV variants maintained by host species in different geographic regions (Smith et al. 1973, Wiktor and Koprowski 1978, Müller and Freuling 2020, Pieracci et al. 2020, Worsley-Tonks et al. 2020). Advances in gene sequencing and phylogenetic analyses enabled a more detailed differentiation of viral lineages, usually host-dependent and, for mesocarnivores as opposed to bats, often circulating in certain geographic areas within host ranges (Smith et al., 1995; Blanton et al., 2011; Troupin et al., 2016, Szanto et al. 2011; Elmore et al. 2017). Our findings in GenBank supported the suggestion that only the raccoon and several bat RV variants were present in the southeastern US from 1978 to 2013 (Fig. 1.4). Additionally, most ADPH reported rabies cases in domestic animals and wildlife since the late 1970s represented individuals infected with the RRV (Fig. 3)(Rupprecht 1995, Wallace et al. 2014). Moreover, all mesocarnivores diagnosed with rabies through ERS operations from 2001 to 2023 represented RRV (Fig. 1.5B).

The raccoon was considered an unimportant rabies host throughout the southeastern US until a raccoon epizootic was recognized in Florida during the early 1950s (Scatterday et al.

1960; CDC 1965a, 1965b, 1981a, 1981b; Held et al. 1967; Bigler et al. 1973). The first confirmed case of rabies in a raccoon was reported during 1954 in Alabama (ADPH n.d.). Reported cases of rabies in raccoons remained sporadic in Alabama (Johnson 1970) until the westward spread of the epizootic into the Florida panhandle reached the southeastern corner of Alabama in the late 1970s (McLean 1971, Arjo et al. 2005, ADPH n.d.). Raccoons were responsible for 53% of all rabies cases reported in Alabama by 1978 (CDC 1977*a*, 1978*a*, *b*, *c*, 1981*a*, *b*, *c*), and the distribution and occurrence of raccoon rabies expanded in v (Fig. 1.3B) (Arjo et al. 2005, ADPH n.d.). Unlike other reservoirs, the raccoon remained the primary species diagnosed with rabies in Alabama with an average of 84 cases reported annually by the ADPH over the next decade (ADPH n.d.). Alabama, along with 15 other eastern states along an established raccoon rabies enzootic zone, accounted for nearly all raccoon rabies cases in the US by 1993 (Krebs et al. 1994, 1996, John W. Krebs et al. 1995, J. W. Krebs et al. 1995) after a second epizootic began in the Mid-Atlantic states during 1977 that resulted in the rapid spread of raccoon rabies beyond the Southeast (Kappus et al. 1970, Nettles et al. 1979, CDC 1981*b*, 1983*a*, Torrence et al. 1992).

The RRV was enzootic in 33 of 67 counties in Alabama that followed a diagonal from the northeast to the southwest corner of Alabama by 2001 (USDA 2015). Both ERS and ADPH surveillance efforts between 2002 and 2003 revealed that the RRV was west of the Alabama River in seven counties (USDA 2015). Southeastern states, including Alabama, consistently accounted for 30% of the total RRV cases reported in the US between 2006 and 2008 (Blanton et al. 2007, 2008, 2009). Between 2013 and 2023, the number of raccoon rabies cases reported in Alabama peaked in 2015 (ADPH n.d.). Despite a century of mandated state vaccination laws, cases of RRV continue to occur in domestic animals (Bruce 2023, Ilha et al. 2024, Jones and

Trotter 2024). Alabama has confirmed cases of rabies in raccoons annually for more than 50 years. The enzootic zone includes most counties to the south and east of the Alabama-Coosa River system (Fig. 1.6). The average of 24 cases per year reported between 2019 and 2023 represented the lowest five year average since 1970 (Ma et al. 2021, 2022, 2023, 2024, ADPH n.d.).

Surveillance and Monitoring

Early records of rabies in the US lacked standardized diagnostic methods and were primarily limited to human and domestic animal mortalities documented by physicians and veterinarians (Huntsville Gazette Company 1890, Declich and Carter 1994, Rupprecht 1995). By the beginning of the 20th century, newspapers in Alabama, such as the 'Auburn Plainsman', 'The Montgomery Advertiser', and 'The Age-Herald', shared prevention methods alongside reported cases (Isbell 1924, Alabama Polytechnic Institute 1936, Barrett et al. 2019). Surveillance throughout the US improved from clinical to laboratory reporting after rabies became a notifiable disease in 1938 (Steele and Tierkel 1949, Biek et al. 2007).

The ADPH initiated public health (i.e., passive surveillance) efforts in 1946 (Held et al. 1967, ADPH et al. 1975, CDC 1983*b*). Prior to the 1950s, detection relied on tests with limited specificity including the mouse inoculation test or histopathological examination of brain tissues for Negri bodies (Nadin-Davis et al. 2023). The DFA test replaced prior techniques and is used to microscopically examine brain samples to confirm the presence of RV antigens at the diagnostic laboratories located in Montgomery and Mobile (Meslin et al. 1996, Ronald et al. 2003). For public health surveillance, the brain of suspected rabid animals that have exposed humans or domestic animals is submitted to county health departments (Declich and Carter 1994, Davis et al. 2019). In the first decade, the ADPH diagnostic laboratories faced challenges such as

receiving decomposed animal brains (Steele and Tierkel 1949). While submitted specimens have typically been received and tested within a day or two of the animal being euthanized, a public health surveillance case of RRV detected 9.7 km west of the ORV zone in September of 2021 but not reported until January 2022 indicated a need for improved communication between state and federal agencies (Reynolds et al. 2023). Given the importance of timely reporting for management actions, the ADPH reviewed data timelines and quality reports provided by the National Syndromic Surveillance (SyS) program to develop additional procedures to offset problems in April 2024 (CDC 2024). The ADPH tests approximately 1,200 animal samples and investigates approximately 7,000 potential rabies virus exposures annually. Diagnostic laboratories in Alabama confirmed a total of 8,513 rabies cases in 21 species between 1950 and 2023 (CDC 1961, Ronald et al. 2003, ADPH 2023).

While the ADPH had not detected any apparent westward movement of RRV by the 21st century, the leading edge of the enzootic remained uncertain (Krebs et al. 2002, Davis et al. 2021). To aid in the development of an effective ORV program in the state, Alabama WS initiated ERS to complement public health surveillance in 2001 (USDA 2015). Alabama WS and the ADPH contacted law enforcement agencies, local veterinarians, and other cooperators to ask for support in reporting sick or unnatural acting raccoons. Raccoons were submitted for testing if they were sick or unusual acting, found dead in unusual places, roadkill, collected by shooting, or collected by trapping. The initial objective of ERS in Alabama was to identify the western front of raccoon RRV enzootic territory and to evaluate threats of spread in counties beyond the Alabama-Coosa River system (USDA 2001). Alabama WS detected RRV west of the river system in at least 7 of the 30 counties sampled between 2001 and 2003. Surveillance conducted by the ADPH and WS during 2002 and 2003 confirmed ten or more RRV cases in domestic and

wild animals where it had not previously been detected, in Cherokee and Dekalb Counties, as well as where it had been detected, in Autauga and Clarke Counties. In response, Alabama WS increased sampling effort within a 1.6 km radius of cases and implemented ORV in subsequent years (Kostrzewski and Slate 2002, 2004, USDA 2003). In 2004, the ADPH identified two RRV cases from Shelby County, but no cases were confirmed by WS through ERS (Kostrzewski and Slate 2004).

The integration of dRIT in 2005 improved ERS efficiency by allowing WS to conduct faster rabies testing by reducing the burden on public health laboratory partners (Smith et al. 1973, Middel et al. 2017, Patrick et al. 2019). While dRIT was used for testing brainstems to diagnose rabies virus in the tissue, collected Sera continued to be sent to CDC or another partner laboratory to evaluate the presence of rabies virus neutralizing antibodies as an index to population immunity. The proportion of specimens tested in Alabama using dRIT increased from 33% in 2005 to 99% in 2006 after no false dRIT-positive results were detected in samples tested by the CDC (Fig 1.5A) (USDA 2015). Alabama WS yielded all negative tests for the animals collected as part of ERS efforts in 2006 (n = 322), 2007 (n = 137), 2008 (n = 208), and 2009 (n = 200). Approximately 50 raccoon rabies cases were detected annually by the ADPH for the same period (ADPH n.d.). Samples collected through ERS efforts in 2012 predominately focused on Mobile County due to a canine distemper outbreak (USDA 2015). Neither ERS nor public health surveillance identified RRV cases in the southwestern corner of Alabama in 2012. However, the ADPH identified RRV in Baldwin County during the three years before and after ERS sampling (ADPH n.d.). Alabama WS intensified focal trapping efforts in 2013 (n = 748) in response to the re-emergence of RRV in Shelby and Chilton counties. Positive cases (n= 21) from 2013 accounted for 35% of the cases detected by ERS from 2001 to 2023 (USDA 2019). In 2014,

Alabama increased ERS sampling through focal trapping efforts following a report from the ADPH of two RRV cases that were identified approximately 20 to 25 km west of the ORV zone in northeast Alabama. Alabama WS identified eight additional rabies positive cases (n = 769) that included one road-killed specimen collected approximately 80 km from the western edge of the ORV zone in Madison County (USDA 2019).

A decade after Alabama implemented a rabies control program, the ADPH continued to confirm about 40 cases of raccoon rabies with at least 10 cases of RRV spillover to other animals each year (ADPH n.d.). Given the spatial significance in preventing further expansion of the raccoon RRV epizootic, Alabama was one of four pilot states selected in 2015 to implement the ERS initiative by USDA WS, which categorized samples into six categories with a stratified point system to improve collection of samples with a greater likelihood of testing positive (Kirby et al. 2017, Davis et al. 2021). Within the first year, Alabama WS increased sampling efforts which resulted in 36.1% greater collection of strange acting animals, the ERS category with the highest likelihood of rabies detection (Kirby et al. 2017). Nuisance-trapped or homeowner derived but healthy (NWCO/Other) samples represented the majority of ERS samples collected in Alabama from 2016 to 2023 (Fig. 1.7) (USDA and TVA 2014). For the same period, the frequency at which samples were collected increased and was more evenly distribution between spring (March-May), summer (June-August), fall (September-November), and winter (December-February) compared to previous years.

Between 2001 and 2023, raccoons accounted for 91% of the ERS samples tested for RV in Alabama. Demographic information collected from raccoons (n = 6959) as part of ERS operations included sex, relative age, lactation status, and weight. From 2001 to 2023, male raccoons accounted for a greater proportion of collected samples annually in 20 of the 23 years,

as well as overall (56.7% male; 43.3% female). Lactation was observed in 54.5% of the females with reproductive status reported. Adult raccoons accounted for 83.5% of samples collected with relative age recorded overall and for each sex. The average weight of sampled male raccoons exceeded females annually from 2001 to 2023. From 2001 to 2023, WS collected 7,640 samples from 12 animal species across 56 of the 67 counties in Alabama (Fig. 1.8). Rabies diagnostics were completed by CDC (18%, n = 1364) or dRIT (82%, n = 6276) and resulted in the identification of 60 RRV cases by ERS. The positive cases detected by ERS were distributed between 15 counties with 22% (n = 13) and 13% (n = 8) of samples collected in Shelby and Autauga Counties, respectively.

Monitoring of rabies virus neutralizing antibody (rVNA) seroprevalence in Alabama raccoons has been a critical component of assessing the effectiveness of ORV programs and is used as an index to population immunity (USDA 2015). The presence of rVNA in blood samples collected from trapped raccoons is measured by the rapid fluorescent focus inhibition test (Meslin et al. 1996; WHO 2018). Raccoons were trapped pre-ORV and post-ORV to compare the post-vaccination antibody prevalence to pre-vaccination levels until 2006, when operations shifted to post-ORV only (USDA 2015). The rVNA seroprevalence observed among raccoons sampled in the Birmingham zone increased from the fall of 2005 (20%) to 2006 (40.3%) (Table 1.1). However, levels of rVNA seroprevalence in Alabama raccoons fluctuated from 2002-2010 but did not indicate that vaccination coverage was sufficient to achieve ideal herd immunity (Thulke and Eisinger 2008; USDA 2015).

Control and Prevention

Broad-scale reduction of reservoir populations below an arbitrary threshold density was the primary management strategy to reduce rabies in wildlife prior to the 20th century (Sikes and

Tierkel 1961, Anderson et al. 1981, Debbie 1991, Fehlner-Gardiner 2018). Guidelines documented in Alabama during 1894 were to muzzle dogs, disinfect or burn kennels where rabid dogs had been confined, and quarantine imported dogs for at least six months. Additionally, domestic animals that had been bitten by another animal and mesocarnivores located in areas where rabies had been suspected were killed (Wilkinson 1894, Johnson 1970, Sheeler 2002, Fisher 2007).

In the 20th century, rabies management strategies shifted from population reduction to preventing rabies through vaccination. A nationwide effort to manage dog rabies through mandatory vaccination began before World War II (Baer 1991, Baker et al. 2022). The city of Montgomery enforced mandatory dog vaccinations beginning in 1937 (Steele 1988, Freire De Carvalho et al. 2018), and a statewide mandate issued in 1939 (Webster 1942) led to the vaccination of 177,039 dogs in the first year and approximately 70,000 dogs annually through the late 1940s (Lederle Laboratories Division 1948, Johnston 1993). The administrative code for rabies control in Alabama was enacted during 1975 (Chapter 420-4-4) (ADPH et al. 1975, Johnston and Jones 2009, USDA and TVA 2014, ADPH 2023). To support the mandated vaccination of dogs and cats three months and older (Johnston 1993, Krebs et al. 1994, John W. Krebs et al. 1995, Smith et al. 1995), the Alabama Veterinary Medical Association, ADPH, and Alabama Cooperative Extension Services collaborated to provide low-cost RV vaccination clinics, and since 1993 designated a week of June as 'Rabies Awareness Week' (Johnston 1993, Johnston and Walden 1996).

Control of rabies in wildlife by ORV became a realistic management objective in the US after research demonstrated the field safety and immunogenicity of a live recombinant vaccinia-rabies glycoprotein (V-RG) vaccine for numerous vertebrate species (Artois et al. 1992, Hable et

al. 1992, Slate and Chipman 2020). The NRMP was established in 1995 (Sterner et al. 2009, Clayton et al. 2010) to prevent the further spread of RRV and work toward elimination of several terrestrial RV variants at the local, regional, and national levels (Slate et al. 2009, Wallace and Blanton 2020). The RABORAL V-RG® vaccine (hereafter, V-RG; is a registered trademark in the USA and elsewhere of Merial, Inc., which is now part of Boehringer Ingelheim Animal Health USA Inc., Athens, Georgia, USA) was fully licensed for raccoons in the US in 1997 and remains the only licensed ORV available in the US (Maki et al. 2017).

Following the establishment of the western front of RRV in Alabama through ERS, the first ORV distribution was implemented in the state during 2003 as part of the Georgia-Alabama-Tennessee (GAT) zone (Fig. 1.9A; Appendix 1.2) (Kostrzewski and Slate 2004). Alabama WS distributed fishmeal polymer (FMP) baits containing the V-RG vaccine to control the spread of RRV from five counties in northeastern Alabama (Kostrzewski and Slate 2004, Blanton et al. 2007, Sterner et al. 2009). In 2004, the ORV zone expanded to include the Selma region in response to three confirmed cases of RRV beyond the Alabama River (USDA 2015). The ORV zone expanded to Birmingham in 2005 after RRV cases were detected in Jefferson and Shelby Counties (Nelson and Slate 2005). The following year, coated sachet (CS) baits containing the V-RG vaccine were incorporated into ORV operations. In 2007, the Birmingham expansion shifted east to create a vaccine barrier in response to the detection of a rabid dog (Fig. 1.9B). During the same year, Alabama WS began deploying both CS and FMP baits concurrently to the GAT zone (USDA 2015). In March 2018, the NRMP assisted the Jefferson County Health Department with a one-time local baiting effort that included the Homewood, Mountain Brook, and Vestavia Hills communities in response to an increase in rabies cases in those areas (USDA 2019). The next significant ORV expansion occurred in 2019 by extending the GAT zone south

where rabies cases were persistent (Fig. 1.9E). In 2022, another standard ORV zone intended for long-term operation was established in Baldwin County to serve as an anchor for future management strategies in southern Alabama (Fig. 1.9F) (Ma et al. 2023, R. B. Chipman et al. 2023). Between 2003 and 2023, WS deployed ORV baits using fixed-wing, ground, and rotary-wing methods. Bait distribution in Alabama has typically occurred in the fall (October or November) but has varied with operations in the spring (March) for the Selma and Birmingham expansions and in the winter (January) for Baldwin County. Standard ORV operations distribute baits at a density of 75 baits/km², while contingency action areas and most urban/suburban regions aimed for a density of 150 baits/km² (Rosatte 2013, Maki et al. 2017).

In addition to standard ORV operations, contingency actions (i.e., emergency responses) may be initiated in response to five situations where rabies case(s) occur: 1) well beyond (>80 km) ORV zones; 2) just beyond established ORV zones; 3) where no ORV zone exists; 4) in hotspots within the ORV zones that represent a high risk of spreading; and 6) in epizootics (large numbers of infected animals in a relatively small area) approaching an established ORV zone that potentially could spread through the treatment area (USDA 2009). Dependent on the situation mentioned above, contingency actions may include: intensified ERS through multiple strategies that could include trap-and-test; increased bait density in an established ORV zone (from 75 baits/km^2 to 150 baits/km^2 or 300 baits/km^2); increased bait distribution frequency (twice a year instead of once a year); and trap-vaccinate-release (Sterner et al. 2009, USDA 2009, Davis et al. 2024). To create a barrier of vaccinated individuals, the contingency or expansion ORV zone is created using a 20 km buffer around detected RRV cases.

Alabama accounted for four of 31 contingency actions (as of 9/13/2024) conducted by the NRMP targeting raccoon rabies in the US from 1999-2024 (Fig. 1.9D, E) (USDA 2015,

2019, 2023). During 2013, samples collected and tested from March to September as part of the contingency action response identified three distinct RRV foci in Central Alabama along a north-south gradient in Columbiana, Leeds, and Clanton but no additional ORV zone was implemented (Fig. 1.9C). In 2014, a contingency action that intensified ERS efforts was initiated after the ADPH detected two raccoon rabies cases approximately 20 km west of the Alabama portion of the GAT zone. During intensified ERS efforts, Alabama WS identified a rabies case approximately 80 km west of the existing ORV zone in Madison County. In response, baits were distributed in an area that was double the size of the Alabama portion of the GAT zone from the fall of 2014 until the zone was scaled back during 2017, after no additional rabid animals were detected in Madison County for three years (USDA 2019). Management operations remained stable until 2020 when a rabid raccoon was reported by ADPH to have attacked a human in Cullman County, approximately 8 km west of the GAT zone. Intensified ERS following the attack led to the detection of two additional RRV cases beyond the ORV zone (total of 3 rabid raccoons from Cullman County) and resulted in the establishment of the Cullman contingency action zone. In 2021, RRV was detected in Madison County, approximately 10 km west of the GAT zone and 53 km northeast of the Cullman contingency zone. A second Huntsville contingency ORV zone was implemented in 2022 as a result of cases detected the previous year. Bait distribution encompassing the Cullman Contingency ORV area continued until 2023, with no additional rabies cases detected for over 3 years. The Huntsville Contingency ORV area is anticipated until at least 2024, depending on the continued detection of RRV cases surrounding the area (Reynolds et al. 2023).

Research and Development in Alabama

One of the earliest attempts to delineate the pathogenesis of rabies in Alabama began in 1907 when Dr. Francis Edwards inoculated rabbits (*Leporidae*) with emulsified brain tissue from a suspected rabid dog (Francis 1907). Additionally, the Street-Alabama-Dufferin (SAD) RV, used to derive several attenuated rabies vaccine strains (Lawson and Crawley 1972, Wandeler et al. 1988, Schumacher et al. 1993), was isolated in Alabama from the salivary glands of a rabid dog in 1935 (Abelseth 1964). However, the only attenuated product used in the US, the Evelyn-Rokitniki-Abelseth (ERA) vaccine strain was not effective in raccoons (Rupprecht et al. 1986, Gilbert and Chipman 2020). In the brief period the US Communicable Disease Center was in Montgomery during the 1940s (CDC 1962). The laboratory approved a live RV vaccine of chicken embryo origin (CEO) which was used to vaccinate dogs until it was later replaced by more effective and safer vaccines (Steele and Tierkel 1949, Steele 1988). Following an outbreak of rabid dogs in Birmingham during 1938, rodent infectivity was evaluated to provide insight into RV transmission routes (Dension and Leach 1940). By 1958, pathogenesis studies were performed on Virginia opossums (*Didelphis virginiana*), raccoons, skunks, and foxes in Alabama at the CDC's Southeast Rabies Station using virus derived from the salivary gland of an infected fox collected by the Alabama State Health Department (CDC 1961, Sikes and Tierkel 1961). These studies determined that the number of median lethal doses of the SAD virus required to result in a 50% population reduction (MLD50) varied among species, with foxes needing the least ($n < 5$ MLD50), followed by skunks ($n = 500$ MLD50), raccoons ($n = 1000$ MLD50), and opossums ($n > 80{,}000$ MLD50) (Sikes 1962). Although raccoon biology was studied in the 1970s (Johnson 1970), rabies field-based research gained prominence in Alabama only with the initiation of management efforts in the early 21st century (USDA 2015).

Thereafter, wildlife rabies research shifted focus to enhancing baits, vaccines, baiting strategies, and distribution methods to address the unique spatial and temporal patterns associated with raccoons and RRV (Perry et al. 1989, Kurup et al. 2023). Between 2003 and 2011, several studies were conducted to index relative densities of raccoon populations at eleven properties in northeast, central, and southwest Alabama (Kostrzewski and Slate 2003, 2004, Arjo et al. 2005, 2008, Fisher 2007, Slate et al. 2020). These studies highlighted the effect of habitat type, including agriculture (8 raccoons/km^2), riverine (8 raccoons/km^2), and forested (5 raccoons/km^2), on raccoon density indices (Arjo et al. 2005). Collaborative efforts between the NRMP, Alabama WS, National Wildlife Research Center (NWRC), and Auburn University evaluated the influence of sex, age, and habitat on raccoon space use and survival (Fisher 2007). The NWRC also assessed the utility of natural barriers to RRV spread by monitoring raccoon movements (Arjo et al. 2008, USDA 2019), which was supported by a study evaluating gene flow (Johnson 2009). These studies confirmed that the Alabama-Coosa River System limited but did not prevent movement of raccoons (Arjo et al. 2008, Johnson et al. 2009). Surveillance data from 2012 to 2014 were used to model raccoon habitat suitability and potential risk corridors for RRV spread in pine-dominated areas of Alabama (Algeo et al. 2017). Alabama WS tested a helicopter pulse baiting strategy in the Prattville area from 2011 to 2013, which involved distributing baits in clusters to target family groups of raccoons instead of the standard baiting method in which baits were distributed evenly along line transects (USDA 2015). An additional bait distribution study compared ground baiting and bait station methods in Birmingham from 2018 to 2020 (USDA 2019).

Challenges and Perspectives

The management of RRV presents an array of complex ecological and logistical challenges today. Retrospectively, Alabama reported cases of rabies in raccoons approximately 30 years before ORV was initiated (McLean 1971). By the time ORV efforts were initiated, an enzootic front was established across 33 counties, from the northeastern to the southwestern corners of the state (USDA 2001). Despite being ecological generalists, raccoons maintain densities that vary due to multiple factors, such as anthropogenic development, resource availability, and landscape features (Arjo et al. 2005, Chamberlain et al. 2007, Hill et al. 2023*a*, *c*). Variable raccoon densities likely contributed to the observed rate of spread throughout Alabama (Houle et al. 2011, Hill et al. 2023*c*).

The basic success of ORV programs is dependent on the adequacy of surveillance. Alabama relies on both public health surveillance and ERS to determine the spatial extent of RRV and assess management effectiveness (Roess et al. 2012, Kirby et al. 2017). Passive surveillance conducted by the ADPH plays an integral role in the management responses made by Alabama WS and the NRMP. Nevertheless, overt biases must be considered when interpreting data from both surveillance sources. Passive surveillance data may not provide a comprehensive picture of rabies prevalence, especially in less populated or rural areas (Slate et al. 2009, Kunkel et al. 2023). From 2018 to 2022, the number of suspect animals submitted to the ADPH was greatest in counties with a city that had a population of 50,000 or more people (Fig. 1.6) (Smith et al. 1995, Ma et al. 2020, 2021, 2022, 2023). Counties with no rabies cases reported to the ADPH may represent true absence, unreported existence, and undetected presence (Slate et al. 2009, Kunkel et al. 2023). For example, the only reported human death during 1963 occurred in western Alabama where no animal rabies cases had been reported for multiple years (CDC 1964, 1965*b*, 1981*b*). Moreover, after the COVID-19 pandemic, public attitudes and

submission of suspect animals may have shifted. Data provided through ERS by design is generally concentrated 80km east and west of the ORV zone and throughout the zone. In contrast, the areas to the east of the Alabama-Coosa River system are considered a lower priority in Alabama. While Alabama ERS is largely focused within and to the west of ORV zone, efforts also extend to areas west of the Alabama-Coosa River system, where no ORV zone exists. The additional coverage by Alabama ERS is unique relative to other states conducting similar surveillance for rabies management. In 2023 and 2024, RRV cases beyond the Alabama-Coosa River system in unvaccinated areas support the continuation of targeted surveillance outside of ORV management areas (ADPH n.d.).

While additional surveillance across Alabama would benefit a comprehensive understanding of rabies epidemiology, targeted efforts in specific northern (Jefferson and Shelby), central (Autauga, Chilton, Dallas), and southern (Clarke, Baldwin, Mobile) counties where RRV activity has been greatest may be integral to management efforts if resources are available to conduct additional sampling and testing. Urban areas in the Greater Birmingham region represent areas with increased human, wildlife, and domestic animal interfaces. Although the western spread of RRV in Alabama is believed to be limited within the central Blackbelt Prairie region, the mechanisms driving the relationship remain unclear. The Alabama-Coosa River system limits but does not prevent raccoon movement and gene flow. Therefore, additional research in the central Blackbelt Prairie region is warranted (Arjo et al. 2008, Johnson et al. 2009, Root et al. 2009). Finally, minimal surveillance coverage in the southwestern counties makes it difficult to ascertain the status of RRV in these areas. In 2023 and 2024, the ADPH reported several cases of RRV spillover to domestic animals that resulted in the exposure of multiple humans and animals (Jones and Trotter 2024, Ma et al. 2024). The consideration of

domestic animals as sentinel species for rabies activity highlights the importance of a One Health approach to rabies management (Pepin et al. 2024, Carpenter et al. 2025). Despite an apparent reservoir shift from domestic dogs to mesocarnivores and bats, vaccination of domestic animals will always be required for thorough rabies prevention control (Bergman et al. 2009).

Evaluation of molecular genetics to characterize the relatedness of host populations and associated RV variants allows the delineation of circumstances leading to outbreaks, identification of animal reservoirs, and elucidation of aspects of the natural history of disease maintenance within specific geographic areas (Dharmarajan et al. 2009, Root et al. 2009, Szanto et al. 2011, Hopken et al. 2023). Although a distinct RRV lineage was identified for Alabama, Georgia, and Florida (Szanto et al. 2011), the development of more fully characterized samples throughout Alabama will require additional genetic sequencing beyond what is currently available in GenBank (Pieracci et al. 2020). The elimination of canine RV variants, characteristics of viral perpetuation, and ecological barriers like the Tennessee river may have prevented the establishment of fox and skunk RV variants in Alabama (Johnson 1969, Sidwa et al. 2005). However, the historical origins and disappearance of the gray fox RV variant in Alabama remain enigmatic. Projected raccoon population dynamics beyond the established ORV and RRV zones are adequate to result in a rapid westward spread beyond Alabama's border, if current ORV barriers are breached and uncontrolled (Duke et al. 2014, USDA and TVA 2014, Hill et al. 2023*a*, *c*). The spread of RRV from enzootic to naïve areas via accidental or intentional translocation remains a major threat to wildlife management (Rosatte et al. 2007, Chipman et al. 2008, Slate et al. 2008, Elmore et al. 2017, Carpenter et al. 2025). Moreover, as the human-wildlife interface becomes more interconnected, the behavior and ecology of reservoirs and pathogens are likely to shift (Wallace et al. 2014, Worsley-Tonks et al. 2020, Van

De Vuurst et al. 2022). Recent documentations of cross species transmission events in the US highlight the threat of novel RV strain emergence (Carey and McLean 1983, Kuzmin et al. 2012, Mollentze et al. 2014, Holmes 2022, Gilbert 2023, Holmes and Harvey 2023, Rupprecht et al. 2023).

Establishment of the ORV zone in southern Alabama during 2022 serves as an anchor for future management efforts towards RRV elimination while also providing valuable data regarding ORV performance in southeastern subtropical coastal areas (R. B. Chipman et al. 2023). However, continued evaluation of ORV performance in these unique habitats is needed. The influence of vertebrate competitors, invertebrate fouling, specific vaccine bait types, and both target and non-target species population densities on the utility of current ORV management strategies have not been thoroughly evaluated in Alabama. Successful implementation of a wildlife rabies control program in Alabama to prevent the westward expansion of the RRV has required cooperation among the ADPH, WS, CDC, MOWA Band of Choctaw Native Americans, animal control, and local veterinarians, among countless other local, state, and federal collaborators and the public (Pepin et al. 2024). In 2024, the USDA approved nearly $19 million in emergency funding to address wildlife rabies outbreaks in multiple states over three years, including Alabama. Funding may be used for the expansion of ORV zones, increased bait densities, and ERS in high-risk areas (Espinosa 2024). A multifaceted approach that includes interagency collaboration, adaptive strategies, continued research, and public education will be critical for effective prevention, control, and eventual elimination of the RRV from Alabama and to the remainder of the eastern US (Rupprecht et al. 2018, Jeon et al. 2019, Chipman 2023, USDA 2023).

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Table 1.1 Rabies virus neutralizing antibody (rVNA) seroprevalence in raccoon samples collected by Wildlife Services at 4-12 weeks post- oral rabies vaccine (ORV) bait distribution in Alabama as part of monitoring efforts from 2002-2011.

Year	**Timing**	**ORV Zones**	**Serum Samples**	**rVNA[1]**	
				Positive	% Positive
2002	Fall	[2]	574	18	3.1%
2003	Fall	GAT[3]	126	41	32.5%
2004	Spring, Fall	GAT, Selma	255	44	17.3%
2005	Spring, Fall	GAT, Selma, Birmingham	398	91	22.9%
2006	Spring, Fall	GAT, Birmingham	387	105	27.1%
2007	Spring, Fall	GAT, Birmingham	383	121	31.6%
2008	Fall	GAT, Birmingham	251	126	50.2%
2009	Fall	GAT, Birmingham	280	63	22.5%
2010	Fall	GAT	122	47	38.5%
2011	Fall	GAT	208	52	25.0%

[1] Count/percent of blood serum samples with rabies virus neutralizing antibody ≥ 0.05 IU/ml
[2] To provide baseline rVNA in ORV-naïve raccoon populations (i.e., collected prior to any ORV)
[3] Georgia-Alabama-Tennessee

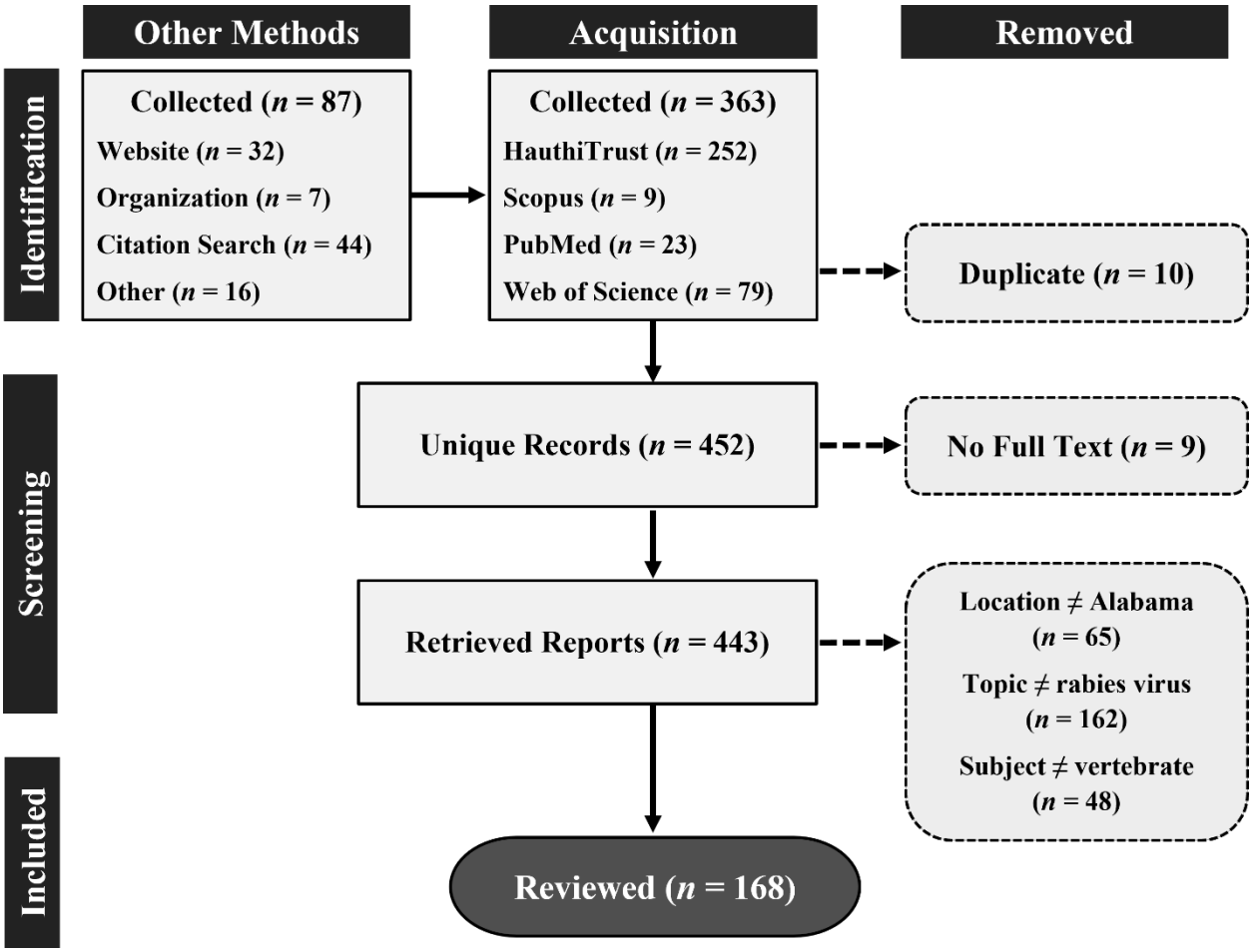
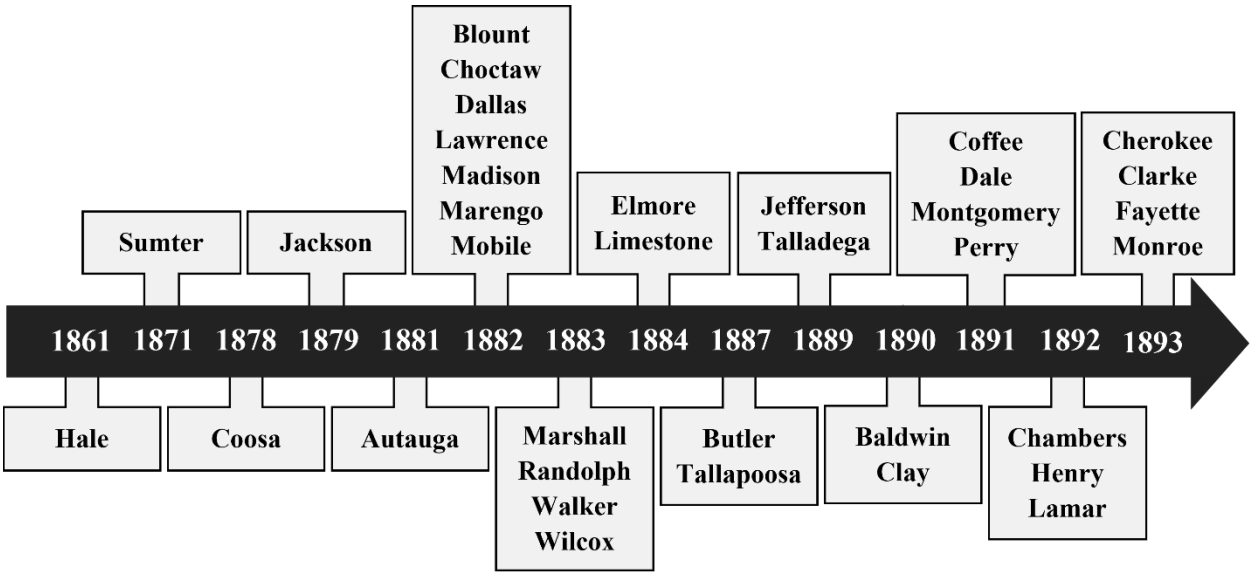


Figure 1.1 The number of records (*n*) identified, included, and excluded for each phase of the preferred reporting items for systematic reviews and meta-analyses (PRISMA) process. Other records originated from state and federal government websites, directly from organizations, and from the references cited section of selected reports.



No Rabies		Rabies: Time Unknown			No Report
Calhoun	Marion	DeKalb	Barbour	Pickens	Colbert
Escambia	Russell	Greene	Bibb	Shelby	Covington
Etowah	St. Clair	Lee	Bullock	Tuscaloosa	Crenshaw
Franklin	Winston	Lowndes	Chilton	Washington	Pike
Geneva		Macon	Cleburne		
Lauderdale		Morgan	Conecuh		

Figure 1.2 Timeline of initial recognition of rabies reported by physicians for each county of Alabama during the 19th century. Counties in the shaded boxes below the timeline reported a 'rabies-free' status, reported rabies presence but did not provide details, or did not report any rabies data.

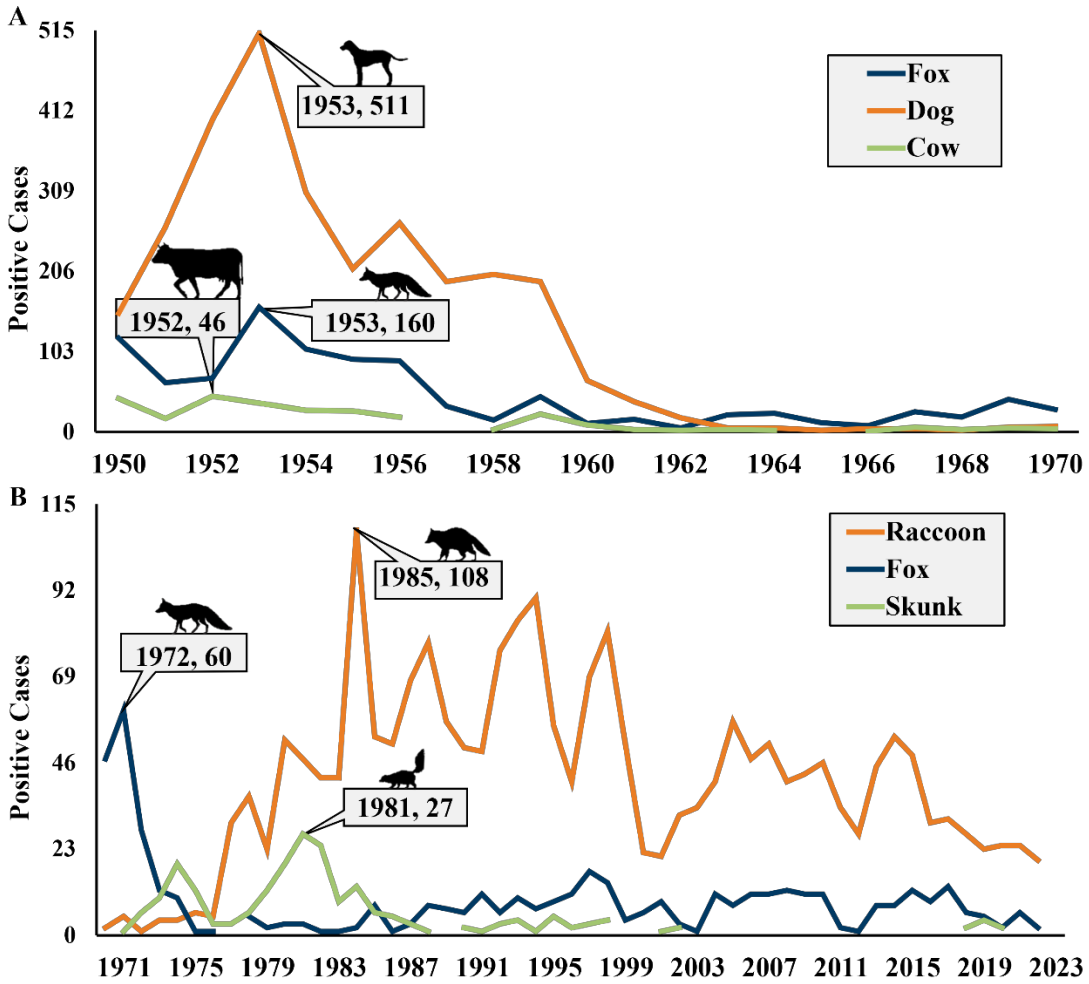


Figure 1.3 Laboratory confirmed positive rabies cases reported by the Alabama Department of Public Health (ADPH) A. Rabies cases reported from 1950-1970 for domestic dogs (*Canis lupus familiaris*), red foxes (*Vulpes vulpes*),gray foxes (*Urocyon cinereoargenteus*), and livestock, primarily cows (*Bos taurus*). B. Rabies cases reported by the ADPH from 1971-2023 for gray and red foxes, raccoons (*Procyon lotor*), and striped skunks (*Mephitis mephitis*). Callouts contain the year in which the number of reported rabies cases for the taxa were greatest within the selected period, followed by the number of cases.

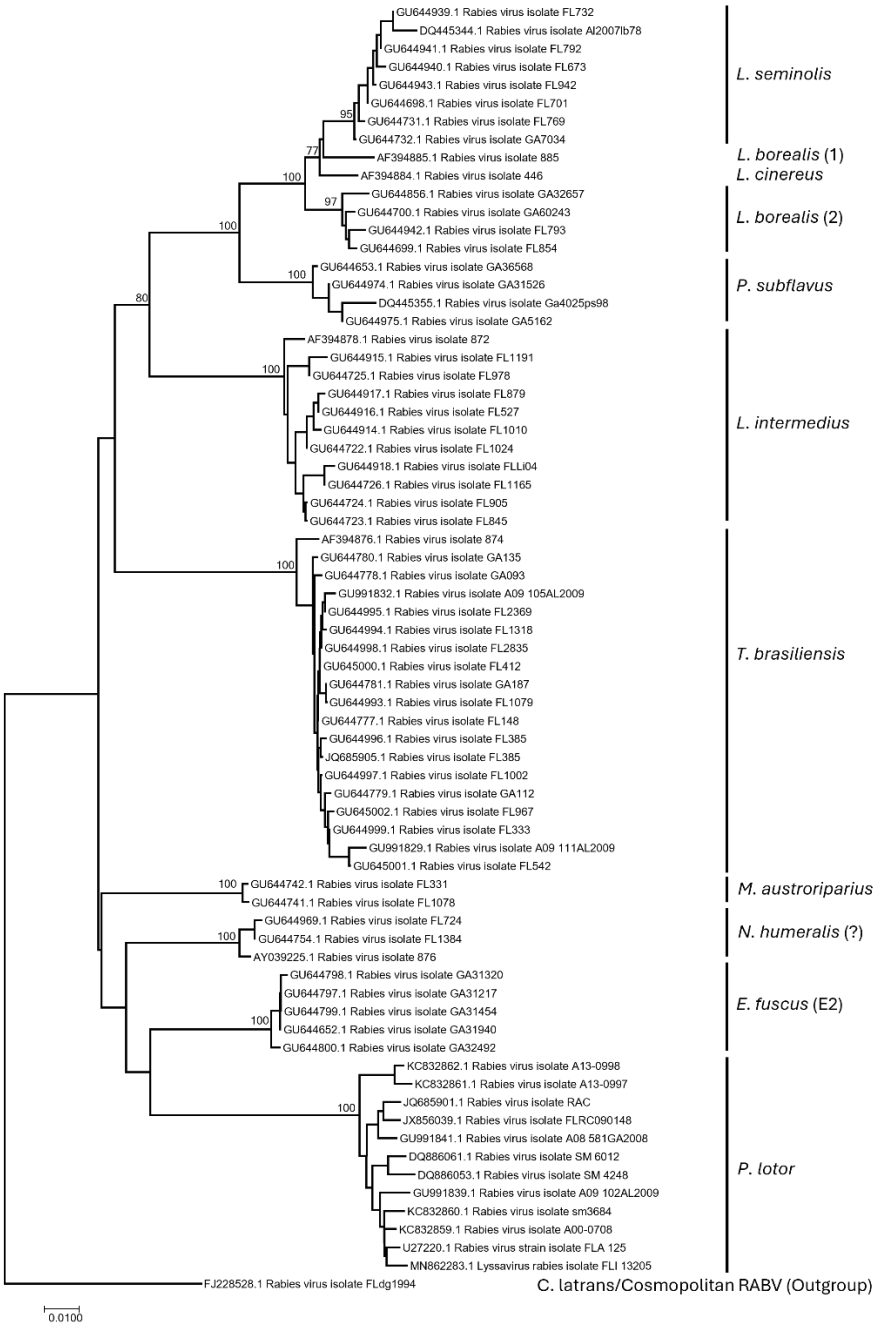


Figure 1.4 Neighbor-joining phylogenetic tree of the complete and partial nucleoprotein (N) genesequences of rabies viruses from the southeastern United States available in GenBank. The analysis was based on p-distances, with pairwise gap deletion, for 1000 bootstrap replicates. The bootstrap values over 70% are indicated at key nodes, and distances are drawn to scale. The tree is rooted with the coyote (*Canis latrans*) – cosmopolitan – rabies virus variant.

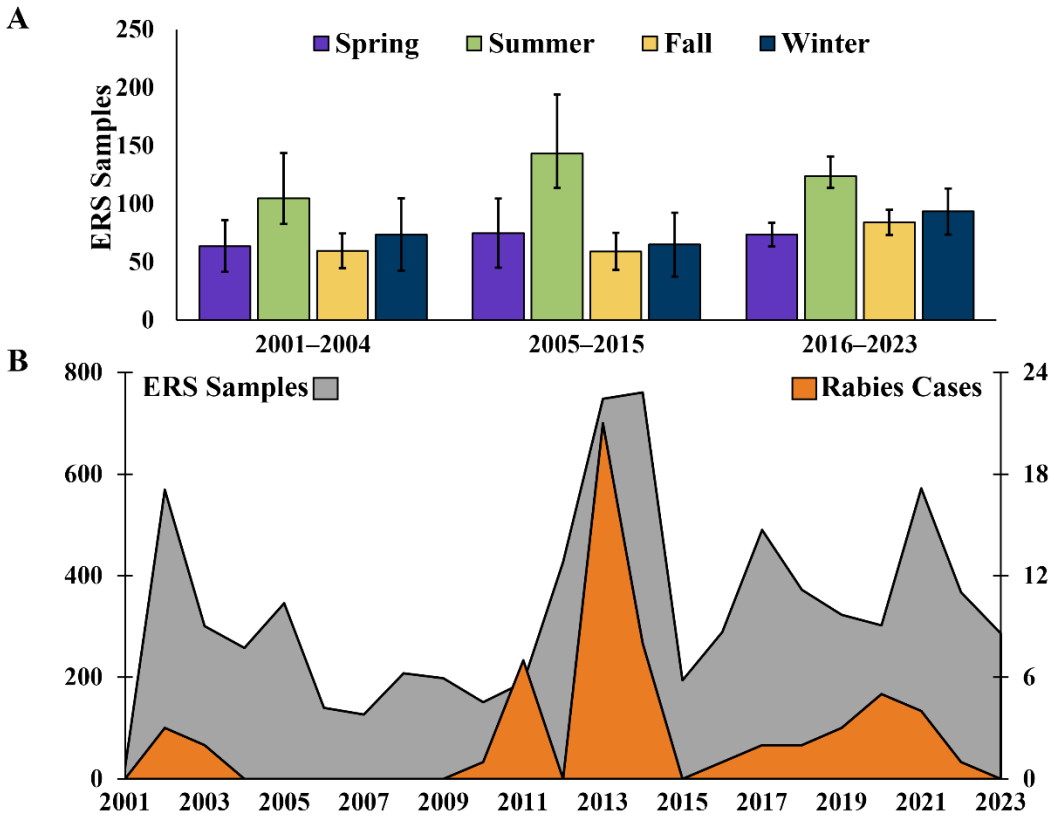


Figure 1.5 Comparison of Alabama enhanced rabies surveillance (ERS) A. animal samples collected and tested during spring (March–May), summer (June–August), fall (September–November), and winter (December–February), across three operational phases: early efforts (2001–2004), implementation of the direct rapid immunohistochemical test (2005–2015), and the advanced initiative (2016–2023). Error bars represent the variation in sample collection. B. Annual number of animal samples collected and annual number of confirmed rabies cases.

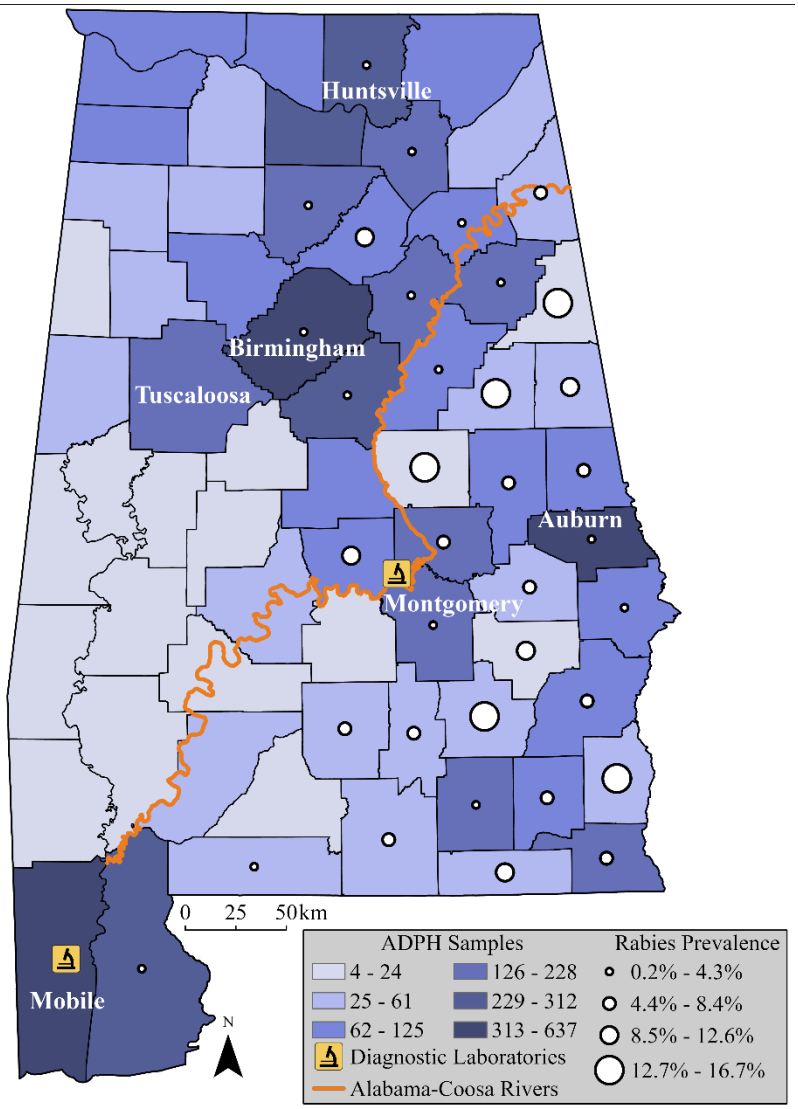


Figure 1.6 Rabies period prevalence by county from 2018-2022, based on both enhanced rabies surveillance (active) and public health surveillance (passive) compared to the number of suspect animals submitted to the Alabama Department of Public Health rabies diagnostic laboratories (yellow square containing a microscope icon). Labels represent cities where the population reported by the United States Census Bureau was equal or greater than 50,000 during this period

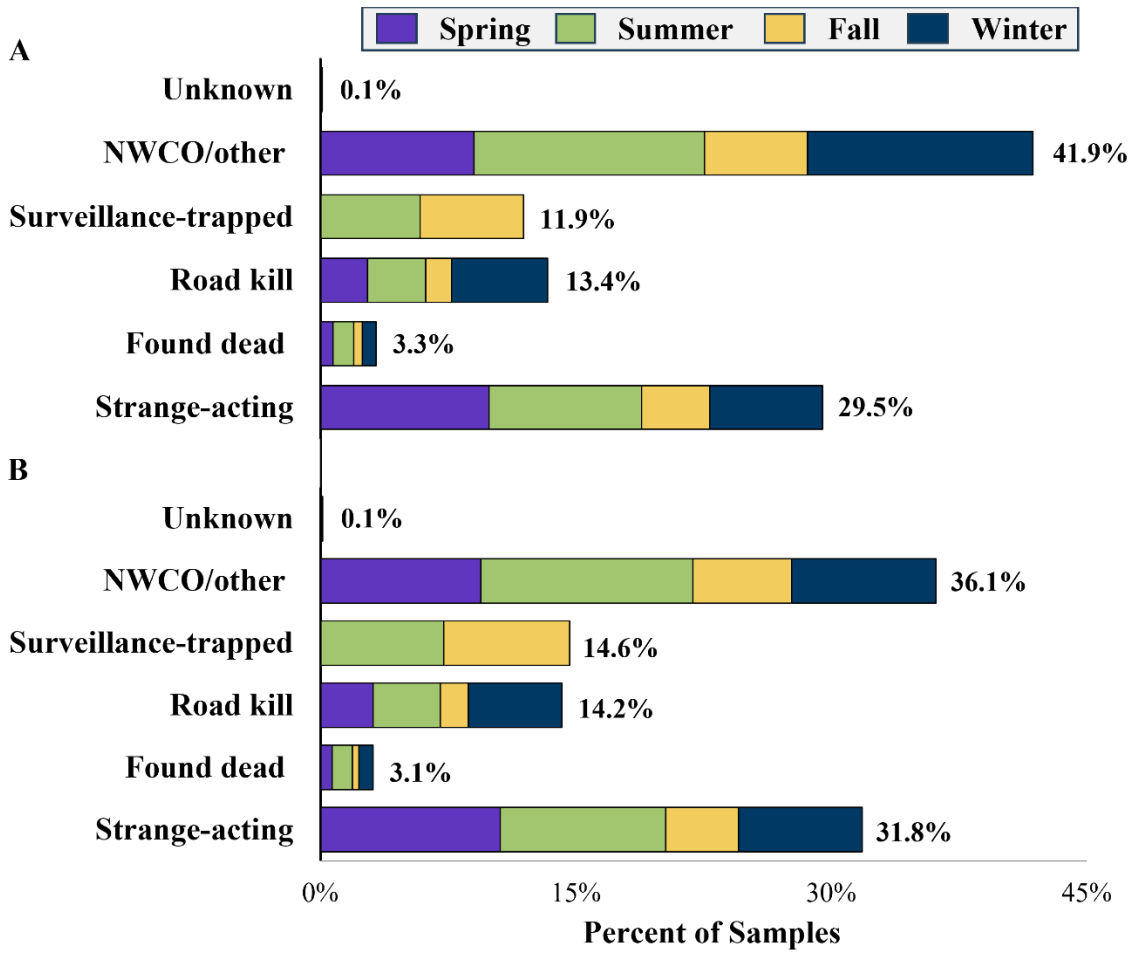


Figure 1.7 Comparison of enhanced rabies surveillance (ERS) samples collected in Alabama from 2016-2023 by collection method and seasons including spring (March, April, May), summer (June, July, August), fall (September, October, November), and winter (December, January, February), for A. all animal samples B. raccoon (*Procyon lotor*) samples.

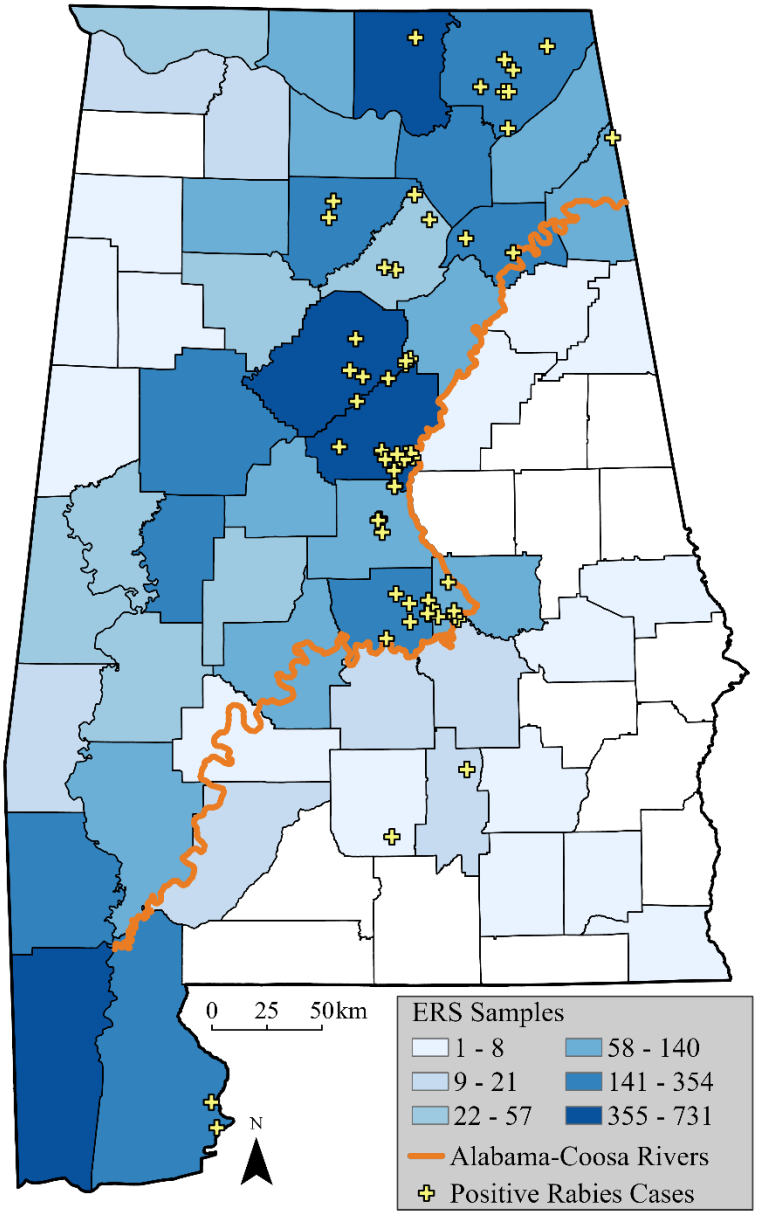


Figure 1.8 County-level count of enhanced rabies surveillance (ERS) samples collected by Wildlife Services and rabies positive cases (yellow cross) in Alabama from 2001-2023.

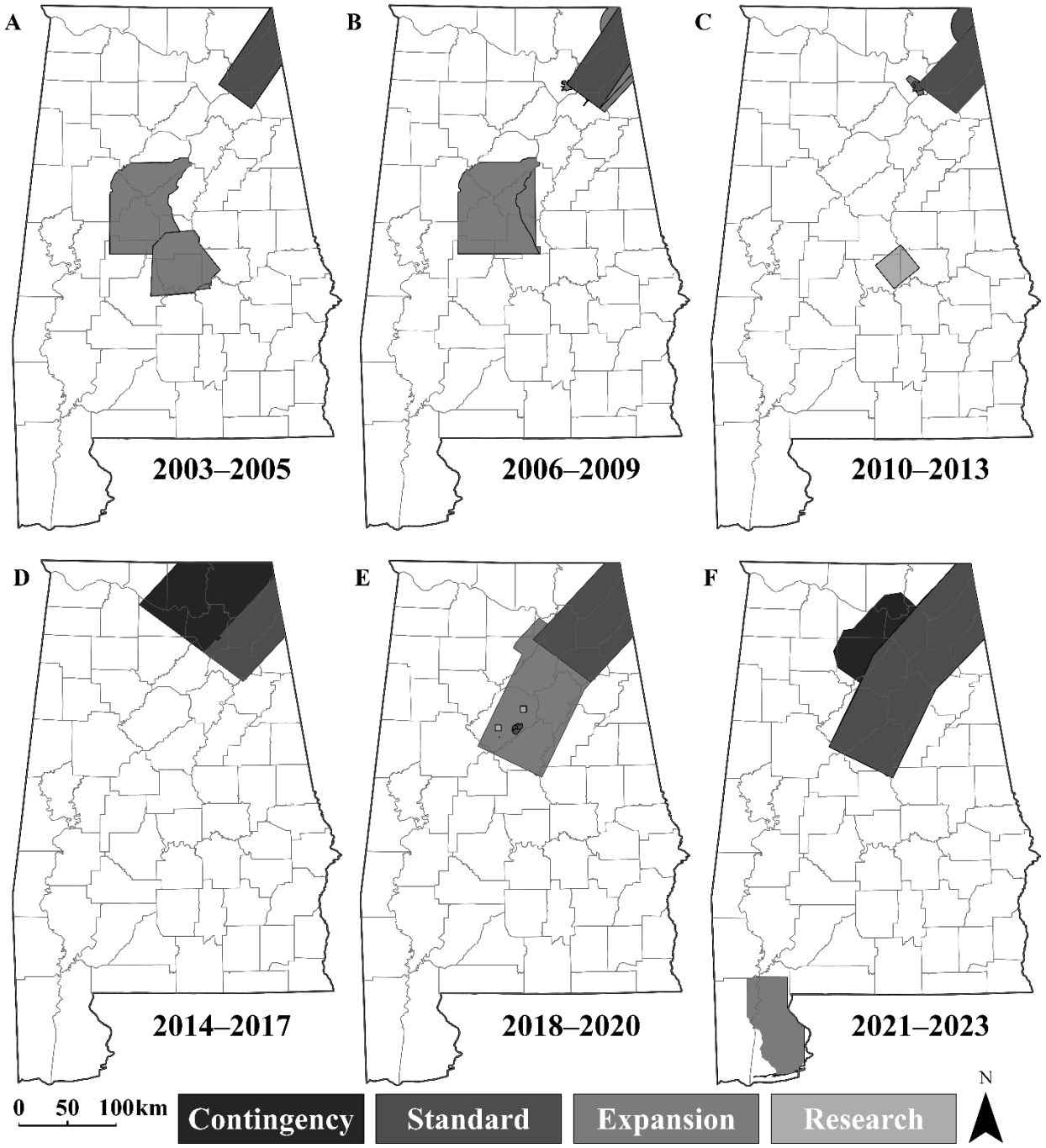


Figure 1.9 Oral rabies vaccine (ORV) zones implemented as part of standard operations, zone expansions, research studies, or contingency actions in Alabama from A. 2003-2005 B. 2006-2009 C. 2010-2013 D. 2014-2017 E. 2018-2020 F. 2021-2023.

Appendix 1.1. Sequences of nucleoprotein (N) gene of rabies viruses from the southeastern US available in GenBank.

GenBank No	State	Year	Host	Lineage
AF394876	FL	1988	T. brasiliensis	T. brasiliensis
AF394878	FL	1988	L. intermedius	L. intermedius
AF394884	GA	1982	L. cinereus	L. cinereus
AF394885	FL	1988	L. borealis	L. borealis (1)
AY039225	FL	1988	M. austroriparius	N. humeralis
DQ445344	AL	1978	L. borealis	L. seminolis
DQ445355	GA	1998	P. subflavus	P. subflavus
FJ228528	FL	1994	Dog	C. latrans
GU644652	GA	2004	E. fuscus	E. fuscus (E2)
GU644653	GA	2004	E. fuscus	P. subflavus
GU644698	FL	2003	L. borealis	L. seminolis
GU644699	FL	2003	L. borealis	L. borealis (2)
GU644700	GA	2005	L. borealis	L. borealis (2)
GU644722	FL	2001	L. intermedius	L. intermedius
GU644723	FL	2001	L. intermedius	L. intermedius
GU644724	FL	2001	L. intermedius	L. intermedius
GU644725	FL	2001	L. intermedius	L. intermedius
GU644726	FL	2004	L. intermedius	L. intermedius
GU644731	FL	2003	L. seminolis	L. seminolis
GU644732	GA	2003	L. seminolis	L. seminolis
GU644741	FL	2001	M. austroriparius	M. austroriparius
GU644742	FL	2001	M. austroriparius	M. austroriparius
GU644754	FL	2001	N. humeralis	N. humeralis (?)
GU644777	FL	2004	T. brasiliensis	T. brasiliensis
GU644778	GA	2004	T. brasiliensis	T. brasiliensis
GU644779	GA	2004	T. brasiliensis	T. brasiliensis
GU644780	GA	2004	T. brasiliensis	T. brasiliensis
GU644781	GA	2004	T. brasiliensis	T. brasiliensis
GU644797	GA	2004	E. fuscus	E. fuscus (E2)
GU644798	GA	2004	E. fuscus	E. fuscus (E2)
GU644799	GA	2004	E. fuscus	E. fuscus (E2)
GU644800	GA	2005	E. fuscus	E. fuscus (E2)
GU644856	GA	2005	L. borealis	L. borealis (2)
GU644914	FL	2002	L. intermedius	L. intermedius
GU644915	FL	2002	L. intermedius	L. intermedius
GU644916	FL	2002	L. intermedius	L. intermedius
GU644917	FL	2002	L. intermedius	L. intermedius
GU644918	FL	2004	L. intermedius	L. intermedius
GU644939	FL	2001	L. seminolis	L. seminolis
GU644940	FL	2003	L. seminolis	L. seminolis
GU644941	FL	2004	L. seminolis	L. seminolis
GU644942	FL	2004	L. seminolis	L. borealis (2)
GU644943	FL	2004	L. seminolis	L. seminolis
GU644969	FL	2003	N. humeralis	N. humeralis (?)
GU644974	GA	2004	P. subflavus	P. subflavus

GU644975	GA	2005	P. subflavus	P. subflavus
GU644993	FL	2001	T. brasiliensis	T. brasiliensis
GU644994	FL	2002	T. brasiliensis	T. brasiliensis
GU644995	FL	2003	T. brasiliensis	T. brasiliensis
GU644996	FL	2003	T. brasiliensis	T. brasiliensis
GU644997	FL	2004	T. brasiliensis	T. brasiliensis
GU644998	FL	2004	T. brasiliensis	T. brasiliensis
GU644999	FL	2004	T. brasiliensis	T. brasiliensis
GU645000	FL	2004	T. brasiliensis	T. brasiliensis
GU645001	FL	2004	T. brasiliensis	T. brasiliensis
GU645002	FL	2004	T. brasiliensis	T. brasiliensis
GU991829	AL	2009	T. brasiliensis	T. brasiliensis
GU991832	AL	2009	T. brasiliensis	T. brasiliensis
JQ685905	FL	2003	T. brasiliensis	T. brasiliensis
JQ685901	GA	2003	P. lotor	P. lotor
U27220	FL	1987	P. lotor	P. lotor
GU991841	GA	2008	P. lotor	P. lotor
GU991839	AL	2009	P. lotor	P. lotor
MN862283	AL	1991	P. lotor	P. lotor
JX856039	FL	2009	P. lotor	P. lotor
KC832860	FL	1997	P. lotor	P. lotor
KC832859	FL	2000	P. lotor	P. lotor
KC832862	FL	2013	P. lotor	P. lotor
KC832861	FL	2013	P. lotor	P. lotor
DQ886061	TN	2003	P. lotor	P. lotor
DQ886053	NC	1992	P. lotor	P. lotor

Appendix 1.2. Oral rabies vaccine zones in Alabama from 2003 to 2023 where fishmeal polymer (FMP) and coated sachet (CS) baits were distributed by methods including fixed wing (FW), rotary wing (RW), ground (G), and bait station (S).

Year	Zone	Timing	Area (km²)	Baits	Bait Style	Distribution Method
2003	GAT[1]	Fall	3187	273478	FMP	FW, G
2004	GAT	Fall	3187	335133	FMP	FW, G
	Selma	Spring	3504	279720	FMP	FW, G
2005	Selma	Spring	3522	254800	FMP	FW, G
	GAT	Fall	3202	172091	FMP	FW, G
	Birmingham	Fall	6536	471855	CS, FMP	FW, G
2006	Birmingham	Spring	6538	442021	CS, FMP	FW, G
	GAT	Fall	3200	179541	FMP	FW, G
	Birmingham	Fall	6538	441448	CS, FMP	FW, G
2007	Birmingham	Spring	6538	434503	CS, FMP	FW, G
	GAT	Fall	3200	618298	CS, FMP	FW
	Birmingham	Fall	6562	445297	CS, FMP	FW, G
2008	GAT	Fall	2784	133875	CS, FMP	FW
	Birmingham	Fall	6557	414571	CS, FMP	FW, G
2009	GAT	Fall	4472	216540	CS, FMP	FW, G
	Birmingham	Fall	3876	220459	CS, FMP	FW, G
2010	GAT	Fall	3607	217057	CS, FMP	FW, G
2011	GAT	Fall	3177	203016	CS, FMP	FW, G
	Prattville*	Fall	1038	81000	CS	RW
2012	GAT	Fall	3109	196687	CS, FMP	FW, G
	Prattville*	Fall	1038	81300	CS	FW
2013	GAT	Fall	3081	164751	CS, FMP	FW, G
	Prattville*	Fall	1038	81000	CS	FW
2014	GAT	Fall	4079	217223	CS, FMP	FW, RW, G
	Huntsville	Fall	7926	832170	CS, FMP	FW, RW, G
2015	GAT	Fall	4087	220634	CS, FMP	FW, RW, G
	Huntsville	Fall	7951	823049	CS, FMP	FW, RW, G
2016	GAT	Fall	4103	272937	CS, FMP	FW, RW, G
	Huntsville	Fall	11001	1012525	CS, FMP	FW, RW, G
2017	GAT	Fall	4288	233857	CS	FW, RW
	Huntsville	Fall	7181	391421	CS	FW, RW
2018	Birmingham[2]	Spring	80	10080	FMP	G
	Birmingham*	Fall	93	10440	FMP	G, BS
	GAT	Fall	6829	350913	CS	FW, RW
2019	Birmingham*	Fall	91	11160	FMP	G, BS
	GAT	Fall	9349	447360	CS	FW, G
2020	GAT	Fall	2960	159300	CS	FW
	Cullman	Fall	14247	865870	CS, FMP	FW, RW, G
2021	GAT	Fall	3189	172050	CS	FW
	Cullman	Fall	15762	1036446	CS, FMP	FW, RW, G
2022	Baldwin	Winter	4046	218640	CS, FMP	FW, RW, G
	GAT	Fall	3327	180661	CS	FW, RW
	Cullman/Huntsville	Fall	17101	1181160	CS, FMP	FW, RW, G

2023	Baldwin	Winter	4104	222480	CS, FMP	FW, RW, G
	GAT	Fall	4148	242400	CS	FW
	Cullman/Huntsville	Fall	16689	1136160	CS, FMP	FW, RW, G

[1] Georgia-Alabama-Tennessee
[2] One-time, localized effort, led by the Jefferson County Health Department
* Studies of bait distribution strategies, led by the National Wildlife Research Center

Chapter 2: Oral Rabies Vaccine Bait Encounters and Uptake by Target and Non-target Species in Alabama

Abstract

The raccoon (*Procyon lotor*) rabies virus (RRV) variant poses significant challenges to public health and wildlife management across the eastern United States (US). Despite efforts by the National Rabies Management Program (NRMP), including strategic distribution of oral rabies vaccine (ORV) baits, vaccination coverage has been insufficient to achieve local elimination in wildlife populations. Alabama, in the southeastern US, is characterized by diverse landscapes and ecological conditions that potentially influence ORV effectiveness. This state represents the westernmost extent of the enzootic RRV front and ORV zone. Nevertheless, the fate of ORV baits has not been examined in this region. From 2022 to 2024, we deployed 1,728 placebo ORV baits across northern, central, and southern Alabama, evaluating the effects of bait type (RABORAL V-RG or ONRAB), habitats (upland or lowland), and physiographic region (north, central, south) on bait uptake by raccoons and non-target species using camera traps. Raccoons accounted for 32.7% of vertebrate bait uptake, while the primary non-target competitor, Virginia opossums (*Didelphis virginiana*), consumed 52.9%. Bait uptake by raccoons declined rapidly throughout the seven day study period, with 82.7% of bait uptake occurring within three days of deployment, potentially due to degradation of bait attractiveness. Future studies in Alabama should include additional habitats and physiographic regions and evaluate the influence of environmental degradation on bait uptake by raccoons. Development of ORV strategies tailored to the dynamic environments of the southeastern US such as bait modifications, habitat specific distribution, and seasonally optimized deployment, may be essential to achieve management goals in this region.

Introduction

In the eastern United States (US), raccoons (*Procyon lotor*) serve as the primary reservoir for the raccoon rabies virus (RRV) variant, accounting for at least 30% of rabies cases reported annually to the Centers for Disease Control and Prevention (CDC) (Ma et al. 2022, 2023, 2024). The US Department of Agriculture's National Rabies Management Program (NRMP), through Wildlife Services, distributes oral rabies vaccines (ORV) strategically to create a vaccination barrier to prevent the westward movement of RRV (USDA 2023). This NRMP has historically been successful in halting the spread of RRV beyond the Appalachian Mountains (Slate et al. 2008). Consequently, management efforts have shifted toward local elimination of RRV and adjusting ORV zones eastward (Slate and Chipman 2020). However, current vaccination efforts in the southeastern US have not yet reached thresholds necessary for elimination of RRV (WHO 2018, Haley et al. 2019, Dixon et al. 2023, Hill et al. 2023*b*).

Alabama is a critical area for RRV management, representing the westernmost extent of the enzootic RRV front and ORV zone (R. B. Chipman et al. 2023, Ma et al. 2024). Bait distribution in central and northern Alabama began in 2003 as part of a regional Georgia-Alabama-Tennessee (GAT) management zone and expanded to include Baldwin County in coastal southwest Alabama during 2022 (Chipman et al., 2023; Kostrzewski & Slate, 2003; Krebs et al., 2004). Historically, the Alabama-Coosa River system was believed to have acted as a partial barrier to RRV spread in south and central Alabama, although this waterway has not fully prevented western movement of the disease to date (Arjo et al. 2008, Johnson et al. 2009). Mechanisms underlying the limited RRV spread observed in the state's central Blackbelt Prairie region, west of this river system, where no ORV operations were conducted, remain unclear. However, the NRMP expanded ORV operations in response to the westward spread of RRV

beyond the GAT zone in 2020 and 2022, highlighting the need for improved understanding of factors affecting ORV vaccination efforts (Espinosa 2024).

Field effectiveness of ORV operations is influenced by multiple factors, including the encounter rate and selective uptake of baits by raccoons (Slate et al. 2009, Davis et al. 2024). Raccoons are adaptable, ecological generalists that inhabit a variety of habitats (Johnson 1970, Daniels et al. 2019). Alabama's transitional landscape is characterized by diverse physiographic regions, climatic conditions, and habitats such as forested mountains, rolling plains, coastal lowlands, and a variety of aquatic environments (Griffith et al. 1989, Omernik 1995, Algeo et al. 2017). Non-target species with similar ecological niches, such as wild pigs (*Sus scrofa*) and Virginia opossums (*Didelphis virginiana*), are abundant throughout Alabama and may compete with raccoons for ORV baits (Duncan 2013, Slate et al. 2020, Helton et al. 2023). Additionally, mild winters, high humidity, and elevated invertebrate activity seasonally in Alabama may accelerate bait degradation and impact ORV performance by reducing bait palatability (Bachmann et al. 1990, Linhart et al. 1993).

This study aimed to evaluate the overall fate of ORV baits in Alabama, with a specific focus on competition for baits between raccoons and non-target species. Additionally, the specific influence of habitats, invertebrate fouling, and different vaccine baits on the utility of current management strategies for RRV have not been evaluated thoroughly in Alabama. Research objectives of this study were to: 1) quantify the uptake of placebo ORV baits by raccoons and competitors; 2) examine the time to bait uptake; and 3) assess the effects of regions, habitats, and bait types on the probability and rate of bait consumption.

Study Area

We sampled three distinct physiographic regions in Alabama for this study (Fig. 2.1). Sampling in the northern region took place in Madison County at Redstone Arsenal (34.69° N, 86.65° W), a 150-km² property within the Eastern Highland Rim region of Alabama (Omernik 1995, Griffiths et al. 2001). The land cover included woody wetlands (25%), pasture and hay (17%), deciduous forest (16%), and evergreen forest (11%), with the remaining 22% consisting of developed land (Dewitz 2024). Elevation varied from 376 m above sea level on Madkin Mountain and Weeden Mountain to an average of 177 m above sea level in lowland habitats.

In the central region, sampling was conducted in Autauga County at the Charles D. Kelley Autauga Wildlife Management Area (32.59° N, 86.55° W), a 33-km² property managed by the Alabama Department of Conservation and Natural Resources. Elevation ranged from 84 m to 184 m above sea level, characterized by rolling hills typical of the Fall Line Hills region (Omernik 1995, Griffiths et al. 2001). The primary land cover included loamy and sandy soils, supporting managed and natural pine forests (51%) and grassland and herbaceous areas (19%) (Dewitz 2024).

In the southern region, sampling was conducted across 44 km² of Baldwin County, encompassing five Forever Wild and ADCNR State Lands Division tracts. These tracts included Weeks Bay Reserve (30.31° N, 87.77° W), Live Oak Landing (30.95° N, 87.87° W), Perdido River Wildlife Management Area (30.68° N, 87.45° W), Splinter Hill Bog (31.03° N, 87.66° W), and the Blackwater River South Special Opportunity Area (30.47° N, 87.47° W). Elevation averaged 150 m above sea level, representing the Southeastern Floodplain and Low Terraces region (Griffiths et al. 2001). The dominant land cover types were evergreen forest (44%) and woody wetland (42%) (Dewitz 2024).

Methods

Placebo Baits

To examine the effect of bait type, we compared the fate of placebo RABORAL V-RG® (V-RG; a registered trademark in the USA and elsewhere of Merial, Inc., which is now part of Boehringer Ingelheim Animal Health USA Inc., Athens, Georgia, USA) baits (Maki et al. 2017) and placebo Ontario Rabies Vaccine (ONRAB®; Artemis Technologies Inc., an indirect, wholly-owned subsidiary of Ceva Sante Animale, S.A., Ontario, Canada) baits (USDA 2009). The V-RG coated sachet (CS) bait consisted of a plastic container coated with wax and fishmeal crumbs. The ONRAB Ultralight bait consisted of a plastic blister pack embedded in a wax-fat-based matrix, coated with artificial flavorings, such as icing sugar and marshmallow flavoring (Rosatte et al. 2009). The packets and attractants of both placebo V-RG and placebo ONRAB baits were identical to the baits distributed by the NRMP. Baits distributed during standard ORV operations contain either a recombinant live human adenovirus type 5 vector (ONRAB) or a recombinant live vaccinia virus vector (V-RG) expressing the glycoprotein gene of the Evelyn-Rotkitniki-Abelseth (ERA) strain of rabies virus (Fehlner-Gardiner et al. 2012). The placebo baits distributed in this study contained at least 1.5 mL of water similar to the volume of vaccine in V-RG and ONRAB baits.

Fieldwork

We examined the fate of baits during the same approximate time of the year that they are distributed as part of ORV programs. In the northern and central Alabama regions, we collected samples in September or October of 2022 and 2023. In the southern region we collected samples in January of 2023 and 2024. We visited study regions in a fixed order each year and completed

sampling approximately two weeks ahead of scheduled NRMP ORV operations in the GAT and Baldwin County zones to ensure that data were not influenced by oversaturation of ORV baits.

In each region, we established a total of twelve sites, evenly divided between upland and lowland habitats. Upland habitats were characterized by greater elevations that supported ecosystems such as pine forests and grasslands (Phillips 2006). Lowland habitats were areas of lower elevation near permanent water sources or areas that were prone to flooding (Kellison and Young 1997). Potential sites were identified using ArcGIS Pro (ESRI 2023) with data from the National Land Cover Data (Dewitz 2024), Shuttle Radar Topography Mission Digital Elevation Models (USGS 2021), and the National Hydrography Dataset (Moore et al. 2019). A grid of 320 m x 500 m cells were generated across Alabama, with each cell matching the dimensions of a study site. The 320-m sides of each cell were oriented north to south, while the 500-m sides were oriented east to west. Cells with >75% forested land cover and no large water bodies along the 320-m sides were recorded. Average elevation for each recorded cell was calculated using the corner (NE, NW, SE, SW) values. Regional averages were used to determine the elevation threshold for classifying cells as upland or lowland habitat relative to the potential cells.

Using a random block design, six upland and six lowland sites were selected per region. Adjacent or touching cells were discarded and systematic selection continued until a set of sites met spatial and habitat requirements. Sites remained static throughout the study, unless major disturbances (e.g., tornadoes, clearcutting) necessitated relocation to adjacent areas. At each site, we deployed 12 tree-mounted cameras (Moultrie 900i, PRADCO, Outdoor Brands Inc., Birmingham, Alabama, USA). Cameras were spaced 64m apart along parallel transects (six cameras each), which aligned with the 320-m sides of the site. Cameras were mounted

approximately one meter high at a 65° angle using stainless steel mounts and programmed to take three motion-triggered rapid-fire photos without a quiet period between events.

Procedures were designed to replicate the conditions of standard ORV operations in Alabama. Site locations were established with approximately 500 m spacing between distribution lines, corresponding to the aircraft maneuvers between flight lines for ORV distribution. Additionally, the grid ensured that the 320 m sides of each site were aligned with specific longitude degrees. To replicate the bait density used in standard ORV operations (75 baits/km^2) targeting raccoons (Slate and Chipman 2020), we placed a single placebo ORV bait on the ground in front of each camera (12 baits per 1,600m^2 site) and we distributed the same bait type to all cameras in a site. The understory vegetation and litter around the baits were left undisturbed. In each region, cameras remained active for two, seven-night sessions per year. For the first session each year, we randomly assigned bait type using a block design to ensure that an equal number of sites (6 sites/bait) were assigned V-RG and ONRAB baits per session. At the start of second session, we visited cameras and deployed the opposite bait type compared to the prior session so that all cameras in the study recorded data for both baits each year.

At the end of each sampling period, we attempted to locate the bait from the previous session within a three-meter vicinity of the deployment location for every rebait and retrieval. We collected baits found at the end of sessions regardless of condition and stored them until we returned from the field and could visually examine bait characteristics. For each collected bait, we recorded the percentage of attractant that remained, and any damage observed to the packet. While the bait characteristics were used to evaluate the palatability of baits that were not taken within the sampling period, the fate of baits was defined based on the volume of water that remained in the packet, as a vaccine surrogate. We defined collected baits as taken or fouled if

the packet was empty or was leaking and contained less than 1-ml of water. Baits that were no longer intact or with perforations were assumed to have been consumed by an unknown vertebrate, while baits not filled and with no visible damage were considered fouled.

We processed camera trap images using Camelot software (Fauna and Flora International, Vietnam; Hendry and Mann 2017). The fate of the bait from images was classified as 'taken' if there was direct oral contact with the bait by an animal or if an interaction with the bait was followed by subsequent images showing the bait as destroyed or missing. We recorded what animal consumed the bait along with the date and time when a bait was considered 'taken'. Bait uptake was recorded one time for each bait even if the bait was consumed multiple times. We classified the bait consumer by species when possible but deferred to genus or family when necessary. For example, we were able to classify rabbits (*Sylvilagus* spp.) by genus, but small rodents were grouped into the *Muridae* family. We compared records from collected baits and camera images to corroborate the fate assigned to baits for analysis.

Statistical Analysis

We analyzed the probability and rate of bait uptake using R version 4.4.1 (R Core Team 2024). Generalized linear mixed-effects models (GLMMs) were implemented using the lme4 package to assess the likelihood of bait uptake (Bates et al. 2003, 2015). The binary response variable indicated whether the bait was 'taken' or 'not taken' by the group being evaluated within each sampling period. We employed a binomial error structure with a logit link function for this analysis (Christ 2009). To evaluate the rate of bait uptake over time, we used the survminer and survival packages (Therneau 2024). Kaplan-Meier (KM) survival curves were constructed to assess the probability of bait uptake across a seven-night sampling period. Baits that were not consumed during this session were right-censored (Kaplan and Meier 1958, Kleinbaum and

Klein 2012). The log-rank test was used to compare survival distributions across different regions, habitats, and bait types (Bland and Altman 2004). Cox proportional hazards (CPH) models were employed to assess the effect of covariates on bait uptake rate (Andersen and Gill 1982, Therneau and Patricia 2001).

We categorized baits into models based on their epizootiological importance and observed bait uptake frequency. Three separate models were developed to evaluate: 1) all taken baits; 2) baits taken by raccoons; and 3) baits taken by opossums. For species-specific bait uptake, two models were built: Model 1 (M1) assumed all baits were available throughout the sampling period, while Model 2 (M2) excluded baits that were fouled or taken by other species. These models provided insight into the effects of competition, bait fouling, and environmental damage on bait uptake by raccoons and their primary competitors. We tested models using fixed effects for region (north, central, south), habitat type (upland or lowland), bait type (ONRAB or V-RG), and session. To account for additional variability in samples, a random effect for year nested within camera nested within site was included in the GLMMs, and a frailty term for camera was used in the CPH models (Therneau and Patricia 2001, Bolker et al. 2009, Harrison et al. 2018). Lasso regression (least absolute shrinkage and selection operator) was applied using the glmnet package to select penalized fixed effects with non-zero coefficients for both GLMMs and CPH models (Friedman et al. 2008, 2010) (Appendix 2.1). Estimated effects and 95% confidence intervals were exponentiated and reported as odds ratios (OR) or hazard ratios (HR). To simplify the interpretation of effects, we calculated estimated marginal means (EMMs) and performed pairwise comparisons using the emmeans package (Lenth 2017, Lenth et al. 2022).

All statistical tests used a significance threshold of $p < 0.05$ (Fisher 1925). Model diagnostics were evaluated using the performance package (Lüdecke et al. 2021). We compared

the Akaike Information Criterion (AIC; Akaike 1974), corrected AIC (AICc), Bayesian Information Criterion (BIC; (Schwarz 1978), and R^2 (Nakagawa and Schielzeth 2013) for M1 and M2, and reported the results of the optimal model for each treatment (Burnham et al. 2002, Barr et al. 2013). Model 2 (M2) outperformed Model 1 (M1) across all analyses (Appendices 2.2, 2.3). Consequently, all statistical results reported for raccoons and opossums were derived from M2.

Results

We deployed a total of 1,728 placebo ORV baits across three regions in Alabama between 2022 and 2024. At the conclusion of the seven-day study periods, we retrieved 60.1% (346/576) of the baits in the northern region, 77.3% (445/576) in the central region, and 49% (282/576) in the southern region. Invertebrate activity on or near collected baits was documented for 7.7% (83/1073) of baits across all regions. Collected baits classified as not taken included 30.9% (107/346) in the northern region, 60.7% (270/445) in the central region, and 63.5% (178/282) in the southern region. For collected baits assigned a not taken fate, greater than 50% of the attractant remained on 43% (46/107) of baits in the northern region, 38.5% (104/270) of baits in the central region, and 82% (146/178) of baits in the southern region. In contrast, a greater proportion of collected baits determined not to be taken retained less than 25% attractants in the northern (43%, 46/107) and central (40.7%, 110/270) regions compared to the southern region (5.1%, 9/178). A total of 408,701 images were collected over 12,096 trap nights, with vertebrates identified in 80,779 images, which represented 80.96% (1399/1728) of the distributed baits. We excluded 56 baits with an unknown fate from all analyses, and all species-specific analyses excluded the 52 baits taken by an unknown vertebrate and the 28 fouled baits (Table 2.1). The remaining baits included 638 baits that were not taken within the seven-day sampling

period and 954 baits that were taken by an identified vertebrate (Fig. 2.2). Raccoons accounted for 32.7% (312/954) of vertebrate uptake and opossums accounted for 54.9% (505/954) of vertebrate uptake across regions (Fig. 2.3). Secondary target species, including coyotes (*Canis latrans*), gray foxes (*Urocyon cinereoargenteus*), red foxes (*Vulpes vulpes*), and striped skunks (*Mephitis mephitis*), represented 2.4% (23/954) of uptake. Aside from opossums, other non-target species that consumed baits included domestic cats (*Felis catus*), domestic dogs (*Canis lupus familiaris*), eastern cottontails (*Sylvilagus floridanus*), eastern gray squirrels (*Sciurus carolinensis*), nine-banded armadillos (*Dasypus novemcinctus*), rodents, and wild pigs. These species accounted for 11.9% (114/954) of vertebrate uptake across all regions. Rodents responsible for bait uptake were primarily from the families *Muridae* and *Cricetidae*, with *Peromyscus* spp. contributing to bait consumption in all regions. Rodents emerged as a substantial competitor against raccoons, particularly in the southern region, where they consumed 10.2% of the 576 distributed baits compared to 13.9% and 28.3% of baits taken by raccoons and opossums, respectively. Within the southern region, Perdido River WMA accounted for 57.6% (34/59) of baits removed by rodents followed by the 25.4% (15/59) of baits at Splinter Hill Bog. Across regions, upland habitat was associated with 90.1% (64/71) of the baits consumed by rodents. However, factors such as bait type, session, and year were not related to differences in rodent uptake in the study.

Probability of bait uptake by raccoons was 27% ($p = 0.003$) and 29.3% greater ($p = 0.002$) in the northern region compared to the central and southern regions, respectively (Table 2.2, 2.3). Probability of raccoon uptake was at least 20.3% greater for V-RG baits compared to ONRAB baits across all regions ($p < 0.001$). Habitat type did not affect bait uptake by raccoons in the northern ($p = 0.092$) or central regions ($p = 0.653$), but uptake was 43.7% greater

($p < 0.001$) in lowland compared to upland habitat in the southern region. Bait uptake by raccoons was not influenced by sampling year or session, except in the southern region, where probability of bait uptake by raccoons was 13% greater ($p = 0.016$) for the first compared to the second session. 82.7% (258/312) of bait consumption by raccoons occurred by day three of the study period and varied from 77.1% (84/109) to 86.3% (69/80) depending on region (Fig. 2.5a). From day four through the end of the study period, raccoons consumed less than 2% of the baits remaining on the landscape each day (Fig. 2.5b). The rate of bait uptake by raccoons was 72.5% and 89.4% greater in the northern compared to the central ($p < 0.001$) and southern ($p < 0.001$) regions, respectively (Table 2.4). The rate at which raccoons consumed V-RG baits was at least two times greater than ONRAB baits across all regions ($\chi^2 = 27.7$, $p < 0.001$) (Fig. 2.6a). Rate of bait uptake by raccoons was 8.8 times greater in lowland compared to upland habitat in the southern region ($p < 0.001$), but habitat did not significantly influence the rate that raccoons consumed baits in the northern ($p = 0.19$) or central ($p = 0.62$) regions (Fig. 2.6b). Sampling year and session did not influence the rate at which raccoons consumed baits, except in the southern region, where the rate of raccoon uptake for the second session was 35% less than the first session ($p = 0.009$).

Probability of bait uptake by opossums was 50.6% ($p < 0.001$) and 24.1% ($p = 0.033$) less in the central region compared to the northern and southern regions, respectively (Table 2.5, 2.6). Probability of opossum uptake was 20.3% greater for V-RG baits compared to ONRAB baits in the central ($p < 0.001$) region and at least 40.3% greater for V-RG baits in the northern ($p < 0.001$) and southern ($p < 0.001$) regions. We did not detect an effect of habitat type on bait uptake by opossums in the northern ($p = 0.111$) or central regions ($p = 0.276$), but opossum uptake was 45.8% greater ($p = 0.005$) in lowland compared to upland habitat in the southern

region. Probability of bait uptake by opossums for the first compared to the second study year increased 10.8% for the central ($p = 0.014$) region but decreased 34% for the southern region ($p < 0.001$). Session did not influence bait uptake by opossums, except in the central region where uptake probability increased 12.4% ($p = 0.007$) for the second session. 75.3% (380/505) of bait consumption by opossums occurred by day 3 of the study period. This varied from 68.3% (69/101) to 78% (188/241) depending on region. From day 5 through the end of the study period, opossums consumed less than 7% of baits remaining on the landscape each day. Bait uptake rate for opossums decreased 28.5% and 58.8% in the central region compared to the northern ($p < 0.001$) and southern ($p < 0.001$) regions, respectively (Table 2.7). The rate at which opossums consumed V-RG baits was at least two times greater than ONRAB baits across all regions ($\chi^2 = 94.8$, $p < 0.001$). Habitat significantly influenced rate of bait uptake by opossums across regions ($\chi^2 = 10.7$, $p = 0.001$). The rate of bait uptake by opossums was 59% ($p = 0.011$) less in lowland compared to upland habitat in the central region, but at least 42.5% greater in the northern ($p = 0.007$) and southern ($p < 0.001$) regions. Year did not significantly influence the rate that opossums consumed baits in the northern region ($p = 0.173$). Opossum uptake rate in first compared to the second year increased significantly for the southern region ($p < 0.001$) and decreased for the central region ($p = 0.048$). Bait uptake rate by opossums was not influenced by session, except in the central region where the rate of opossum uptake was 52.3% less in the first compared to the second session ($p = 0.002$).

Discussion

Bait uptake by raccoons was relatively low across Alabama, regardless of habitat or bait type, with raccoons consuming only 18.1% of distributed baits and 32.7% of baits taken by vertebrates. This finding is consistent with similar studies in the southeastern US, where

raccoons consumed less than 25% of baits across habitat types (Berentsen et al. 2023, Dixon et al. 2023). Similarly, the proportion of raccoons consuming baits containing biomarker reported in the literature ranged from 12% to 35% (Arjo et al. 2008, Hill et al. 2023*b*). Moreover, the proportion of raccoons sampled with levels of rabies virus neutralizing antibodies (rVNA) indicating vaccination in several southeastern studies support estimates of lower raccoon bait uptake (Pedersen et al. 2019, Helton et al. 2023). However, raccoon uptake rates have been greater in studies conducted farther south. The proportion of baits consumed by raccoons in Florida have been at least 34% (Olson and Werner 1999, Haley et al. 2019) and 69% of trapped raccoons were found to have consumed baits containing biomarkers (Olson et al. 2000). A target vaccination rate of 60% is generally considered the minimum threshold to interrupt rabies virus transmission in raccoon populations (Rees et al. 2013; Berentsen et al. 2018). While our study did not evaluate vaccination, bait consumption rates we documented suggest that current vaccination rates may be insufficient to achieve local elimination in raccoon populations throughout Alabama.

In our study, opossums were the primary competitors for ORV baits in all regions and habitats, consuming approximately 29.2% of the distributed baits and accounting for 52.9% of all baits taken by vertebrates. Uptake of ORV baits by non-target species, like opossums, reduces bait availability for raccoons and does not advance rabies management efforts, particularly for species that are less likely to be infected with or transmit rabies virus due to lower susceptibility (Wallace et al. 2014, Slate et al. 2020, Hill et al. 2024). Numerous studies evaluating ORV bait programs throughout the eastern US, from Florida (Olson et al. 2000) to Massachusetts (Bjorklund et al. 2017), reported similar results as ours and determined that opossums were the primary non-target bait consumers (Heinlein et al. 2020, Berentsen et al. 2023). Additionally,

opossums represented a substantial portion of trapped individuals in multiple studies conducted throughout the eastern US (Sattler et al. 2009, Pedersen et al. 2019, Slate et al. 2020). In contrast, studies conducted in South Carolina at the Savannah River Site since 2020 have consistently reported only 5% to 12% of baits being taken by opossums (Dixon et al. 2023, Helton et al. 2023, Hill et al. 2024). Bait uptake by a variety of secondary target and other non-target species, excluding opossums, accounted for 8% of distributed baits in our study. Bait fate studies across the RRV enzootic zone reported similar results. Raccoon and opossum uptake generally account for at least 90% of vertebrate bait uptake (Linhart et al. 2002; Smyser et al. 2010; Helton et al. 2023). Rodents were identified as the second greatest bait competitor against racoons following opossums. This is similar to a study in a coastal region of Puerto Rico, where rodents were responsible for the removal of up to 50% of distributed V-RG baits (Berentsen et al. 2020). In contrast, rodents were responsible for taking only about 5% of ONRAB baits in Tennessee, which is comparable to the 1.7% of distributed baits taken by rodents in the northern region in our study (Berentsen et al. 2023). Wild pigs were not significant non-target bait competitors. Bait uptake by wild pigs was not recorded in the northern or central regions and accounted for less than 1% of distributed baits. Wild pig uptake represented only 2.4% of baits in the southern region, where we documented pigs at three of the five properties in the study. This finding differs from prior bait fate studies in the southeastern US that found at least 10% of baits were consumed by wild pigs (Fletcher et al. 1990, Haley et al. 2019). Wild pigs are a highly social species and generally move together as a sounder (a group of related females and young) (Sparklin et al. 2009). We speculate that this clumped distribution of wild pigs limits their overall encounter rate with baits, and thus consumption. Additionally, bait consumption by wild pigs only occurred in lowland habitats, where competition with opossums and raccoons may

have prevented additional bait uptake (Dixon et al. 2023). Secondary target species such as coyotes, red and gray foxes, and striped skunks were each responsible for consuming less than 1% the distributed baits throughout the study. While raccoon uptake is the greatest priority, bait uptake by these species helps reduce the circulation of RRV and is considered beneficial to management programs (Slate et al. 2009). Studies identifying skunks and foxes as competitors are primarily limited to the mid-Atlantic and northeastern states (Mainguy et al. 2013, Beasley et al. 2024). Bait uptake by coyotes represented less than 1% of the baits distributed in each region which was less than studies conducted in Tennessee and Florida where coyote uptake accounted for 2% to 15% of baits (Haley et al. 2019, Webster and Beasley 2019, Berentsen et al. 2023). The overall low rate of bait consumption by secondary target species and non-target species (with the exception of opossums) suggests that these species are likely not important factors in most areas of Alabama with regards to ORV management goals.

Bait uptake by raccoons varied across the three physiographic regions sampled, with 22% of baits consumed in the northern region, 19% in the central region, and 14% in the southern region. Raccoon populations in Alabama fluctuate across the state, with the greatest densities observed in the southern region and lowest density in the central region (Johnson 1970, Kostrzewski and Slate 2004, Nelson and Slate 2006). This regional variation in bait uptake likely reflects differences in population characteristics of raccoons, as well as season, habitat characteristics, and landscape composition (Gehrt and Fritzell 1997, Chamberlain et al. 2000, Byrne and Chamberlain 2011, Lüdecke et al. 2021, Hill et al. 2023*c*). Reduced bait uptake in the southern region is also likely influenced by the timing of sampling, which was conducted during January. During winter, raccoon activity typically declines relative to fall, when racoon activity

is elevated due to juvenile dispersal and increased foraging (Rosatte et al. 1992, Yarrow et al. 1999, Hirsch et al. 2016, Baker et al. 2019).

While raccoons consumed a greater proportion of baits in lowland compared to upland habitat across regions, the effect of habitat type on raccoon uptake probability was not significant for the northern and central regions. Studies completed in South Carolina at the Savannah River Site reported consistent findings, with greater uptake estimated in bottomland hardwood compared to upland pine forests (Dixon et al. 2023, Helton et al. 2023, Hill et al. 2023*b*). Foraging behaviors that increase the likelihood of bait encounter by raccoons are often concentrated in lowland habitats because they typically provide more abundant resources such as water, food sources, and denning sites compared to upland habitats (Childs et al. 2000, Arjo et al. 2005, Chamberlain et al. 2007, Fisher 2007, Byrne and Chamberlain 2011). In contrast, pine-dominated uplands generally support lower raccoon densities and increased movement, which can limit bait encounters (Algeo et al. 2017, Elmore et al. 2017). Overall bait uptake by opossums was also greatest in lowland habitats, likely reflecting the overlap in foraging and habitat niches between the two species (Kissell and Kennedy 1992, Beasley et al. 2015, McClure et al. 2022).

We observed a preference for V-RG baits over ONRAB baits by raccoons across all regions regardless of habitat type. While ONRAB baits resulted in greater raccoon uptake and seroconversion rates compared to V-RG baits in Canada (Rosatte et al. 2009, Fehlner-Gardiner et al. 2012, Mainguy et al. 2012), studies comparing bait type are not currently available for comparison in the southeastern US. Baits with fish-flavored coatings have typically resulted in greater raccoon uptake compared to sweet coatings such as cherry essence, persimmon oil, and sweet corn oil (Linhart et al. 1993, 2002, Avrin et al. 2021). Local availability of similar food

items has been found to drive bait preferences for raccoons (Beasley et al. 2015). For example, wild grapes and persimmon are widely available for raccoon consumption throughout Alabama in the fall (September-November), which may have influenced the uptake of the sweet-flavored ONRAB baits by raccoons in our study (Johnson 1970, Yarrow et al. 1999).

The majority of bait uptake by raccoons occurred early during each sampling period and then rapidly decreased after the first few days. Between 78% and 87% of raccoon bait consumption took place within the first three days of the study period. This is consistent with previous studies where over 90% of baits were consumed within the first three days following bait deployment (Olson and Werner 1999, Blackwell et al. 2004, Smyser et al. 2013, Wolf et al. 2022). Olson and Werner (1999) observed that 59% of baits were contacted on the first night and 73% by the second night. Studies with study periods extending beyond one week have reported that the majority of bait uptake occurred within seven days (Roscoe et al. 1998, Blackwell et al. 2004, Hermann et al. 2011). Hence, it is unlikely that bait uptake by raccoons would have grew substantially if we increased the number of days within study sessions (Slate et al. 2020, Dixon et al. 2023). Rate of bait uptake also decreased with time following bait deployment, with raccoons consuming less than 2% of available baits in each region from day four onward.

Despite the decline in bait uptake over time, baits not taken within the seven-day sampling period accounted for a significant portion of distributed baits, representing 36.9% (638/1728) of baits in the study. Baits not taken throughout the study may reflect bait shyness, decreased attractiveness, and resource availability in the landscape. Bait shyness, where individuals that consume one bait do not return for additional baits, can limit overall uptake and reduce the effectiveness of bait distribution efforts (Wandeler 1991, USDA 2023). Bait shyness for raccoons has previously been linked to baits with unfamiliar textures or flavors (Rosatte et al.

1992, Webster and Beasley 2019). This may also reflect local resource availability or individual preferences (Heinlein et al. 2020). Attractants are crucial for both bait encounter and palatability, directly influencing raccoon uptake rates (Linhart et al. 2002, Boyer et al. 2011). However, resource abundance, particularly of natural food sources, such as mast or anthropogenic food, could reduce raccoon reliance on baits (Prange et al. 2004, Bozek et al. 2007, Beasley et al. 2015).

Contrary to our initial expectations developed from studies completed in South Carolina (Dixon et al. 2023, Helton et al. 2023), we found a low rate (<2%) of fouled baits in our study. Fouled baits represented either environmental degradation from precipitation, humidity, and temperature or invertebrate activity (Linhart et al. 1993, Berentsen et al. 2020). Determination of fouling in our study relied upon visual examination of baits collected after the conclusion of the seven-day sampling period. Empty or leaking baits were defined as fouled if we were unable to visually identify perforations to the packet. If the rates we documented are accurate, then fouling of baits by weather and invertebrates is not a significant factor affecting ORV programs in Alabama. However, environmental factors and increased invertebrate activity can reduce bait encounter and uptake by raccoons, even when they are not fouled (Linhart et al. 1993, Berentsen et al. 2020). We were unable to measure palatability or scent production of collected baits, nor the manner in which invertebrates may have influenced the bait coatings. Environmental degradation of baits may be accelerated by climatic factors such as increased temperature, humidity, and precipitation in the southeastern US relative to other regions of the RRV enzootic zone (Linhart et al. 2002, Hermann et al. 2011, Maki et al. 2017, Avrin et al. 2021). Hable et al. (1990) found that rainfall caused the fishmeal portion of the bait to become soggy or the gelatin capsule to dissolve. Similar effects were observed after seasonal flooding events in lowland

habitats in other studies conducted in the southeastern US (Cagle 1949, Hill et al. 2023*b*). Masson et al. (1999) found that placing baits in direct sunlight resulted in a greater titer loss ($10^{2.2}$ $TCID_{50}$/mL) compared to shaded areas ($10^{0.8}$ $TCID_{50}$/mL). Invertebrate activity, especially by fire ants (*Solenopsis invicta*), has been shown to reduce bait palatability substantially and bait availability for raccoons and raccoon uptake in studies from Ohio to Puerto Rico (Olson and Werner 1999, Linhart et al. 2002, Berentsen et al. 2020, Dixon et al. 2023, Helton et al. 2023). Invertebrates were observed on or next to approximately 8% of collected baits in each region of our study but were not identified as major competitors against raccoons. While invertebrates were recorded evenly between upland and lowland habitats, ONRAB baits accounted for 80% of the baits with invertebrates present, compared to 20% for V-RG baits. The reduced observation of invertebrate presence in the southern region may have been related to the timing of sampling. In Texas, ORV operations are conducted during the winter to minimize competition from invertebrates, primarily fire ants (Fearneyhough et al. 1998). As we described previously, the rate of bait uptake decreased throughout the sampling period, supporting the idea that attractiveness of baits decreased throughout the study. Additional studies should evaluate the effects of invertebrate activity and environmental damage on the encounter and selective uptake of baits.

There are limitations to consider when interpreting the findings of this study. First, the study areas sampled may not fully capture the diversity of ecosystems across Alabama and the broader southeastern region, restricting the spatial and ecological generalizability of the results. Alabama encompasses a wide range of ecological regions, each with distinct topography, resource availability, and wildlife community assemblages (Griffiths et al. 2001, Duncan 2013). The properties sampled in this study represent only a subset of these ecosystems, and the patterns

observed may not fully reflect bait uptake dynamics across all regions. Second, the reliance on motion-triggered camera traps without supporting video or timelapse data limited our ability to determine the fate of every distributed bait. In particular, invertebrate activity and bait fouling were difficult to evaluate, as small ectothermic organisms do not generate sufficient heat to activate camera sensors (O'Connell et al. 2011, Ortmann and Johnson 2021, Gao et al. 2024). Finally, habitat was classified into two broad categories (upland, lowland) due to sample size constraints, which may have masked potential variation in bait uptake by raccoons and non-target species. A more detailed habitat classification could have accounted for additional habitat-specific factors, such as varying levels of human development and resource availability, that influence bait encounter rates and competition among species (Helton et al. 2023, Hill et al. 2023*c*, 2025). Addressing these limitations in future research could enhance our understanding of the ecological and environmental mechanisms shaping bait uptake and fate across Alabama's heterogeneous landscapes.

Management Implications

Our study provides critical insights into optimizing ORV programs in Alabama and similar southeastern U.S. landscapes. The relatively low rates of bait uptake by raccoons, particularly in the central and southern regions, suggest that current baiting strategies may not achieve the vaccination coverage necessary to eliminate RRV in wildlife populations. Substantial bait competition by opossums underscores the need for modifications to bait attractant or the adoption of alternative distribution strategies, such as bait stations, to deter non-target consumption and prioritize raccoon uptake (Stanton et al. 2024). Findings from this study also indicate that targeted bait distribution in lowland habitats, paired with adjustments to bait density and timing during periods of peak raccoon activity, such as juvenile dispersal in the fall or spring

breeding season, may result in greater bait uptake by raccoons. Given that both raccoons and opossums were more likely to consume baits in lowland habitats, targeting bait distribution based solely on habitat type may not yield substantial improvement. Rapid decline of bait uptake by raccoons indicates that adaptation of bait attractants and coatings to enhance their resilience to environmental degradation may extend attractiveness following deployment. Site-specific evaluation of raccoon and non-target population densities, movement patterns, and resource selection may be necessary to refine strategies and maximize the field effectiveness of ORV efforts in Alabama and elsewhere.

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Table 1.1 Data sources used to determine the fate of ORV baits Alabama, USA, from 2022-2024.

	Fate of bait				
	Unknown	Not taken	Taken, sp known	Taken, sp unknown	Fouled, unknown
Bait collected; images of animal(s) tagged	—	361	439	12	12
Bait collected; images captured-no animals	—	184	—	15	12
Bait collected; no pictures-technical error	—	12	—	25	4
Bait not collected; images of animal(s) tagged	4	62	515	—	—
Bait not collected; images captured-no animals	8	19	—	—	—
Baits not collected; no pictures-technical error	44	—	—	—	—

— Not applicable

Table 2.2 Likelihood of oral rabies vaccination bait uptake by raccoons (*Procyon lotor*) in relation to region (north, central, south), bait type (ONRAB, V-RG), habitat type (upland, lowland), and other factors (session, year) in Alabama, USA, 2022-2024.

Region	Parameter	OR[1]	Lower CI[2]	Upper CI	*p*-value
All	Region [north]	3.292	1.568	6.910	0.002
	Region [south]	0.887	0.410	1.919	0.760
	Region [south]*	0.269	0.122	0.596	0.001
	Bait [V-RG]	3.422	2.432	4.815	<0.001
	Habitat [lowland]	2.843	1.528	5.288	0.001
	Session [b]	0.849	0.616	1.170	0.317
	Year [2]	0.93	0.67	1.29	0.67
North	Bait [V-RG]	3.097	1.723	5.566	<0.001
	Habitat [lowland]	1.610	0.903	2.872	0.107
	Session [b]	1.102	0.645	1.883	0.723
	Year [2]	0.716	0.418	1.226	0.224
Central	Bait [V-RG]	2.867	1.799	4.568	<0.001
	Habitat [lowland]	1.114	0.701	1.771	0.648
	Session [b]	0.992	0.625	1.575	0.974
	Year [2]	1.038	0.593	1.819	0.895
South	Bait [V-RG]	6.266	2.518	15.592	<0.001
	Habitat [lowland]	24.980	3.194	195.341	0.002
	Session [b]	0.372	0.166	0.833	0.016
	Year [2]	1.237	0.546	2.805	0.610

[1] Odds ratio
[2] 95% confidence interval
* Releveled from central to north reference
─ Predictor excluded from model

Table 2.3. Pairwise comparison of oral rabies vaccination bait uptake by raccoons (*Procyon lotor*) for region (north, central, south), bait type (ONRAB, V-RG), and habitat type (lowland, lowland) conducted in Alabama, USA, 2022-2024.

Region	Contrast	OR[1]	SE[2]	z-ratio	*p*-value
All	Central / north	0.304	0.115	-3.149	0.005
	Central / south	1.128	0.444	0.305	0.950
	North / south	3.712	1.504	3.237	0.004
North	ONRAB / V-RG	0.323	0.097	-3.779	<0.001
	Upland / lowland	0.621	0.183	-1.613	0.107
	Upland ONRAB / lowland ONRAB	0.621	0.183	-1.613	0.371
	Upland ONRAB / upland V-RG	0.323	0.097	-3.779	0.001
	Upland ONRAB / lowland V-RG	0.201	0.088	-3.649	0.002
	Lowland ONRAB / upland V-RG	0.520	0.208	-1.637	0.358
	Lowland ONRAB / lowland V-RG	0.323	0.097	-3.779	0.001
	Upland V-RG / lowland V-RG	0.621	0.183	-1.613	0.371
Central	ONRAB / V-RG	0.349	0.083	-4.431	<0.001
	Upland / lowland	0.898	0.212	-0.457	0.648
	Upland ONRAB / lowland ONRAB	0.898	0.212	-0.456	0.968
	Upland ONRAB / upland V-RG	0.349	0.083	-4.431	<0.001
	Upland ONRAB / lowland V-RG	0.313	0.104	-3.486	0.003
	Lowland ONRAB / upland V-RG	0.389	0.131	-2.801	0.026
	Lowland ONRAB / lowland V-RG	0.349	0.083	-4.431	0.000
	Upland V-RG / lowland V-RG	0.898	0.212	-0.456	0.968
South	ONRAB / V-RG	0.160	0.074	-3.945	<0.001
	Upland / lowland	0.040	0.042	-3.067	0.002
	Upland ONRAB / lowland ONRAB	0.040	0.042	-3.067	0.012
	Upland ONRAB / upland V-RG	0.160	0.074	-3.945	0.001
	Upland ONRAB / lowland V-RG	0.006	0.008	-4.049	<0.001
	Lowland ONRAB / upland V-RG	3.987	4.138	1.332	0.542
	Lowland ONRAB / lowland V-RG	0.160	0.074	-3.945	0.001
	Upland V-RG / lowland V-RG	0.040	0.042	-3.067	0.012

[1] Odds ratio
[2] Standard error

Table 2.4 Rate of oral rabies vaccination bait uptake by raccoons (*Procyon lotor*) in relation to region (north, central, south), bait type (ONRAB, V-RG), habitat type (upland, lowland), and other factors (session, year) in Alabama, USA, 2022-2024.

Region	Predictor	HR[1]	Lower CI[2]	Upper CI	*p*-value
All	Region [north]	2.252	1.725	2.940	<0.001
	Region [south]	1.189	0.882	1.603	0.257
	Region [south]*	0.528	0.395	0.705	<0.001
	Bait [V-RG]	2.256	1.801	2.825	<0.001
	Habitat [lowland]	1.854	1.472	2.335	<0.001
	Session [b]	0.837	0.670	1.046	0.118
	Year [2]	0.956	0.759	1.203	0.700
North	Bait [V-RG]	1.999	1.405	2.844	<0.001
	Habitat [lowland]	1.267	0.889	1.803	0.190
	Session [b]	—	—	—	—
	Year [2]	—	—	—	—
Central	Bait [V-RG]	2.46	1.665	3.635	<0.001
	Habitat [lowland]	1.102	0.752	1.614	0.620
	Session [b]	—	—	—	—
	Year [2]	—	—	—	—
South	Bait [V-RG]	2.638	1.684	4.133	<0.001
	Habitat [lowland]	8.875	4.687	16.805	<0.001
	Session [b]	0.546	0.346	0.860	0.009
	Year [2]	—	—	—	—

[1] Hazard ratio

[2] 95% confidence interval

* Releveled from central to north reference

— Predictor excluded from model

Table 2.5. Likelihood of oral rabies vaccination bait uptake by Virginia opossums (*Didelphis virginiana*) in relation to region (north, central, south), bait type (ONRAB, V-RG), habitat type (upland, lowland), and other factors (session, year) in Alabama, USA, 2022-2024.

Region	Predictor	OR[1]	Lower CI[2]	Upper CI	*p*-value
All	Region [north]	14.257	4.598	44.201	<0.001
	Region [south]	4.009	1.367	11.756	0.011
	Region [south]*	0.334	0.131	0.852	0.022
	Bait [V-RG]	7.488	4.729	11.857	<0.001
	Habitat [lowland]	2.002	0.852	4.705	0.111
	Session [b]	1.293	0.915	1.827	0.146
	Year [2]	—	—	—	—
North	Bait [V-RG]	8.809	3.929	19.752	<0.001
	Habitat [lowland]	1.901	0.813	4.441	0.138
	Session [b]	1.302	0.718	2.359	0.385
	Year [2]	—	—	—	—
Central	Bait [V-RG]	4.539	2.414	8.537	<0.001
	Habitat [lowland]	0.503	0.149	1.695	0.268
	Session [b]	2.553	1.400	4.657	0.002
	Year [2]	2.258	1.249	4.081	0.007
South	Bait [V-RG]	7.711	3.960	15.013	<0.001
	Habitat [lowland]	7.547	1.459	39.043	0.016
	Session [b]	0.827	0.458	1.495	0.530
	Year [2]	0.234	0.126	0.437	<0.001

[1] Odds ratio
[2] 95% confidence interval
* Releveled from central to north reference
— Predictor excluded from model

Table 2.6 Pairwise comparison of oral rabies vaccination bait uptake by Virginia opossums (*Didelphis virginiana*) for region (north, central, south), bait type (ONRAB, V-RG), and habitat type (upland, lowland) in Alabama, USA, 2022-2024.

Region	Contrast	OR[1]	SE[2]	z-ratio	*p*-value
All	Central / north	0.070	0.041	-4.603	<0.001
	Central / south	0.249	0.137	-2.530	0.031
	North / south	3.556	1.927	2.342	0.050
North	ONRAB / V-RG	0.114	0.047	-5.281	<0.001
	Upland / lowland	0.526	0.228	-1.483	0.138
	Upland ONRAB / lowland ONRAB	0.526	0.228	-1.483	0.448
	Upland ONRAB / upland V-RG	0.114	0.047	-5.281	<0.001
	Upland ONRAB / lowland V-RG	0.060	0.039	-4.362	0.000
	Lowland ONRAB / upland V-RG	0.216	0.118	-2.813	0.025
	Lowland ONRAB / lowland V-RG	0.114	0.047	-5.281	<0.001
	Upland V-RG / lowland V-RG	0.526	0.228	-1.483	0.448
Central	ONRAB / V-RG	0.220	0.071	-4.694	<0.001
	Upland / lowland	1.990	1.230	1.109	0.268
	Upland ONRAB / lowland ONRAB	1.989	1.233	1.109	0.684
	Upland ONRAB / upland V-RG	0.220	0.071	-4.694	<0.001
	Upland ONRAB / lowland V-RG	0.438	0.300	-1.204	0.624
	Lowland ONRAB / upland V-RG	0.111	0.079	-3.091	0.011
	Lowland ONRAB / lowland V-RG	0.220	0.071	-4.694	<0.001
	Upland V-RG / lowland V-RG	1.989	1.233	1.109	0.684
South	ONRAB / V-RG	0.130	0.044	-6.008	<0.001
	Upland / lowland	0.133	0.111	-2.410	0.016
	Upland ONRAB / lowland ONRAB	0.133	0.111	-2.410	0.075
	Upland ONRAB / upland V-RG	0.130	0.044	-6.008	<0.001
	Upland ONRAB / lowland V-RG	0.017	0.016	-4.263	<0.001
	Lowland ONRAB / upland V-RG	0.979	0.836	-0.025	1.000
	Lowland ONRAB / lowland V-RG	0.130	0.044	-6.008	<0.001
	Upland V-RG / lowland V-RG	0.133	0.111	-2.410	0.075

[1] Odds ratio
[2] 95% confidence interval

Table 2.7. Rate of oral rabies vaccination bait uptake by Virginia opossums (*Didelphis virginiana*) in relation to region (north, central, south), bait type (ONRAB, V-RG), habitat type (upland, lowland), and other factors (session, year) in Alabama, USA, 2022-2024.

Region	Predictor	HR[1]	Lower CI[2]	Upper CI	*p*-value
All	Region [north]	3.509	2.671	4.610	<0.001
	Region [south]	2.062	1.548	2.747	<0.001
	Region [south]*	0.588	0.460	0.750	<0.001
	Bait [V-RG]	2.727	2.271	3.275	<0.001
	Habitat [lowland]	1.475	1.235	1.761	<0.001
	Session [b]	1.134	0.950	1.354	0.164
	Year [2]	0.808	0.653	1.000	0.050
North	Bait [V-RG]	2.557	1.963	3.331	<0.001
	Habitat [lowland]	1.425	1.103	1.840	0.007
	Session [b]	—	—	—	—
	Year [2]	0.811	0.600	1.096	0.173
Central	Bait [V-RG]	2.818	1.869	4.248	<0.001
	Habitat [lowland]	0.591	0.395	0.884	0.011
	Session [b]	1.890	1.252	2.853	0.002
	Year [2]	1.674	1.006	2.786	0.048
South	Bait [V-RG]	3.151	2.278	4.358	<0.001
	Habitat [lowland]	2.766	2.006	3.814	<0.001
	Session [b]	—	—	—	—
	Year [2]	0.503	0.366	0.692	<0.001

[1] Hazard ratio

[2] 95% confidence interval

* Releveled from central to north reference

— Predictor excluded from model

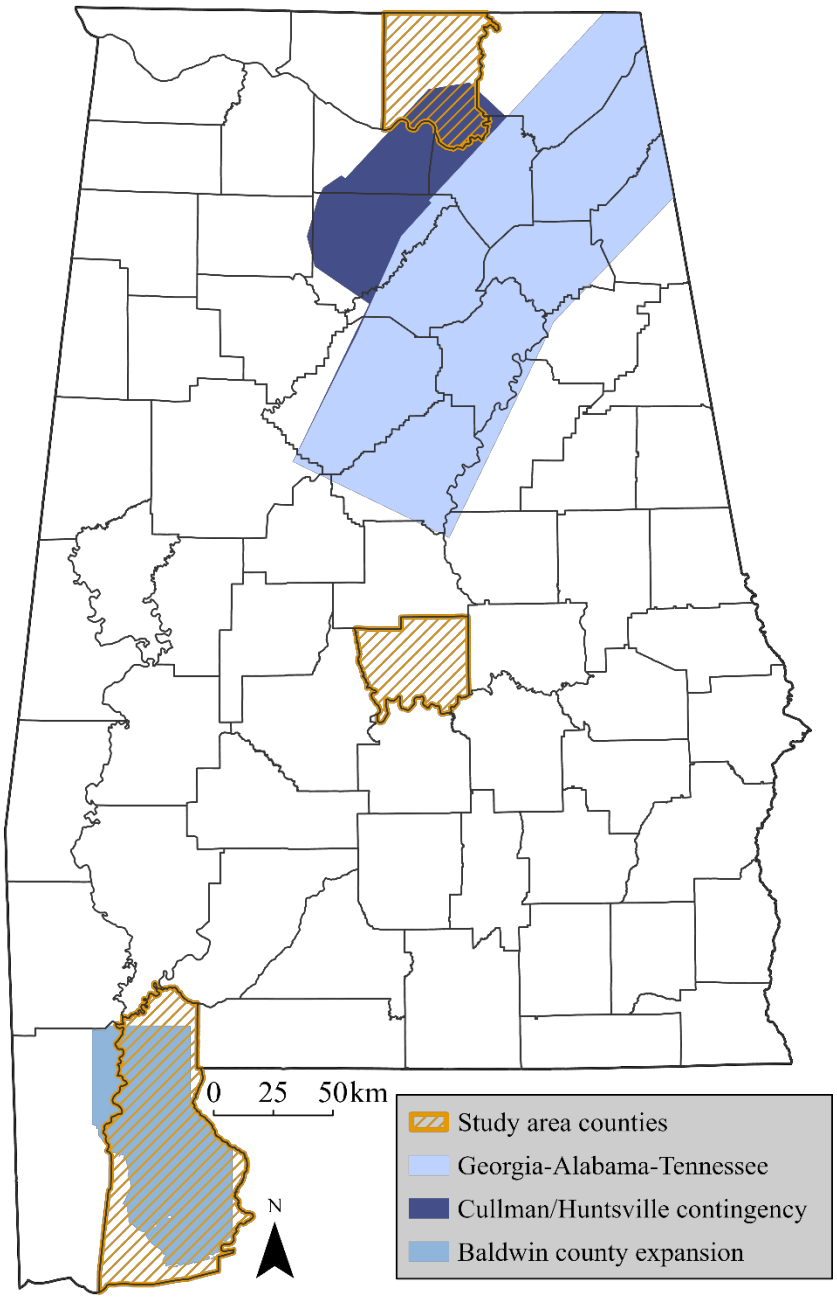

Figure 2.1 Counties in Alabama, USA, where camera traps were deployed (stripes) from 2022-2024 to evaluate the fate of oral rabies vaccine (ORV) baits, and the ORV distribution zones (shaded) implemented by the National Rabies Management Program.

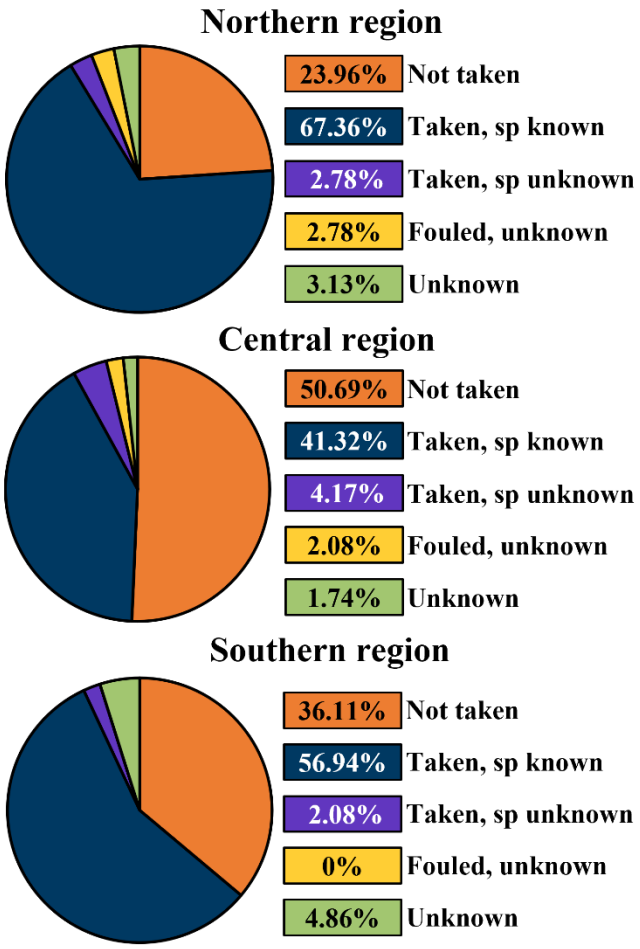

Figure 2.2 Cumulative percentage of bait fate in the northern, central, and southern regions of Alabama, USA, sampled from 2022 to 2024. Baits were classified as 'Not taken' if they remained intact at the end of the seven-day sampling period. Baits were classified as 'Taken, sp. known' if they were consumed by an identifiable animal in images. Baits were classified as 'Taken, sp. unknown' if collected baits showed signs of vertebrate damage (e.g., tooth indentations, destruction, crumbling) but no images were available to identify the animal. Baits were classified as 'Fouled, unknown' if collected baits were empty or leaked without signs of damage (e.g., perforations) and no images of animal contact were available. Baits were classified as 'Unknown' if neither images nor collected bait provided information on the fate.

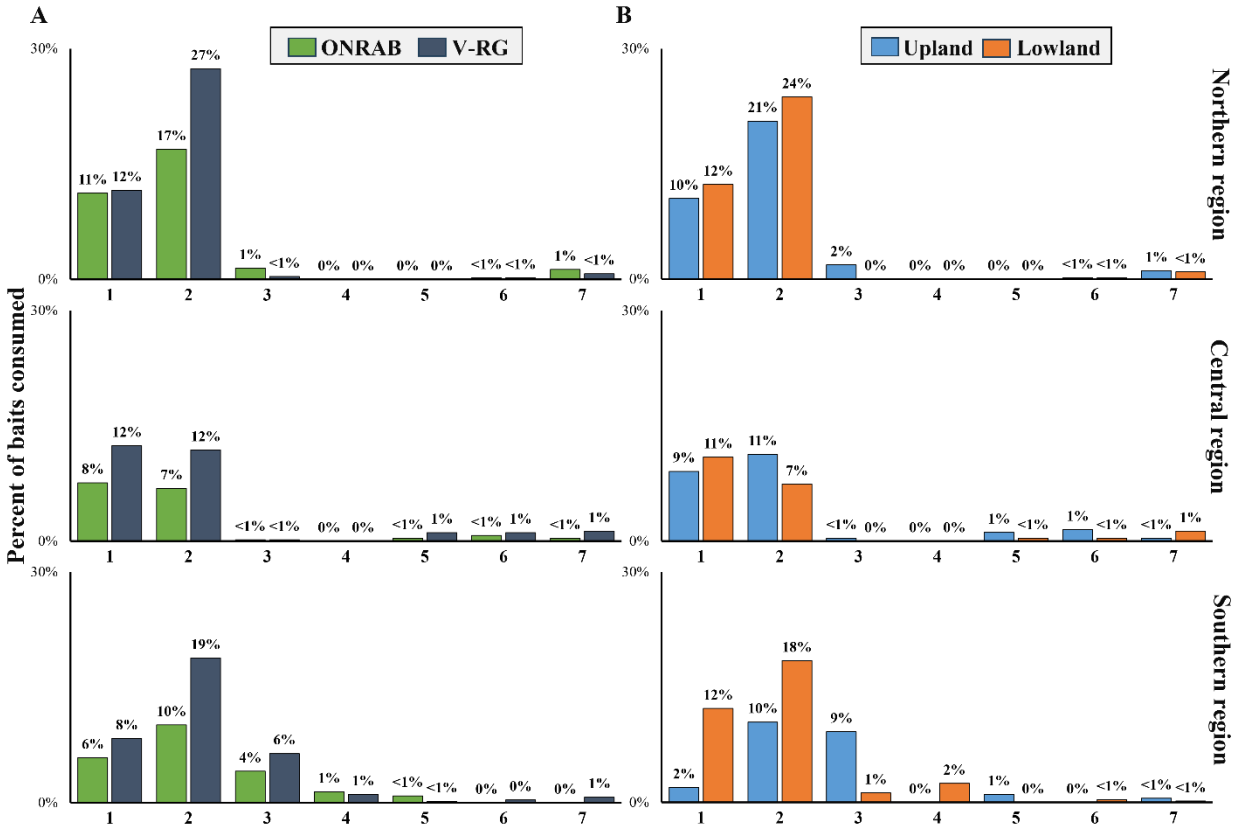

Figure 2.3 Influence of A. bait type (ONRAB, V-RG) and B. habitat type (lowland, upland) on percentage of ORV baits consumed by raccoons (*Procyon lotor*) and competitors of baits distributed with known fate in northern (n = 544), central (n = 540), and southern (n = 564) Alabama, USA, from 2022-2024. Species described as 'Other' consumed less than 1% of distributed baits and included the coyote (*Canis latrans*), domestic cat (*Felis catus*), eastern cottontail (*Sylvilagus floridanus*), eastern gray squirrel (*Sciurus carolinensis*), nine-banded armadillo (*Dasypus novemcinctus*), and striped skunk (*Mephitis mephitis*).

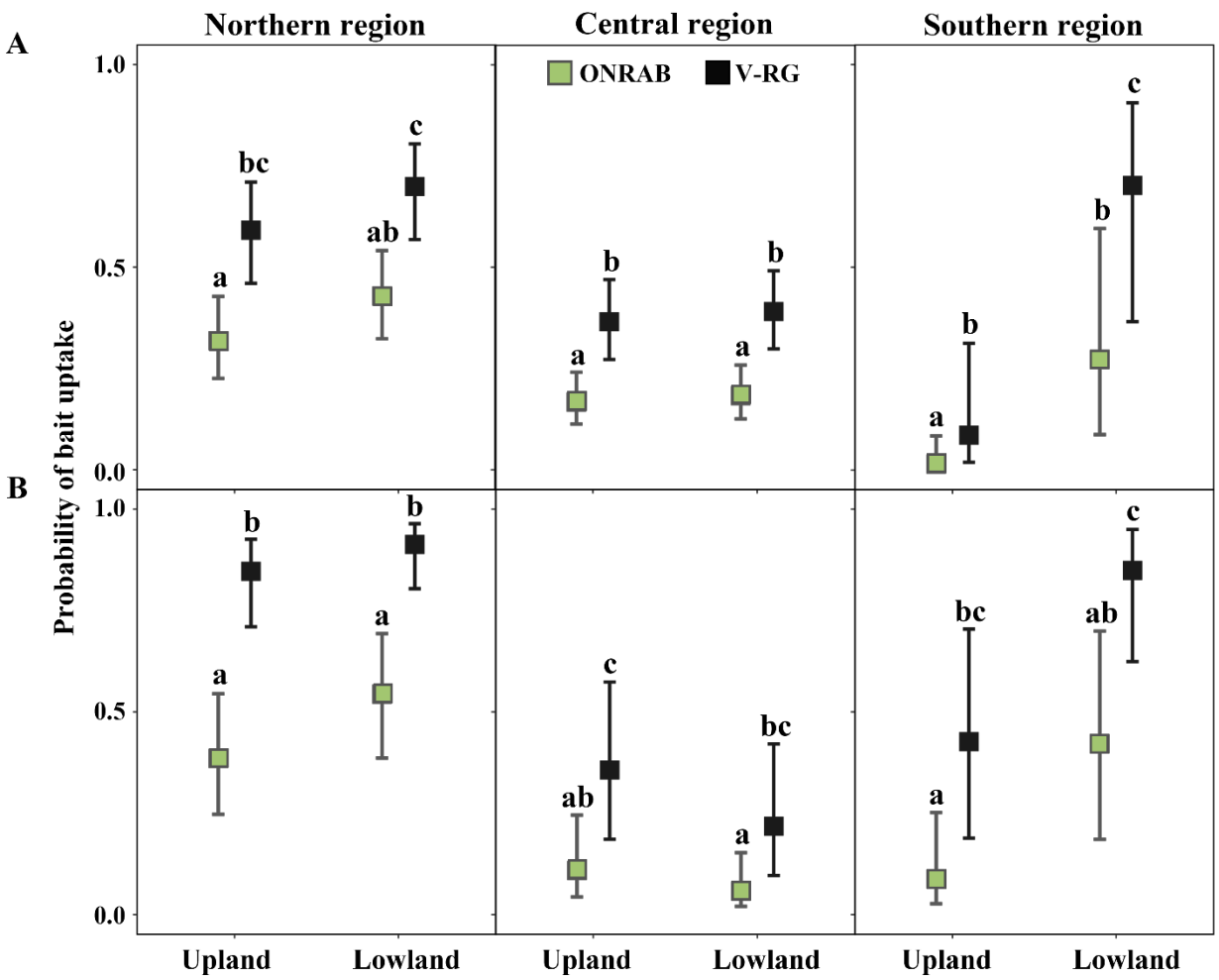

Figure 2.4 Estimated marginal mean and 95% confidence interval depicting the probability that baits in northern, central, and southern Alabama, USA were A. taken by raccoons (*Procyon lotor*) or B. taken by Virginia opossums (*Didelphis virginiana*) for each bait and habitat type from 2022-2024. Within each panel, means sharing a common letter are not significantly different at α = 0.05.

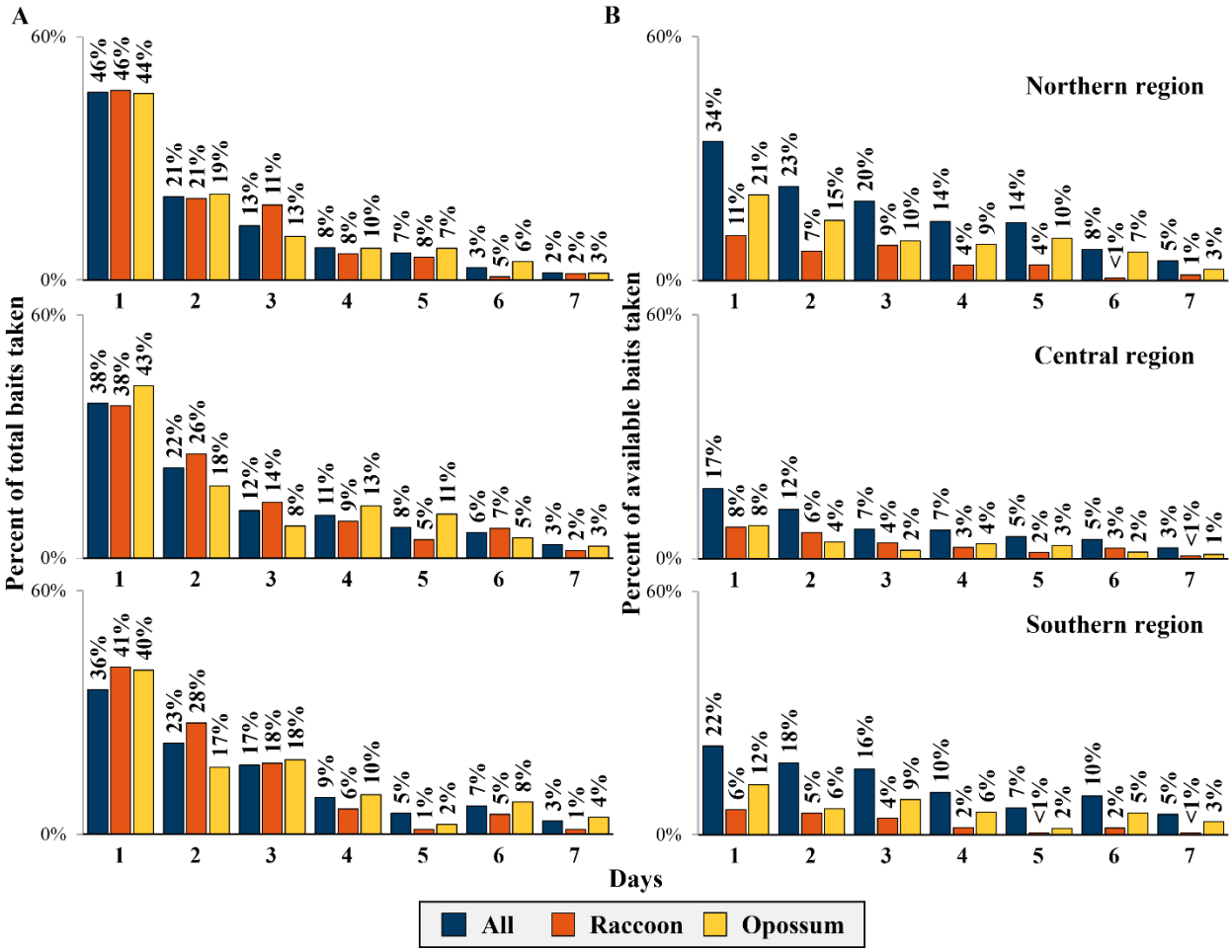

Figure 2.5 Daily percentage of baits consumed by all species, raccoons (*Procyon lotor*), and Virginia opossums (*Didelphis virginiana*) in northern, central, and southern Alabama, USA, from 2022-2024 out of A. the cumulative number of baits taken per group, and B. the baits remaining available (i.e., not taken).

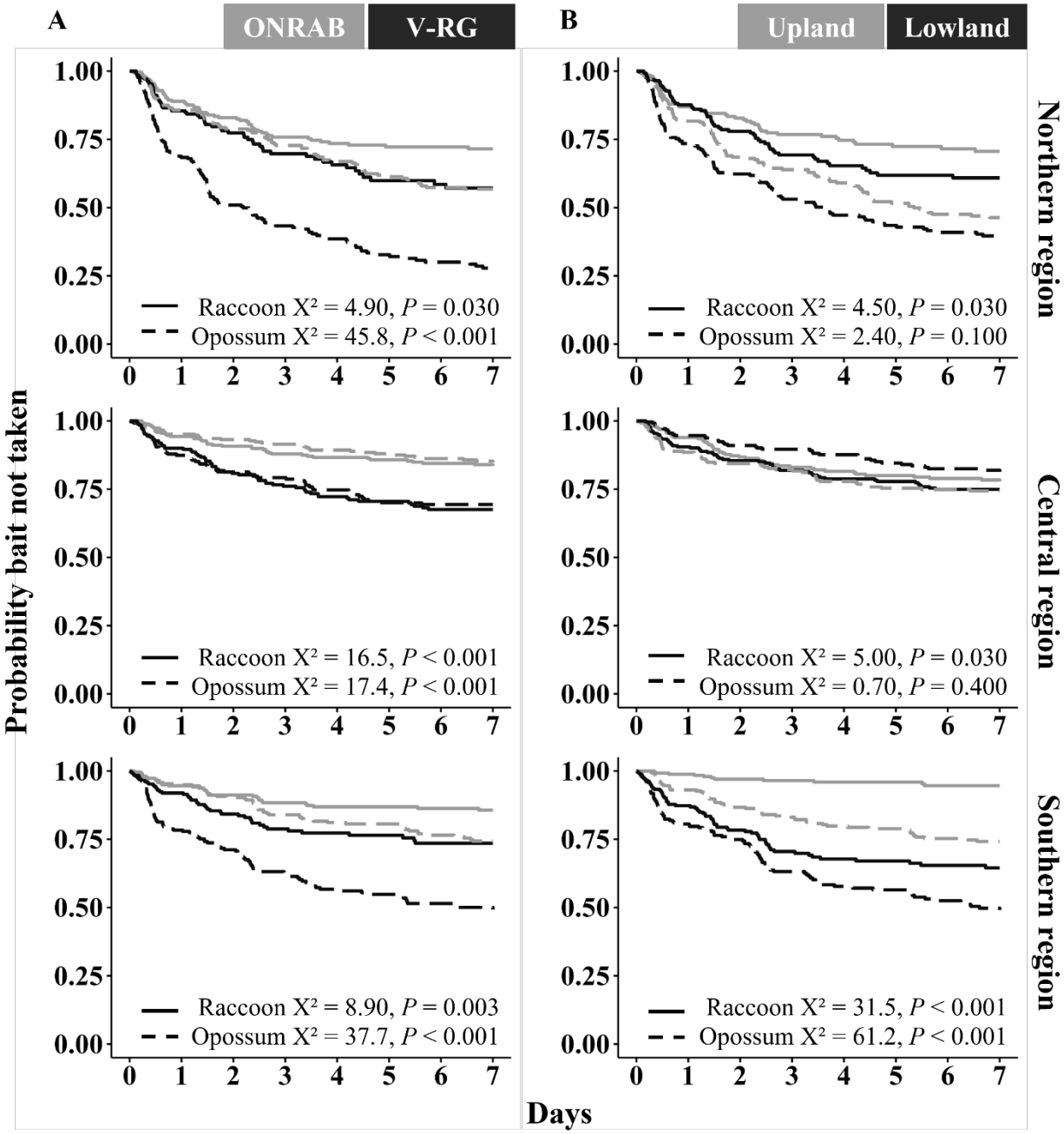

Figure 2.6 Bait survival (i.e., not taken) function estimated by the Kaplan-Meier method for each A. bait type and B. habitat type by raccoons (*Procyon lotor*) (solid line) and Virginia opossums (*Didelphis virginiana*) (dotted line) within the northern, central, and southern regions in Alabama, USA from 2022-2024. The log-rank test values in each panel indicate a significant difference between the curves for each species.

Appendix 2.1. Selected models for generalized linear models and Cox proportional hazards models analyzing the effects of region (north, central, south), bait type (ONRAB, V-RG), habitat (upland, lowland), session (a, b), and year (1, 2) on oral rabies vaccination bait uptake by Virginia opossums (*Didelphis virginiana*) and raccoons (*Procyon lotor*) in Alabama, USA, 2022-2024.

Generalized linear mixed effects | generalized linear models				
Raccoon	All	M2	Region + bait + habitat + session + year	(1 | Site/camera)
	North	M2	Bait + habitat + session + year	(1 | Site/camera)
	Central	M2	Bait + habitat + session + year	(1 | Camera)
	South	M2	Bait + habitat + session + year	(1 | Site/camera)
Opossum	All	M2	Region + bait + habitat + session	(1 | Site/camera/year)
	North	M2	Bait + habitat + session	(1 | Site/camera/year)
	Central	M2	Bait + habitat + session + year	(1 | Site/camera)
	South	M2	Bait + habitat + session + year	(1 | Site/camera)
Cox proportional hazards models				
Raccoon	All	M2	Region + bait + habitat + session + year	(1 | Camera)
	North	M2	Bait + habitat	(1 | Camera)
	Central	M2	Bait + habitat	(1 | Camera)
	South	M2	Bait + habitat + session	(1 | Camera)
Opossum	All	M2	Region + bait + habitat + session + year	(1 | Camera)
	North	M2	Bait + habitat + year	(1 | Camera)
	Central	M2	Bait + habitat + session + year	(1 | Camera)
	South	M2	Bait + habitat + year	(1 | Camera)

Appendix 2.2. Model performance metrics for generalized linear models evaluating oral rabies vaccination bait uptake by Virginia opossums (*Didelphis virginiana*), raccoons (*Procyon lotor*), and all taken baits (including fouled) in relation to region (north, central, south) and bait type (ONRAB, V-RG) in Alabama, USA, 2022-2024.

Model		Baits	AIC[1]		AICc	BIC[2]	Conditional R^2	Marginal R^2
Raccoon	All	M1	1592	1499.138	1499.251	1547.492	0.271	0.084
	All	M2	950	1060.798	1060.990	1104.506	0.369	0.182
	North	M1	526	586.741	586.957	616.598	0.027	0.005
	North	M2	262	355.813	356.254	380.791	0.183	0.094
	Central	M1	530	529.989	531.184	602.627	0.148	0.145
	Central	M2	400	456.277	456.491	480.226	0.116	0.075
	South	M1	536	354.489	354.701	384.478	0.571	0.255
	South	M2	288	224.441	224.841	250.082	0.674	0.393
Opossum	All	M1	1592	1736.737	1736.851	1785.092	0.459	0.157
	All	M2	1143	1246.609	1246.768	1291.982	0.630	0.274
	North	M1	526	690.724	690.941	720.581	0.291	0.086
	North	M2	379	445.360	445.662	472.923	0.543	0.176
	Central	M1	520	468.504	468.719	498.414	0.584	0.110
	Central	M2	393	398.348	398.639	426.165	0.482	0.169
	South	M1	536	549.663	549.875	579.652	0.477	0.180
	South	M2	371	370.971	371.280	398.384	0.595	0.335

[1]Akaike information criterion
[2]Bayesian information criterion

Appendix 2.3. Model performance metrics for Cox proportional hazard models evaluating oral rabies vaccination bait uptake by Virginia opossums (*Didelphis virginiana*), raccoons (*Procyon lotor*), and all taken baits (including fouled) in relation to region (north, central, south) and bait type (ONRAB, V-RG) in Alabama, USA, 2022-2024

Model			Baits	Events	AIC[1]	AICc	BIC[2]	R^2	Concordance Index
Raccoon	All	M1	1592	312	4300.821	4300.880	4334.942	0.054	0.65
	All	M2	950	312	4057.926	4058.115	4101.369	0.123	0.65
	North	M1	526	124	1426.046	1426.069	1434.577	0.015	0.57
	North	M2	262	124	1298.188	1298.235	1305.325	0.061	0.62
	Central	M1	530	108	1281.167	1281.536	1320.926	0.070	0.67
	Central	M2	400	108	1241.418	1241.985	1281.375	0.101	0.68
	South	M1	536	80	874.591	874.670	892.199	0.176	0.79
	South	M2	288	80	795.531	795.616	806.520	0.288	0.80
Opossum	All	M1	1592	505	6828.114	6828.949	6962.834	0.164	0.71
	All	M2	1143	505	6588.156	6589.297	6712.920	0.237	0.72
	North	M1	526	241	2716.187	2716.356	2742.342	0.107	0.65
	North	M2	379	241	2602.622	2603.085	2637.130	0.179	0.66
	Central	M1	520	101	1177.708	1178.305	1228.705	0.120	0.73
	Central	M2	393	101	1137.321	1138.148	1185.178	0.161	0.73
	South	M1	536	163	1832.028	1832.073	1844.881	0.167	0.73
	South	M2	371	163	1744.628	1744.693	1756.377	0.249	0.74

— Not applicable
[1]Akaike information criterion
[2]Bayesian information criterion