Influence of oral rabies vaccine bait density on rabies seroprevalence in wild raccoons

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Abstract

The effect of different oral rabies vaccine (ORV) bait densities (75, 150, and 300 baits/km²) on the seroprevalence of rabies virus neutralizing antibodies (RVNAs) in raccoons (Procyon lotor) was assessed at a 15% seroprevalence difference threshold in rural areas of northeast Ohio. Results (n = 588 raccoons) indicated that seropositivity for RVNAs was associated with both bait density and bait campaign frequency. Associations were not detected for raccoon gender, age, or macro-habitat. The odds of being seropositive were greater for raccoons originating from 300 bait/km² treatment areas relative to those coming from the 75 bait/km² areas (odds ratio [OR] = 4.4, probability [P] < 0.001, 95% confidence interval [CI] = 2.4–7.9), while accounting for cumulative ORV campaigns. No statistical advantage in seroprevalence was detected when comparing 150–75 baits/km². These results indicate that a relatively extreme bait density when evenly distributed may be necessary to obtain a significant increase in seroprevalence. Higher bait densities may be more appropriate and less costly to address focused outbreaks than labor intensive trap-vaccinate-release and local population reduction campaigns. Finally, dramatic increases in seroprevalence of RVNA were not observed in raccoons between sequential, semi-annual campaigns, yet cumulative ORV campaigns were associated with gradual increases in seroprevalence.

1. Introduction

Since 1994, oral rabies vaccination (ORV) has emerged as an increasingly important strategy for controlling the raccoon variant of the rabies virus in the eastern United States of America and Canada [1–8]. The vaccinia-rabies glycoprotein recombinant virus vaccine (Raboral V-RC®, Merial Limited, Athens, GA) used in these programs was developed in 1983 [9] and was subsequently shown to be both safe and effective in raccoons (Procyon lotor) and in a variety of non-target species [10–14]. However, adequate seroconversion with V-RC has not been attained in striped skunks (Mephitis mephitis) [15]. The Raboral V-RC® vaccine is typically dispensed in a plastic sachet that is inserted into the hollow core of a fishmeal polymer bait, or with a fishmeal coating on the sachet.

Strategic use of ORV helped block the expansion of the raccoon rabies epizootic on peninsulas in Massachusetts [6] and New Jersey [7], and in northeastern Ohio, where ORV baits (baits) were distributed in defined geographic areas using pre-determined distribution strategies, and at target bait densities, to form ORV “barriers” of immune animals in conjunction with favorable landscape features (e.g., the Cape Cod Canal). However, raccoon rabies breached the New Jersey ORV barrier after approximately 2 years and the barriers in Massachusetts and Ohio were both breached during 2004. Contingency efforts are currently underway in Massachusetts [16], and Ohio [17] to contain raccoon rabies, eliminate it from the newly affected areas, and reestablish the ORV zones.

The raccoon rabies epizootic, which began along the Virginia–West Virginia border in 1977 and continued to spread
through the 1990s, was detected in northeast Ohio on March 5, 1997. In response to this threat, the Ohio ORV Program was initiated during the spring of 1997 [5]. The goals of the Ohio ORV Program were to stop further spread of the raccoon variant of rabies into Ohio, and the remainder of the Midwest regions of the USA and Canada, and to reduce the incidence of raccoon rabies in northeastern Ohio [18]. The Ohio ORV Program began incorporating efforts to evaluate the recommended protocols for vaccinating wild, free-ranging raccoons by the Spring of 1998 [19].

In this paper, we evaluated the relationship of Raboral V-RG® at bait densities of 75, 150 and 300 baits/km² to population rabies antibody seroprevalence in free-ranging raccoons (P. lotor) in rural areas of northeast Ohio following uniform bait distribution using fixed-wing aircraft. This investigation was conducted concurrent with the fifth Ohio Raccoon ORV Campaign during Spring, 1999.

2. Materials and methods

2.1. Bait

Raboral V-RC® (Merial Limited, Athens, GA) sachets inserted into fishmeal polymer bait blocks (FMP—5.1 cm × 3.2 cm × 1.9 cm) were used exclusively in this study. Tetracycline (150 mg/bait) was incorporated into the FMP as a biomarker to facilitate bait uptake assessments.

2.2. Experimental ORV zone

Three experimental ORV treatments were conducted as part of the 5th Spring Ohio ORV Campaign. The ORV zone included major portions of Ashtabula, Trumbull, Mahoning, and Columbiana counties, as well as smaller areas of the two adjacent counties to the south (Jefferson and Carroll). Ashtabula, Trumbull and Mahoning are relatively flat and located in the Allegheny Glacial Plateau. Columbiana, Jefferson and Carroll counties transition to rolling hills and are in the southern unglaciated Allegheny Plateau [20]. The 1999 Ohio ORV zone was comprised of >85% rural and <10% urban landscape types. The three largest cities within the ORV zone were: Youngstown (pop. 82,026); Warren (pop. 46,832); and Ashtabula (pop. 21,472) [21]. The ORV zone was between two presumed natural barriers expected to prevent or inhibit rabies spread, Lake Erie and the Ohio River (Fig. 1). The eastern edge of the barrier was the Ohio–Pennsylvania border located along the eastern edges of Ashtabula, Trumbull, Mahoning, and Columbiana Counties and the Ohio–West Virginia border marked by the Ohio River along a portion of the eastern edges of Columbiana and Jefferson Counties. The width of the barrier varied from 16.1 km in the north to approximately 40.2 km in the south. The total area of the 1999 ORV barrier was 4458 km².

2.3. Bait distribution

Bait distribution occurred during April 25–May 1, 1999. Specific habitats were targeted for bait distribution in urban areas by vehicle, helicopter or on foot. Relatively uniform bait distribution occurred using fixed-wing aircraft (Ontario Ministry of Natural Resources [OMNR] de Havilland Twin Otter aircraft crewed by OMNR, and Ohio state government and United States Department of Agriculture [USDA], Animal and Plant Health Inspection Service [APHIS], Wildlife Services [WS] personnel) over rural regions of the ORV zone. Baits were distributed along predetermined global positioning system (GPS)-determined flight lines spaced at 500 m intervals. Aerial ORV activity ceased over areas of potentially greater risk of baits striking people, property, and large bodies of water.

2.4. Establishing three bait density study areas

ORV treatment areas baited at target bait densities of 75 baits/km² (2317 km²), 150 baits/km² (499 km²) and 300 baits/km² (1642 km²) were established in rural areas of the Ohio ORV zone (Fig. 2a). Urban areas, water-bodies and other factors had an effect on the, actual bait densities achieved for the three treatment areas.

2.5. Post-ORV sampling

2.5.1. Sample size determination

The desired sample size of 525 (175/treatment area) raccoons was based on power analysis for the detection of 15% differences between treatments (α = 0.05, β = 0.20, assumed prevalence = 32% based on Ohio Spring 1998 seroconversion rates) [19].

2.5.2. Trap site selection

Trap sites within each treatment area were located in rural habitats, at least 4.8 km from: (1) other ORV treatments; (2) areas...
Table 1
Unique raccoon capture results. Capture data sorted by bait density area, age, number of raccoons captured per week and trap success.

<table>
<thead>
<tr>
<th>Week following bait distribution</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
<th>7th</th>
<th>8th</th>
<th>9th</th>
<th>10th</th>
<th>11th</th>
<th>12th</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raccoons captured (all age classes)</td>
<td>19</td>
<td>11</td>
<td>31</td>
<td>25</td>
<td>43</td>
<td>17</td>
<td>31</td>
<td>41</td>
<td>218</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raccoons captured (&gt;1 year old)</td>
<td>19</td>
<td>11</td>
<td>28</td>
<td>22</td>
<td>23</td>
<td>16</td>
<td>22</td>
<td>34</td>
<td>175</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trap nights</td>
<td>135</td>
<td>90</td>
<td>128</td>
<td>96</td>
<td>124</td>
<td>120</td>
<td>264</td>
<td>237</td>
<td>1194</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trap success</td>
<td>14%</td>
<td>12%</td>
<td>24%</td>
<td>26%</td>
<td>35%</td>
<td>14%</td>
<td>12%</td>
<td>17%</td>
<td>18%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 vaccines/km²</td>
<td>Raccoons captured (all age classes)</td>
<td>39</td>
<td>21</td>
<td>24</td>
<td>8</td>
<td>24</td>
<td>17</td>
<td>36</td>
<td>210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raccoons captured (&gt;1 year old)</td>
<td>39</td>
<td>21</td>
<td>24</td>
<td>8</td>
<td>22</td>
<td>13</td>
<td>29</td>
<td>21</td>
<td>177</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trap nights</td>
<td>158</td>
<td>120</td>
<td>83</td>
<td>114</td>
<td>120</td>
<td>195</td>
<td>140</td>
<td>1050</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trap success</td>
<td>25%</td>
<td>18%</td>
<td>20%</td>
<td>10%</td>
<td>21%</td>
<td>14%</td>
<td>21%</td>
<td>26%</td>
<td>20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 vaccines/km²</td>
<td>Raccoons captured (all age classes)</td>
<td>33</td>
<td>28</td>
<td>31</td>
<td>25</td>
<td>33</td>
<td>26</td>
<td>13</td>
<td>189</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raccoons captured (&gt;1 year old)</td>
<td>33</td>
<td>28</td>
<td>29</td>
<td>20</td>
<td>33</td>
<td>25</td>
<td>10</td>
<td>178</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trap nights</td>
<td>120</td>
<td>87</td>
<td>120</td>
<td>90</td>
<td>112</td>
<td>76</td>
<td>57</td>
<td>662</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trap success</td>
<td>28%</td>
<td>32%</td>
<td>26%</td>
<td>28%</td>
<td>20%</td>
<td>34%</td>
<td>23%</td>
<td>29%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 3rd week was defined as 21–25 days following middle of bait distribution period.  
  b One trap night equals 1 trap placed for 1 night of trapping effort.  
  c Trap success equals number of raccoons captured per week divided by total number of trapping nights of effort.

where habitat targeting was used; (3) untreated areas with raccoon rabies (along Pennsylvania–Ohio border); and (4) raccoon rabies-free areas west of the Ohio ORV zone to reduce sampling bias (Fig. 2). Trap site habitats were classified, based on gross visual examination, as either “agricultural” (includes pastures, buildings, and cultivated areas) or non-agricultural “forested” (deciduous or coniferous).

The cumulative number of previous ORV campaigns at each trapping site ranged from 3 to 5 and was determined by the number of previous ORV campaigns at each trap site using overlay maps created with ArcView® GIS Version 3.2 (1999 Environmental Systems Research Institute, Inc.) software (Fig. 1).

2.5.3. Trapping schedules and protocols
Tomahawk® live traps (Tomahawk Live Trap Company, Tomahawk, WI, 54487) baited with marshmallows coated with a mixture of honey, anise oil, and vanilla extract were set 3–12 weeks post-baiting. During weeks 3–5 and 9–12, limited resources constrained trapping to two of the three ORV treatment areas each week, and trapping efforts were rotated among the three treatment areas on a weekly schedule (Table 1). Trapping occurred concurrently in all three ORV treatment areas during weeks 6–8. To meet sampling goals, the cumulative number of serum samples from raccoons within each treatment area was monitored weekly so that sampling could be adjusted accordingly.

2.5.4. Handling, sampling and data collection of individual raccoons
Captured (3–12 weeks post-baiting) raccoons were anesthetized with 7.2–14.4 mg/kg of a 5:1 mixture of ketamine hydrochloride (100 mg/ml–Ketaset®, Fort Dodge, Fort Dodge, IA, 50501) and xylazine hydrochloride (100 mg/ml–TranquiVed®, VEDCO, Inc., St. Joseph, MO, 64504) and observed for signs of clinical disease (cachexia, abnormal body discharges, and excessive alopecia) and evidence of trauma secondary to aggressive behavior (bite wounds, lacerations, or abscesses). Each raccoon was ear-tagged for identification, and sex, age category, weight, capture location (GPS coordinates and mailing addresses), macro-habitat, and ORV history were recorded for each capture location. Blood samples (3–7 ml, depending on age, were collected; sera were frozen at 4°C initially and at –70°C long-term) and first upper premolar teeth were collected for ageing and bait uptake analysis through tetracycline marking. An intra-muscular injection of commercial, inactivated rabies vaccine (IMRAB3®, Merial Limited, 115 Trans Tech Drive, Athens, GA, 30601–1649) was administered. Upon recovery from anesthesia, raccoons were released. Recaptured raccoons were not blood-sampled again. Data collected from raccoons were entered into a Microsoft Access® database (Microsoft Inc.).

2.5.5. Determining relative raccoon abundance
Trap night success was used as a crude measure of relative raccoon abundance [22]. Trap success was calculated for each treatment area (unique raccoons/trap nights × 100). Recaptured raccoons were excluded, and trap night adjustments made for sprung traps and non-target captures.

2.5.6. Seroprevalence of rabies virus neutralizing antibody
Raccoon sera were analyzed for rabies virus neutralizing antibodies (RVNAs) using the rapid fluorescent focus inhibition test (RFFIT), as described [23]. Sera were screened for RVNA at dilutions of 1:5 and 1:25. At a magnification factor of 200, 20 fields were examined on each slide for the presence of specific fluorescence. Titers were recorded as <1:5 (1–10 positive fields of 20 fields using a 1:5 dilution of serum), ≥1:5 (1–10 positive fields of 20 fields using a 1:5 dilution of serum), ≥1:12 (0 positive fields of 20 fields using a 1:5 dilution of serum) and ≥1:56 (0 positive fields of 20 fields using a 1:25 dilution of serum).

2.5.7. Data analysis
Success of the ORV program was based on the percent of sampled raccoons classified as seropositive. A seropositive raccoon was defined as having a serum RVNA titer of ≥1:5, ≥1:12, or ≥1:56 in the RFFIT. Simple descriptive analysis was conducted by sorting seropositive and seronegative raccoons within each of five categorical variables (age, sex, capture location macro-habitat, bait density, and cumulative bait campaign frequency).

A multivariable logistic regression model was used to identify the relationship between bait density and seroprevalence, while adjusting for potential confounding factors [24]. The model was constructed using a forward stepwise procedure, based on the likelihood ratio chi-square test statistic. Entry of a variable into the model required an association between the independent variable and positive titer, α ≤ 0.05.
3. Results

3.1. Bait distribution

A total of 750,000 baits were distributed over 4458 km² as part of the Spring 1999 Ohio ORV campaign. Approximately 633,260 baits were distributed from fixed-wing aircraft over rural areas, and ground and helicopter crews distributed approximately 116,740 more baits in urban areas, villages, parks, edges of major waterbodies, and along major roadways. Actual bait densities for the 75, 150, and 300 baits/km² within study treatment areas were 89, 131, and 270 baits/km² (Fig. 1).

3.2. Trapping results

Post-ORV sampling resulted in a total of 695 raccoons captured (617 unique, 78 recaptured) over 2906 trap nights at 95 multi-trap sites (33, 36, and 26 sites each in the 75, 150, and 300 baits/km² areas) (Fig. 2b). Unique raccoons included 530 adult and yearling raccoons in approximately equal numbers from each of the bait density treatment areas, and 87 juveniles (<1 year old) based on cementum annuli analysis (Table 1). Non-target species captured included: opossum (Didelphis virginianus) (n = 51), woodchuck (Marmota monax) (n = 43), eastern cottontail rabbit (Sylvilagus floridanus) (n = 6), domestic cat (Felis catus) (n = 5), skunk (Mephitis mephitis) (n = 4), and an unspecified turtle (n = 1).

Trap success was 18%, 20%, and 29% for the 75, 150 and 300 baits/km² treatment areas, respectively (Table 1). Trap success in the 300 baits/km² treatment area was higher than in the other two treatment areas (χ² = 30.4, P < 0.001) suggesting a higher relative abundance of raccoons in that treatment area. Lower trapping successes (<15%) during weeks 3, 5 and 9 in the 75 baits/km² treatment area and during weeks 7 and 9 in the 150 baits/km² treatment area made increased trapping efforts in these areas necessary during weeks 10, 11 and 12 (Table 1).

3.3. Serologic results and descriptive statistics by categorical variables

RVNA seroprevalence (n = 597) (Table 2) was 29%, 22% and 14% (with titers of ≥5, ≥12, and ≥56) (Table 3). A rise in overall weekly RVNA seroprevalence occurred beginning week 4, peaked during week 5, followed by a decline, and returned to week 3 levels by week 8 (Fig. 3).

Fig. 3. Overall weekly RVNA seroprevalence for raccoons sampled following the Ohio spring 1999 oral rabies ORV campaign.

From among individual categorical variables, the largest difference in RVNA seroprevalence was found for bait density (Table 3). Internal differences within all other individual categorical variables were ≤11%. The maximum seroprevalence value by treatment area occurred during week 5 from raccoons in the 300 baits/km² treatment area (weekly seroprevalence values ≥20% for 5 of 7 weeks), while weekly seroprevalence values from the 75 and 150 baits/km² treatment areas were not sustained for as long (≥20% for 2 weeks

3.4. Multivariable model

No statistically significant difference in seroprevalence was observed between 75 and 150 baits/km² treatment areas; however, actual baiting densities differed only by 42 baits/km² (131–89 baits/km²) not the 75 targeted. However, raccoons from the targeted 300 baits/km² treatment area were more likely to be seropositive than raccoons from the 75 baits/km² treatment area (odds ratio [OR]₉₅₆ = 5.1, 95% confidence interval [CI] 2.7–9.8) (Table 4). Raccoons from trap sites with three or four cumulative ORV campaigns had similar seroprevalence values when using all three threshold titers (Table 4). However, raccoons from areas with five cumulative ORV campaigns yielded a higher seroprevalence when all three threshold titers were compared (OR₉₅₆ = 4.5, 95% CI 1.9–10.3).

The multivariable logistic regression modeling of data using each of the three threshold titers, ≥5, ≥12, and ≥56 yielded similar results. Therefore, only the results at a threshold titer of ≥56 were selected for both variables associated with positive RVNA seroprevalence (bait density and bait campaign frequency) (Table 4). Raccoon sex, age class, and macro-habitat at trap site were not asso-

![Fig. 4. Weekly RVNA seroprevalence for raccoons sampled in respective bait density areas (titer ≥ 56).](image-url)
Table 3

Table 3 Frequency of rabies virus neutralizing antibody-positive sera with respect to categorical variables.

<table>
<thead>
<tr>
<th>Categorical variable</th>
<th>Number of raccoon sera</th>
<th>% RVNA positive (titer ≥ 5)</th>
<th>% RVNA positive (titer ≥ 12)</th>
<th>% RVNA positive (titer ≥ 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>597</td>
<td>29</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>Bait density</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>211</td>
<td>22</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>150</td>
<td>204</td>
<td>27</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>300</td>
<td>182</td>
<td>41</td>
<td>36</td>
<td>25</td>
</tr>
<tr>
<td>Cumulative vaccination campaigns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3×</td>
<td>70</td>
<td>27</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>4×</td>
<td>175</td>
<td>25</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>5×</td>
<td>352</td>
<td>32</td>
<td>23</td>
<td>16</td>
</tr>
<tr>
<td>Macro-habitat of trap sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agricultural</td>
<td>366</td>
<td>25</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>Forested</td>
<td>231</td>
<td>36</td>
<td>27</td>
<td>18</td>
</tr>
<tr>
<td>Sex of captured raccoon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>304</td>
<td>29</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>293</td>
<td>30</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>Age class of captured raccoon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile (born previous spring)</td>
<td>81</td>
<td>25</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Sub-adult</td>
<td>171</td>
<td>30</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>Adult</td>
<td>345</td>
<td>30</td>
<td>23</td>
<td>15</td>
</tr>
</tbody>
</table>

4. Discussion

This investigation was conducted during an ongoing ORV program for combating raccoon variant rabies. Under these conditions, the bait density and cumulative bait campaign frequency variables influenced the seroprevalence of RVNAs in raccoons (Table 4). The strength of these associations suggested both parameters may influence the outcome of ORV campaigns. No association was found between seroprevalence and macro-habitat at trap sites (agriculture or forest), sex or age.

Increasing bait density from the target of 75 baits/km² treatment area exceeded the targets due, at least in part, to relatively more aerial baiting off-time for metropolitan areas and all remaining baits on a given flight being distributed on baitable portions. The actual densities in both the 150 and 300 baits/km² treatment areas were slightly lower than the target due to proportionately fewer metropolitan areas in those treatment areas leading to the above situation less frequently.

To counter the introduction of raccoon rabies into Ontario, Canada, the OMNR implemented their raccoon rabies contingency plan which consisted of a point of infection control (PIC) approach [8], which included intensive population reduction, trap-vaccinate-release and an ORV program at 70 baits/km². It has been estimated that the OMNR contingency action expense of $604,000 Cdn could potentially result in annual savings of $8–12 million Cdn if raccoon rabies could be prevented from becoming established [8].

No statistical difference in seroprevalence was detected for the target 75–150 baits/km² density treatments, but this could have been influenced by the less disparate actual densities of 89 and 131 baits/km² or edge effect in the relatively smaller 150 bait/km² treatment area (499 km²) versus the 75 baits/km² treatment area (2317 km²) or other factors. However, that a 47% increase in the bait density had no apparent effect on seroconversion raises the possibility that even lower bait distribution densities might result in similar seroprevalence levels, while realizing a cost savings. Given that target bait densities are rarely achieved across all landscapes,
the lower limit for bait density should be determined for specific sets of environmental conditions and raccoon abundance.

In Massachusetts, comparisons between the 93 and 135 baits/km² treatment areas following the first and fifth ORV campaigns found no significant differences in seroprevalence of RVNAs for each respective year [6]. However, treatment areas were small, greatly increasing the potential for edge effects between study areas. In Virginia, no increase in tetracycline deposition in raccoon mandibles between 133 and 428 placebo baits/km² treatment areas was noted [25].

A comparison of effects of 3 cumulative ORV campaigns to those of 4 cumulative ORV campaigns on seroprevalence yielded equivalent results. However, the following (5th) cumulative ORV campaign appears to have influenced RVNA seroprevalence (OR_Titer=1:56 = 4.5, 95% CI 1.9–10.3) (Table 4).

One factor possibly influencing these findings is the relative effectiveness of fall and spring ORV campaigns. The three ORV campaign areas included two spring and one fall campaign, while the four campaign areas included two spring and two fall campaigns. The five ORV campaign areas included three spring and two fall campaigns. Both the Massachusetts [6] and this Ohio investigation suggest that after the initial ORV campaign, dramatic or significant increases in seroprevalence may not occur between sequential, semi-annual campaigns, yet accumulating ORV programs resulted in gradual increases in the seroprevalence of RVNAs in raccoons.

Field investigations related to rabies control must contend with inherent and introduced variables, such as wide variations in reservoir population densities between treatment areas. A lower relative population density might result in less competition for baits than that of the 300 baits/km² trapping sites (Table 1). Therefore, the significant increase in RVNA seroprevalence in the 300 baits/km² treatment area was unlikely due to differing levels of competition for baits.

Non-target species competition for baits represents another potentially confusing variable in ORV [26–29]. Tracking studies in Florida indicated that following raccoons, opossums were the next most common species to have bait contact [29]. Opossums are indigenous to northeastern Ohio and represented the largest number of non-targets captured there (n = 51), while woodchucks were the second most common non-target trapped (n = 43). For the 75, 150, and 300 baits/km² treatment areas, 23, 19, and 9 opossums and 10, 21, and 12 woodchucks were captured. The variation in opossum captures between ORV treatment areas may raise concern over differing levels of competition with raccoons for bait, which could impact seroprevalence.

Macro-habitat was not retained by the model. While others [30] reported high tetracycline uptake in raccoons following targeted bait distribution in forested settings (76%) during small scale baiting trials in Pennsylvania, no comparison was noted with raccoons from nearby non-forest regions. Our preliminary seroprevalence values indicate there was higher RVNA seroprevalence in raccoons from forested relative to agricultural areas (Table 3), but no association was identified following adjustment for confounding factors (Table 4).

No differences in seroprevalence were related to sex or age in this study. However, in post-ORV surveillance following the spring 1998 Ohio ORV campaign, males had a higher RVNA seroprevalence than females (OR = 1.6, 95% CI 0.02–19) [19]. Conversely, females had a slightly higher, but insignificant, percent of RVNA positive individuals in Ohio in 1999. No association was found between age and RVNA seroprevalence during 1998 or 1999. Positive RVNA titers in juvenile raccoons following spring ORV campaigns in 1998 and 1999 are encouraging. Whether these titers represent passive antibodies or an active antibody response remains in question.

4.1. Study limitations

During 1998, 63 juvenile raccoons were captured, vaccinated and released, as part of a different study, without ear tags from nine trap sites, which were eventually assigned to the 300 baits/km² treatment area in 1999. Four of these nine trap sites were used in 1999 and 19 one-year-old raccoons were captured at these trap sites.

To achieve the goal of 300 baits/km², two sets of lines were flown in that zone, each approximating a 150 baits/km² density pattern, with the second set of lines flown perpendicular to the first set of flight lines, resulting in a grid pattern for bait distribution, rather than the typical north–south parallel flight lines used in the 75 and 150 baits/km² treatment areas. Others [31] presented computer modeling results indicating that distances (intervals) between flight lines may be a crucial factor in efficiency of ORV campaigns using uniform spacing, indicating that adjusting flight line spacing relative to expected raccoon home ranges may be more effective at potentially reducing the bait densities required to achieve control. The grid pattern of bait distribution in this study represents a variation of the previous [31] model with the added influence of an increase in bait density. The independent effect of the grid pattern used for bait distribution could not be evaluated in this investigation, but warrants future investigation.

5. Summary

Clearly, bait density is a consideration to maximize seroprevalence of RVNAs in the target populations, but cost-effectiveness will continue to warrant consideration in operational ORV programs. Regardless, it appears that bait density and cumulative bait campaign frequency are only two of several important variables in ORV strategies that must be carefully balanced against the current costs of control with the long-term costs and risks associated with continued spread of raccoon rabies and its attendant impacts.

References