West Nile virus dissemination among individual wild mosquito vectors in Northern Colorado, 2023

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Abstract

West Nile virus (WNV) transmission risk is typically estimated from pooled whole-mosquito infection data, which may overestimate the proportion of mosquitoes capable of transmission. To assess natural viral dissemination in field-collected *Culex tarsalis*, we tested infection rates in tissues of 1,793 individual mosquitoes collected from Northern Colorado in August, 2023. Fifteen mosquitoes had detectable abdominal infections, but WNV RNA was detected in only 53% (8/15) of their thorax and head tissues, while another 27% (4/15) had WNV RNA detected in either the thorax or the head alone. Logistic regression suggests an inconsistent relationship between abdominal viral RNA load and virus dissemination, whereas receiver operating characteristic analysis identifies a threshold of ~59,000 RNA copies in the abdomen predictive of dissemination (AUC 0.80, 95% CI: 0.545, 1). These results suggest whole-body RNA detection may overestimate transmission potential from field-captured mosquitoes, and that incorporating infection data could refine surveillance-based risk indices for WNV.

Introduction

West Nile virus (WNV) is the most widespread arthropod-borne virus in the United States and poses a persistent public health threat and economic burden *(1-4)*. Since its introduction to the country in 1999, thousands of cases of West Nile disease (WND) have been diagnosed in humans annually *(5)* despite evidence of significant underreporting of infections and disease *(6)*. Some estimates put the true number of human infections in the US since the virus emerged at roughly 7 million *(4,7)*. Mosquitoes from the genus *Culex* are the primary vectors for WNV, and *Culex tarsalis* (Coquillett) is one of the most significant enzootic and epidemic vectors in the western U.S., playing a key role in virus amplification via the enzootic cycle among birds and transmission to humans *(8,9)*. Because there is no human vaccine or specific antiviral treatment for WND, disease prevention relies heavily on entomological surveillance, vector control, and personal protective measures *(10)*. These Integrated Vector Management (IVM) strategies include WNV surveillance through mosquito trapping & WNV testing, public communications of WNV transmission risk and recommendations for personal protection measures such as behavior modifications and repellent use when risk is determined to be high, and vector control operations that focus on draining water from larval sites, as well as larvicide and adulticide applications *(11,12)*. This approach may incur significant costs to taxpayers where local and state governments pay to implement it. Furthermore, the efficacy of some of these approaches can be hampered by weather and insecticide resistance in the mosquito populations *(13)*, and there may be concern from individuals or groups about the effect of widespread insecticide use on pollinators and other non-target insect populations and the environment *(14-16)*. Accordingly, IVM-connected WNV surveillance strategies should be employed in at-risk areas using the most precise WNV detection methods, as these provide critical insight for mosquito control

districts/programs, public health authorities and elected officials about contemporary and temporal risks of WNV transmission risk in humans and helps to guide decision making around public communications and control efforts.

Current WNV surveillance programs rely on pooled mosquito testing, whereby whole female mosquitoes in pools (usually up to 50 per pool) are homogenized in a buffer solution. The buffer is then tested for either WNV antigen with a rapid test or WNV RNA through reverse-transcription quantitative PCR (RT-qPCR) to estimate pooled infection rates and the Vector Index (VI), an estimate of the abundance of infected mosquitoes in an area, which can be used to guide public health interventions *(17,18)*. The RT-qPCR method to quantify WNV RNA is considered to be the most sensitive and accurate method *(19,20)* but is more resource-intensive. WNV positive pools are determined from amplification of WNV RNA in pools of mosquitoes captured in standard surveillance traps – meeting a predetermined threshold cycle threshold (CT) value or, ideally, a threshold CT value determined by comparing amplification to a standard curve of known viral RNA quantities *(21)*. This method assumes that one or more mosquitoes from a pool determined to be below this CT threshold is infected with WNV and has the potential to transmit WNV to humans. Data from tested mosquito pools over an area are then used to calculate the mosquito infection rate via either the minimum infection rate (MIR), which assumes only one infected mosquito per positive pool, or via the maximum likelihood estimation (MLE), which probabilistically (via a binomial distribution of infected individuals in a positive pool) estimates the mosquito infection rate given the observed number of positive mosquito pools and their pool sizes (22). The mosquito infection rate multiplied with the species capture rate forms the VI for each captured and tested species *(23)*. These mathematical estimates have been shown to be acceptable measures of true mosquito infection rates, with VI correlating with

human WNV infections in the following weeks. Therefore, they are used as action thresholds to initiate specific vector control interventions to mitigate human risk *(24,25)*. Importantly, the mosquito infection rate variable used in the VI equation naturally varies among mosquito species due to differences in species vector competence for WNV.

Following ingestion of an infectious bloodmeal, the vector competence of a mosquito species or strain for WNV depends on the ability of the virus to infect the midgut and foregut tissues, replicate and spread in those tissues, disseminate into hemocoelic tissues, infect the salivary gland tissue in the thorax, and finally disseminate into the saliva. The vector competence is variable depending on the virus titer ingested in the blood meal *(26)*, and the ingested and newly produced progeny viruses must pass through infection/escape barriers in the midgut and salivary gland tissues before the mosquito can become infectious *(27)*. The time that this process takes is known as the extrinsic incubation period (EIP) and has been characterized rigorously under laboratory conditions *(28,29)*. This period is temperature-dependent but generally lasts between 8-18 days under summer conditions in temperate regions *(30)*.

Much less is known about WNV dissemination rates in individual wild-caught mosquitoes, as testing individual field-derived mosquitoes is often not economical due to low prevalence within mosquito populations, which typically necessitates pooled testing *(21,31,32)*. Condotta et al. tested individual and pooled mosquitoes to evaluate the actual infection rate in WNV positive mosquitoes for *Cx. pipiens* and *Cx. restuans* as well as to evaluate if the MIR and MLE overestimate or underestimate WNV infection *(25)*. However, the authors did not account for the possibility of non-disseminated infections. If a substantial proportion of these mosquitoes harbor only localized midgut infections, risk assessments based solely on infection prevalence may overestimate actual transmission potential. Improved understanding of viral dissemination

in wild mosquitoes is therefore essential to refine entomological risk indices and enhance the accuracy of outbreak predictions.

The 2023 summer in Northern Colorado had unusually high mosquito abundance and infection prevalence, which allowed for a meaningful sample size of individual WNV-positive mosquitoes to be found over a short two-week time frame. Using these naturally infected field-derived mosquitoes, we tested the hypothesis that traditional approaches to assessing WNV risk based on testing of mosquito pools overestimates actual risk. In particular, we sought to determine the extent to which WNV-positive, wild *Culex tarsalis* mosquitoes lack viral RNA in the head and thorax, indicating a lower-than-expected potential for transmission. By dissecting individual mosquitoes and estimating viral loads in specific tissues, we aimed to quantify the proportion of infections that are non-disseminated and assess the implications for surveillance-based risk assessment.

Materials and Methods

Adult *Cx. tarsalis* from Larimer, Weld, and Morgan counties, Colorado, USA were trapped using CDC miniature traps, using dry ice but no light as the attractant, during the weeks of August 7th and 14th 2023. 1791 mosquitoes were captured and stored at –80°C until dissection and testing began in 2024.

Mosquito samples were dissected into abdomen, thorax, and head tissue compartments. Each mosquito and dissection forceps were surface sterilized by dipping it into 70% EtOH and then rinsed twice in PBS before dissection. Each mosquito was dissected separately in depression wells of a glass depression well plate and the plate was sterilized between dissections to avoid contamination among samples. Dissected abdomen and head tissues were stored individually in

microfuge tubes before testing, while thorax tissues were pooled (n=11 per pool) for testing, except for samples 19, 76, 124, and 172 where the thoraces were tested individually while the heads were pooled.

One sterile ball bearing and 300 µL of mosquito diluent was added to each abdominal tissue sample. Mosquito diluent was created using phosphate buffered saline (PBS), fetal bovine serum (FBS), penicillin streptomycin, Fungizone, and gentamicin, and was stored at 4°C. Abdominal tissues were homogenized at 24 Hz frequency for 60 seconds and centrifuged. 15 µL of supernatant from each homogenate was pooled into groups of 11, the RNA was extracted from the pooled homogenate using the Omega Bio-Tek Mag-Bind Viral DNA/RNA 96 Kit and was then tested using RT-qPCR to identify the presence of WNV RNA in each homogenate pool. If a pooled supernatant was positive, the 11 individual abdomen homogenates were subsequently tested for the presence of WNV. Once an individual mosquito was identified as having an abdomen positive for WNV, the corresponding thorax pool and individual head and thorax tissues were tested.

Infection of the midgut is a prerequisite for dissemination of the entire mosquito. Therefore, only individuals with RNA presence in the abdomen were included in this study. CT values were obtained from raw PCR output at a consistent fluorescence threshold of 34,000. Aliquots of varying concentrations of WNV RNA standards were cloned from viral RNA isolated from stocks and stored at –80°C until their use in a standard curve run alongside the samples. RNA concentrations in these aliquots were 100, 10,000, and 1,000,000 copies, and the number of copies in each positive sample was estimated from a linear regression of the standard curve CT values to account for fluctuations in CT estimates between runs. Whole-body CT estimates were created by summing the RNA estimates from the standard curves of the three

separate tissues and converting them back into a CT value. Based on previous studies that showed WNV-infected mosquito cells consistently produced greater than 10^2 RNA copies for each infectious Plaque Forming Unit (PFU) *(33,34)*, a conservative binary infection threshold of 200 RNA copies was created to distinguish weakly infected tissue. This cutoff minimizes false positives from non-infectious viral RNA while maintaining sensitivity for biologically relevant infections which are likely to contain an infectious unit.

Statistical analysis was performed in R version 4.5.1 using packages ggplot2 *(35)* for visualizations, patchwork (36) for organizing plots, corrplot *(37)* for correlation analysis, and pROC *(38)* for receiver operator characteristics analysis. Logistic regression was performed using a generalized linear model with a logit link function, and statistical significance was determined as a p value below 0.05.

Results

To provide historical context for the 2023 dissections, combined *Culex pipiens* and *Culex tarsalis* mosquito abundance and the vector index (VI) in Northern Colorado regions from long-term (2012–2024) surveillance data are displayed relative to 2023 data (Figure 1). These elevated VI and mosquito abundance numbers are consistent with high human West Nile Virus (WNV) case counts from the same surveillance period (Figure 2).

RNA levels in sampled mosquitoes varied widely from nearly undetectable to millions of copies in each tissue compartment but were generally higher in the abdominal tissues (Table 1). Corresponding thorax and head CT values suggest variable dissemination efficiency, with several mosquitoes exhibiting high abdominal RNA but limited or no evidence of dissemination to secondary tissues. Overall, the abdominal infection rate was approximately 1 per 120

mosquitoes, or 0.84%. Of those infected, 8/15 (53.33%) had viral RNA in both the thorax and head tissue.

Mosquitoes with disseminated infections exhibited consistently low CT values across all compartments, reflecting high RNA loads (Figure 3). In contrast, those with failed or incomplete dissemination showed sharp CT increases (lower RNA) between abdomen and head/thorax tissues. Weak infections showed uniformly high CT values or undetectable values.

To assess whether RNA concentrations in one tissue could predict viral presence in others, we conducted correlation analyses using two approaches: raw CT values and log□□-transformed RNA values (Figure 4). Raw CT values (Panel A) showed a strong positive association between thorax and head tissues (r = 0.70), moderate correlation between abdomen and head (r = 0.49), and weak correlation between abdomen and thorax (r = 0.23). RNA values that were estimated from CT outputs were then log-transformed (Panel B) and yielded intermediate to strong correlations: r = 0.63 for thorax–head, r = 0.50 for abdomen-head, and r = 0.21 for abdomen–thorax.

A logistic regression was then conducted to evaluate the relationship between presence or absence of a disseminated WNV infection in mosquitoes and the log-transformed abdominal RNA levels (Figure 5). A binary infection threshold of 200 RNA copies in both the head/thorax was selected to classify individuals as “infected” or “uninfected” in the target tissue. For every log increase in abdominal RNA, the odds of WNV successfully disseminating to a mosquito’s head and thorax increased by approximately 2.5 times (OR = 2.45, 95% CI: 0.81, 7.36, p = 0.11).

Lastly, we evaluated whether RNA concentration in the abdomen could serve as a predictor for the presence of virus in the head and thorax, which is a key indicator of

dissemination and potential transmission competence. The same threshold of 200 copies of RNA from the previous analysis was used. The receiver operating characteristic (ROC) curve analysis revealed good overall discriminative ability, with an area under the curve (AUC) of 0.80 (95% CI: 0.545–1.000; Figure 6). This suggests that abdomen RNA levels can moderately to strongly differentiate mosquitoes with disseminated infections from those without. The optimal abdomen RNA threshold for predicting head/thorax infection was estimated from the AUC using the same RNA copy threshold and measured ~59,000 RNA copies. At this threshold, sensitivity was 1.00, indicating that all mosquitoes with ≥200 copies in the head and thorax were correctly identified. Specificity was 0.625, meaning that 62.5% of mosquitoes without complete dissemination were correctly classified as negative.

Discussion

Our study provides novel insights into WNV infection dynamics in wild, field-collected *Cx. tarsalis*, highlighting that detection of WNV in pools of mosquitoes does not explicitly equate to transmission potential in the wild, because many of these positive mosquitoes do not have completely disseminated infections. Of the 15 mosquitoes that were positive in abdominal tissue, nearly half failed to demonstrate complete viral dissemination into the thorax and head. This indicates that a substantial proportion of naturally infected mosquitoes may not contribute to transmission at the time of capture, despite being classified as “infected” under current surveillance standard methods. The addition of whole-body estimates to our analysis provides a comparison to what likely would have been observed had the whole mosquito been tested by itself, or in a pool of non-infected mosquitoes, mimicking the standard approach used in pool-based arbovirus surveillance.

Importantly, this study used mosquitoes that were collected during the unique conditions of 2023, when Northern Colorado experienced unusually high mosquito abundance, WNV activity, and human cases. These coincided with elevated mosquito abundance and vector index values during mid-August, when field collections were performed. The human cases were often severe, neurological cases, which are the cases most likely to be reported to the health system. Relative to the preceding decade, both mosquito abundance and VI in 2023 ranked among the highest observed allowing us to obtain enough naturally infected individuals (0.83%) that allowed for relatively cost-effective screening and testing of individual mosquitoes and tissue-specific analyses of these mosquitoes, which is rarely economically feasible under typical field conditions *(23)*.

Our correlation analysis suggests that viral burden in head and thorax tissues generally increases with abdominal RNA, but the relationship is not strictly proportional. Mosquitoes with failed or incomplete dissemination showed sharp CT increases between abdomen and head/thorax, suggesting potential midgut escape or salivary gland infection barriers or a mosquito that was captured early in the extrinsic incubation period for the virus. Overall, correlations between WNV infection in abdomen and thorax tissues were lower than abdomen and head tissues. Correlation statistics were relatively similar between CT value and log-RNA estimates.

The relationship between abdominal viral RNA burden and dissemination was not statistically significant in a logistic regression, though the directionality of the relationship suggests that higher abdominal titers increased the odds of dissemination. According to our model, for every log increase in abdominal RNA, the odds of WNV successfully disseminating in a mosquito increased by approximately 2.45 times. Although not statistically significant, the

observed trend is consistent with prior laboratory-based studies that suggest higher viral titer in the infectious bloodmeal, and therefore a lower CT value, leads to an increased chance of a fully disseminated infection *(25)*.

ROC curve analysis further explored the predictive utility of abdominal RNA levels for dissemination. We identified an abdominal RNA threshold of approximately ~59,000 copies that discriminated between disseminated and non-disseminated infections with high sensitivity (1.0) but moderate specificity (0.625), yielding an AUC of 0.80 (95% CI: 0.55–1.0). This finding suggests that increased abdominal RNA values strengthen predictions of a disseminated infection in mosquitoes. The relatively low specificity indicates that a subset of mosquitoes with high abdominal titers may still fail to disseminate or could have recently taken an infectious bloodmeal and are early in the EIP. These results reinforce the biological complexity of WNV dissemination and the limitations of using whole-body RNA measurements as proxies for vector competence.

Several methodological considerations of our data warrant further discussion. First, the rarity of naturally infected mosquitoes necessitated pooling strategies in our initial screening, and while this improved efficiency, it introduced challenges in interpreting thorax RNA values when multiple individuals were combined. In two cases, pooled thorax homogenates might have masked heterogeneity between individuals, diluting strong infections or inflating weak ones. Second, viral RNA quantification by RT-qPCR is inherently variable at low copy numbers, such as the binary cutoff of 200 RNA copies that we applied *(39)*. Although the threshold approach provides a consistent framework, different cutoffs could shift classification, underscoring the need for standardized dissemination definitions for field caught mosquitoes. Finally, our sample size, though substantial for field collections, yielded only 15 positives. This limits our ability to

detect subtle trends and necessitates cautious interpretation of the statistical associations we performed.

Nonetheless, our results align with and extend prior laboratory studies of *Culex spp.* vector competence. Experimental infections in colony mosquitoes often report higher dissemination rates than observed here, possibly reflecting differences in viral dose, mosquito age, or environmental stressors between controlled and field conditions *(28,29)*. The lower dissemination rates in wild mosquitoes may most obviously reflect the timing of their capture before the EIP was complete. Alternatively, they could also reflect a combination of midgut escape barriers, immune priming, or ecological pressures not captured in laboratory systems *(27)*. These findings suggest that extrapolating laboratory-based competence data to field populations may overestimate true transmission risk at the time of mosquito capture and testing.

From a surveillance perspective, these findings have important implications. Current arboviral monitoring programs typically classify any WNV-positive mosquito sample below a cycle threshold value of ~36-39 as potentially infectious, regardless of dissemination status *(21,40)*. Our data suggests this approach may inflate estimates of vectorial capacity by failing to account for the proportion of mosquitoes with unsuccessfully disseminated infections restricted to the midgut, or weak disseminated infections that may be mosquitoes captured early in the EIP or that can only transmit few infectious virions. Similarly, a previous modeling study that incorporated WNV infection dynamics in mosquitoes into transmission models found that a potential explanation for the variability of CT values observed in WNV surveillance pools is due to a substantial proportion of mosquitoes that are exposed to WNV do not develop a productive infection *(41)*. Incorporating tissue-specific viral dissemination data into predictive risk indices could improve their accuracy and reduce overuse of scarce resources. While the logistical

challenges of dissecting large numbers of mosquitoes are arduous, statistical adjustment factors that incorporate CT values as a proxy for predicting the potential dissemination and infectiousness of a mosquito informed by this study and future studies like it may offer a solution.

Future research should focus on validating dissemination thresholds in larger sample sets and across temporal and ecological contexts. Our sampling occurred within a limited two-week period in mid-August, representing only a narrow temporal window of mosquito activity. Seasonal or age-related changes in mosquito physiology may alter dissemination efficiency, and longitudinal studies with broader sampling could illustrate these dynamics to better capture seasonal variation in infection prevalence or dissemination efficiency. An important future step will be integrating dissection-based dissemination data from laboratory studies with salivary secretion of infectious virions, which would directly establish the link between tissue-level infection and actual transmission capability *(42)*. Finally, exploring viral genetic strain variation in dissemination success would provide valuable insight into the evolutionary pressures shaping WNV transmission.

In summary, our findings demonstrate that a substantial fraction of field-collected *Culex tarsalis* harbor WNV infections restricted to the abdomen, without evidence of dissemination to the thorax and head. Abdominal viral RNA levels showed predictive value for dissemination, but with limited specificity. These results demonstrate the importance of distinguishing between infection and transmissibility in surveillance-based risk models. Refining entomological risk indices to include CT values as a proxy for transmission likelihood to account for incomplete dissemination will improve the accuracy of WNV outbreak predictions and guide more efficient public health responses.

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Biographical Sketch

Jackson DeCook is a RaHP VEC fellow and Colorado School of Public Health graduate research fellow at Colorado State University's Center for Vector-borne Infectious Diseases, where he studies transmission dynamics of vector-borne diseases and implications on public health. His research focuses on improving surveillance and Integrated Vector Management strategies as well as understanding mosquito competence for arboviruses, including West Nile virus.

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Table

Table. Infection data from all wild *Culex tarsalis* mosquitoes that tested positive in their abdomens for West Nile virus RNA

Mosquito ID	Abdomen CT	Abdomen RNA	Thorax CT	Thorax RNA	Head CT	Head RNA
A3	15.86	5,985,545.67	25.24	8,461.57	23.39	30,850.83
A4	32.81	42.27	25.24	8,461.57	33.84	20.52
D8	17.54	1,845,606.78	23.85	22,353.08	24.37	15,554.08
D10	16.28	4,471,412.28	23.85	22,353.08	28.74	732.22
19	22.54	68,236.08	21.76	118,408.02	24.41	18,157.96
76	22.99	49,615.82	Negative	Negative	Negative	Negative
124	23.37	37,916.02	Negative	Negative	Negative	Negative
182	37.00	2.46	Negative	Negative	Negative	Negative
259	33.42	137.19	28.14	4,593.69	Negative	Negative
703	21.71	334,350.00	Negative	Negative	33.71	112.79
928	16.98	7,788,455.58	32.01	349.40	Negative	Negative
1103	19.20	1,767,845.07	Negative	Negative	33.87	101.73
1150	18.54	2,752,642.96	30.14	1,212.83	30.59	898.81
1186	23.50	100,984.11	27.16	8,863.06	24.67	46,551.64
1341	18.17	3,358,847.22	25.08	109,088.94	31.48	2,683.82

1,793 mosquitoes were tested in pools, and 143 mosquitoes from positive pools were tested as individuals. RNA numbers refer to estimated RNA copies estimated from a linear regression of CT values from a standard curve. Tissues with less than 200 copies of RNA were considered negative. A prerequisite for inclusion in the analysis and further testing was the presence of viral

RNA in the abdomen tissue.

Figure Legends

Figure 1: Historical West Nile virus mosquito vector abundance and VI in Northern Colorado regions during the 2023 season compared to other seasons. The years 2012-2024 (excluding 2023) are highlighted in grey, while the 2023 season is highlighted in red. BC: Boulder County, CO; BE: Berthoud, CO; FC: Fort Collins, CO; LV: Loveland, CO.

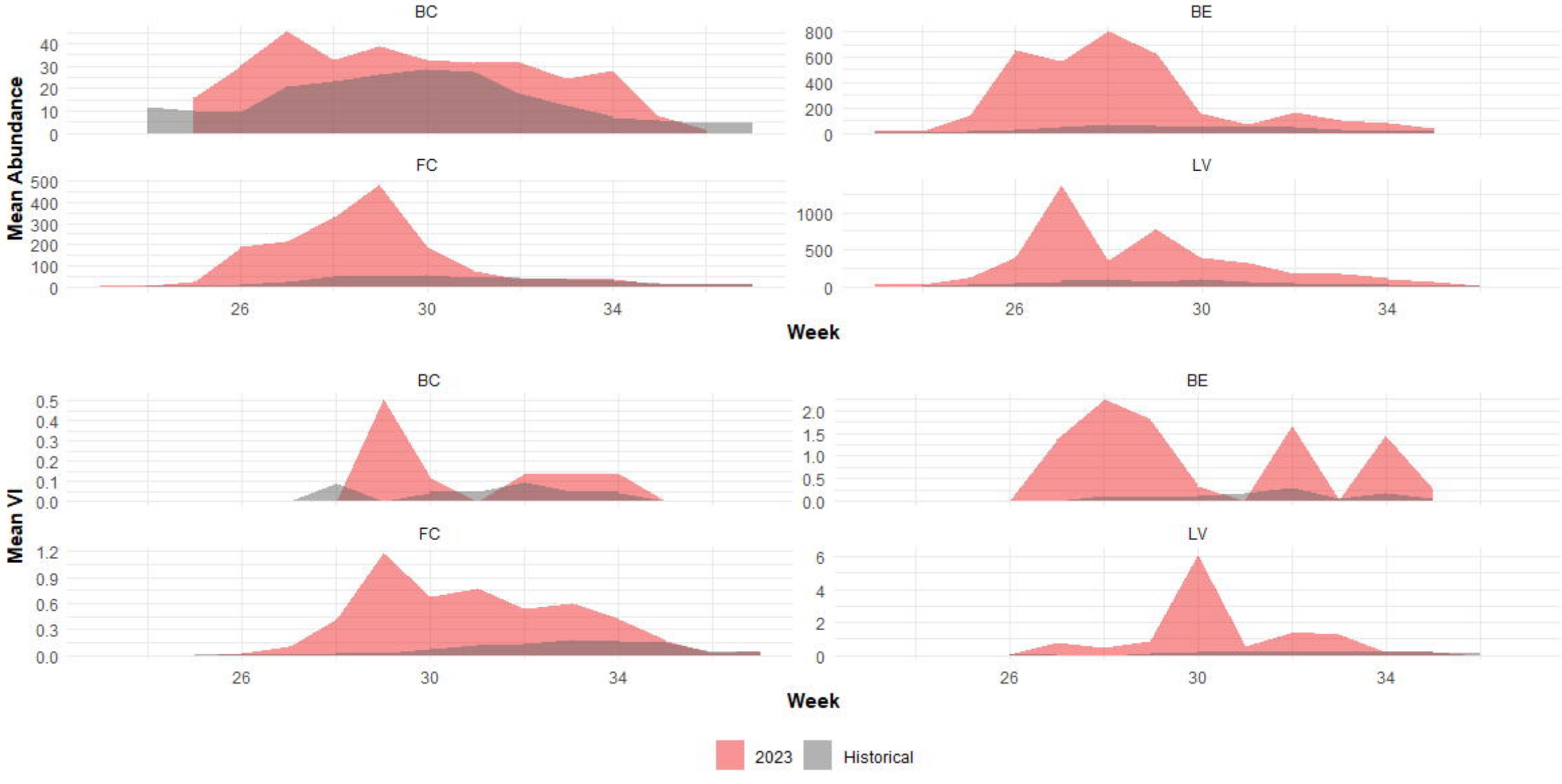
Figure 2: Confirmed human West Nile virus cases in Colorado during 2023 compared to other years. The 2023 season, highlighted in yellow, showed a large spike in human cases compared to other seasons from 2012-2024 highlighted in blue.

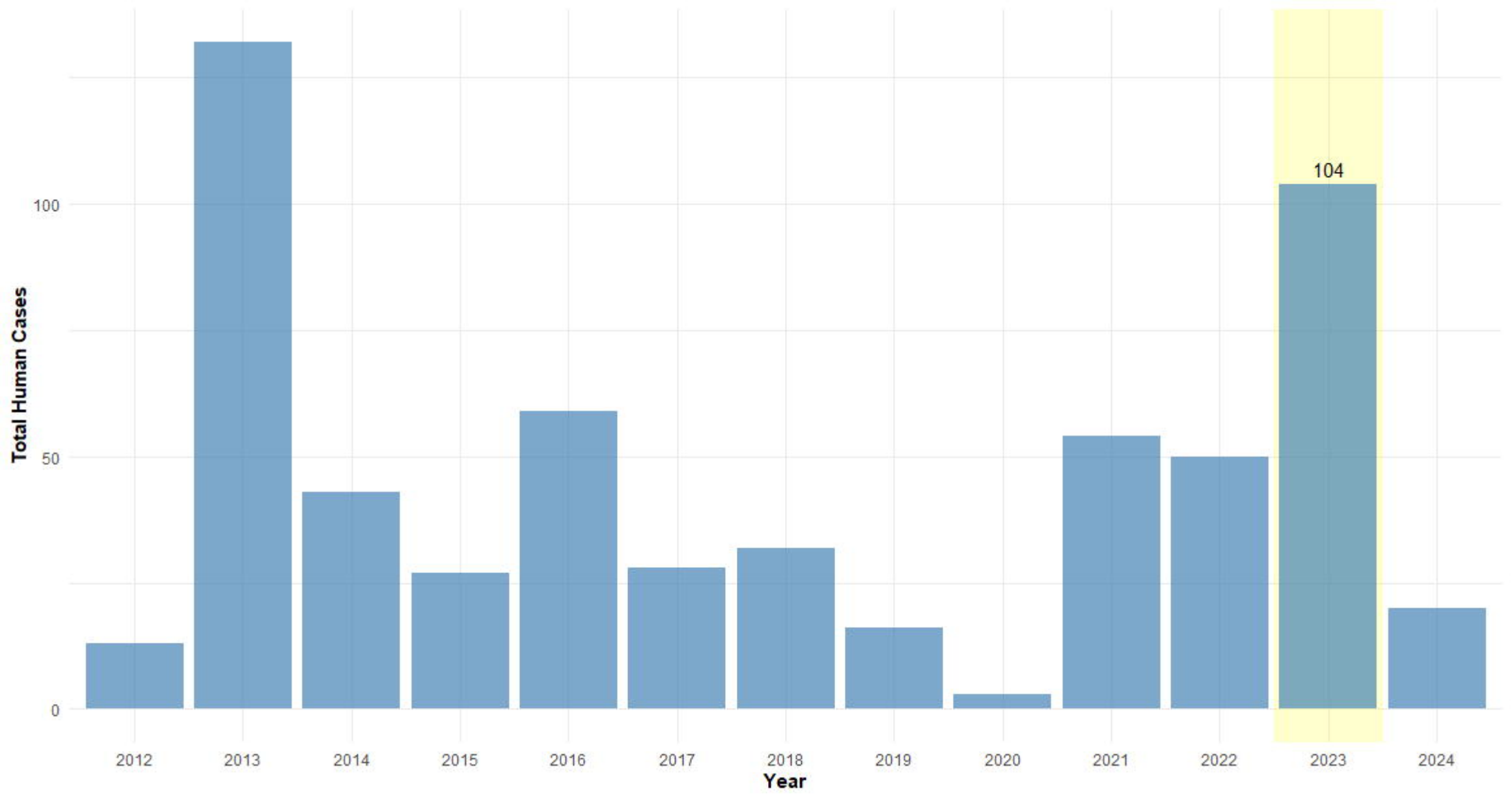
Figure 3: Paired CT values for abdomen, thorax, and head compartments of WNV-positive mosquitoes revealed distinct patterns by dissemination category. WNV-infected mosquitoes were grouped into four phenotypes: disseminated infection (n = 8), failed dissemination (n = 2), incomplete dissemination (n = 4), and weak midgut infection (n = 1). Plots include corresponding whole body mosquito CT estimates determined by adding together and combining RNA estimates from given tissue CT values, then inverting them back into one whole body estimate CT estimate. *Denotes multiple positive samples in one thorax pool, which artificially deflated the CT estimate. **Denotes a weaker midgut infection that had disseminated into the thorax but not the head.

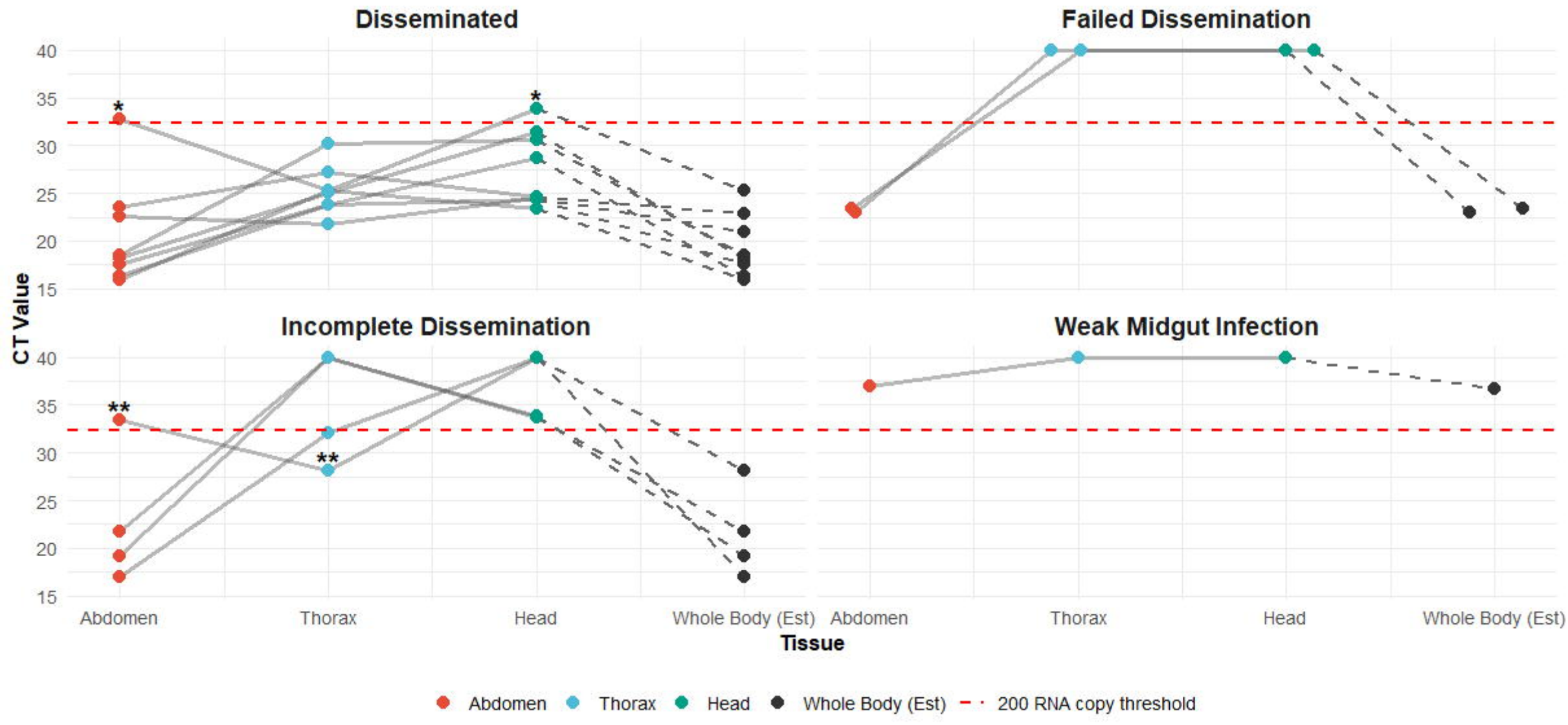
Figure 4: Correlation analyses of RNA values in paired tissue compartments of mosquito samples. Correlation matrices for raw CT values (Panel A) and log-transformed RNA estimates (Panel B).

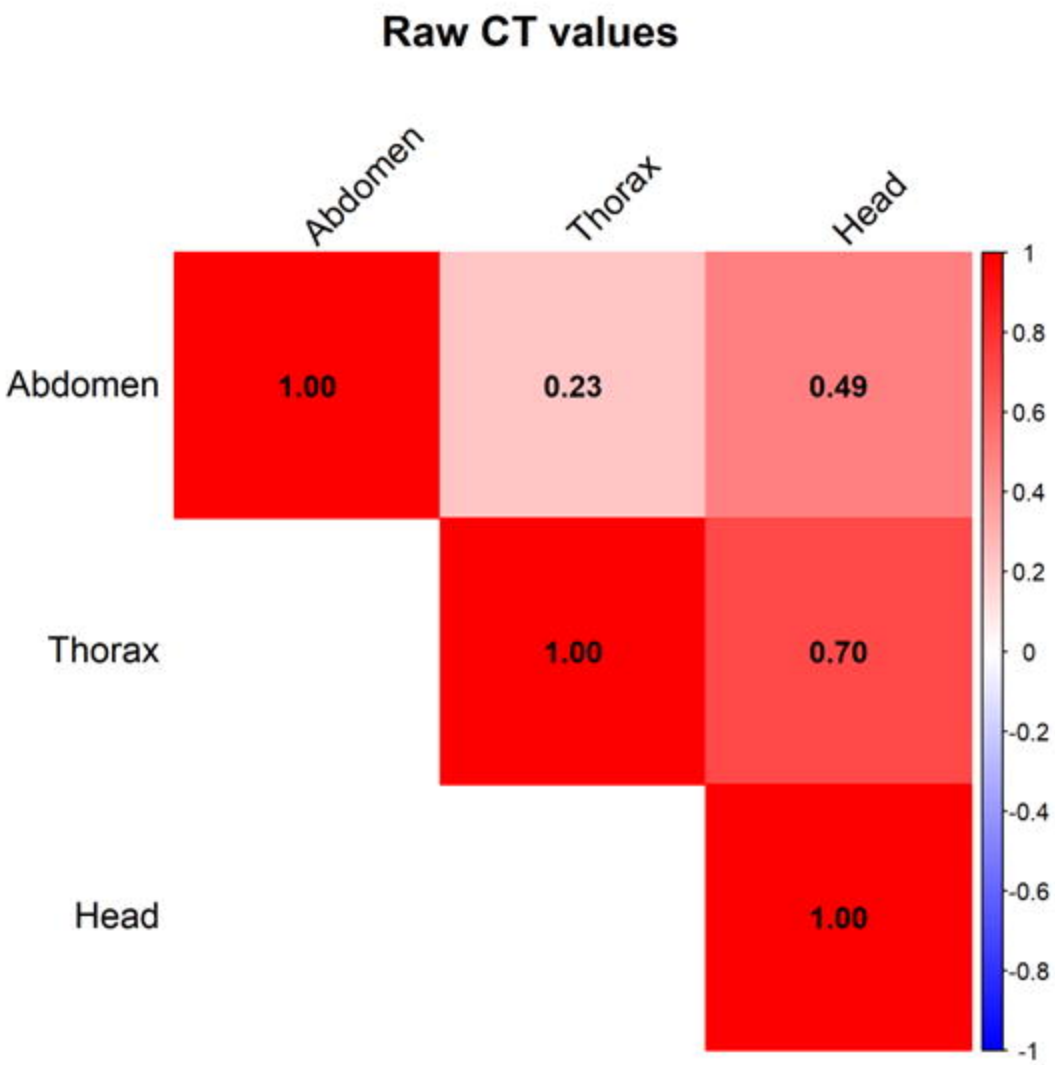
Figure 5: Logistic Regression model predicting complete dissemination of viral RNA via log RNA estimates. Blue dots represent WNV-positive mosquitoes. The red line represents the regression line and the shaded red area the 95% CI of the regression line. For every log increase in abdominal RNA, the odds of WNV successfully disseminating to the head and thorax increased by approximately 2.5 times (OR = 2.45, 95% CI: 0.81, 7.36, p = 0.11)

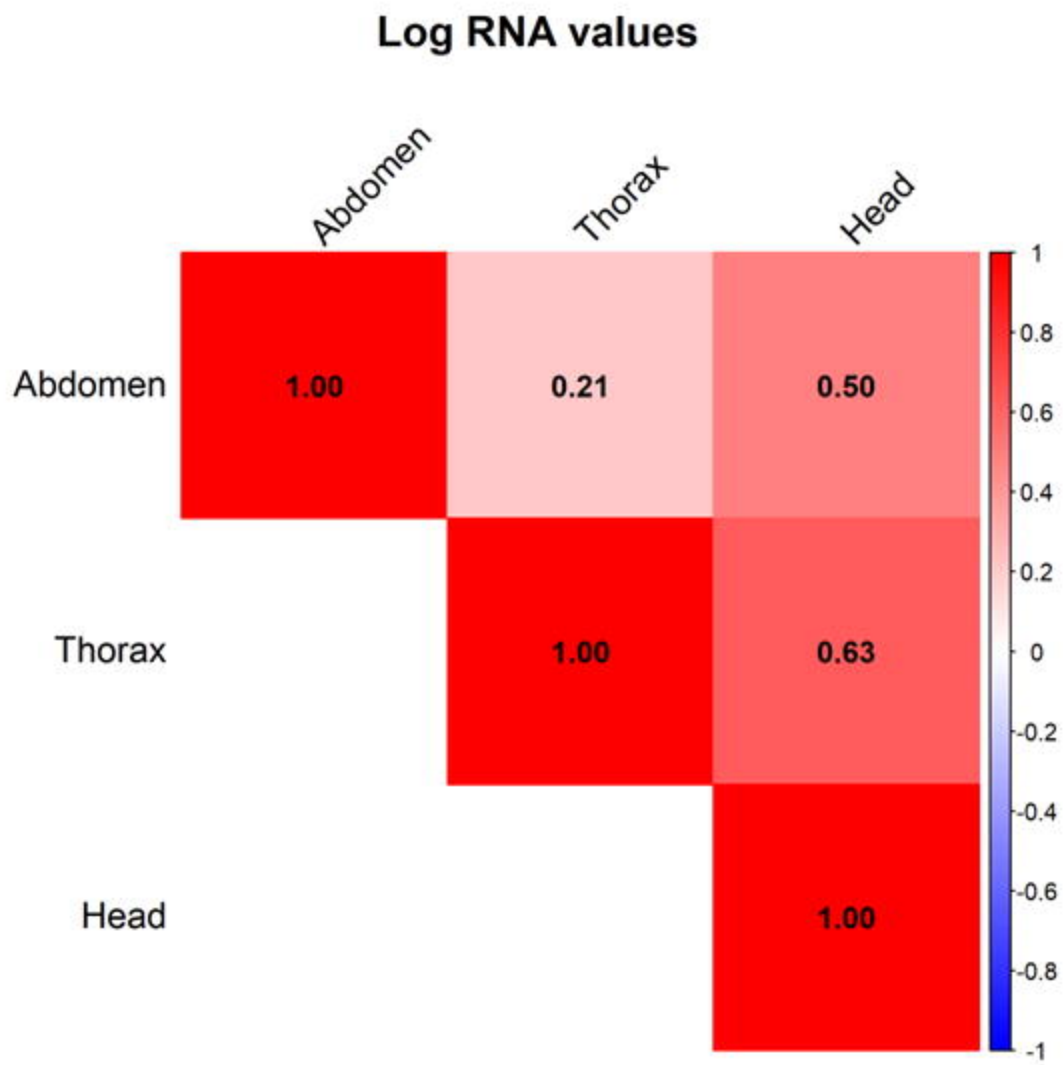
Figure 6: Receiver Operating Characteristic (ROC) curve analysis for abdomen RNA value that predicts binary threshold for complete dissemination. The binary infection threshold was set at 200 copies of viral RNA. The area under the curve (AUC) for prediction of infection was 0.8 (95% CI: 0.545, 1). The optimal threshold for prediction of complete dissemination at 200 copies of RNA by abdomen RNA was ~59,000 copies with a sensitivity of 1 and a specificity of 0.625.

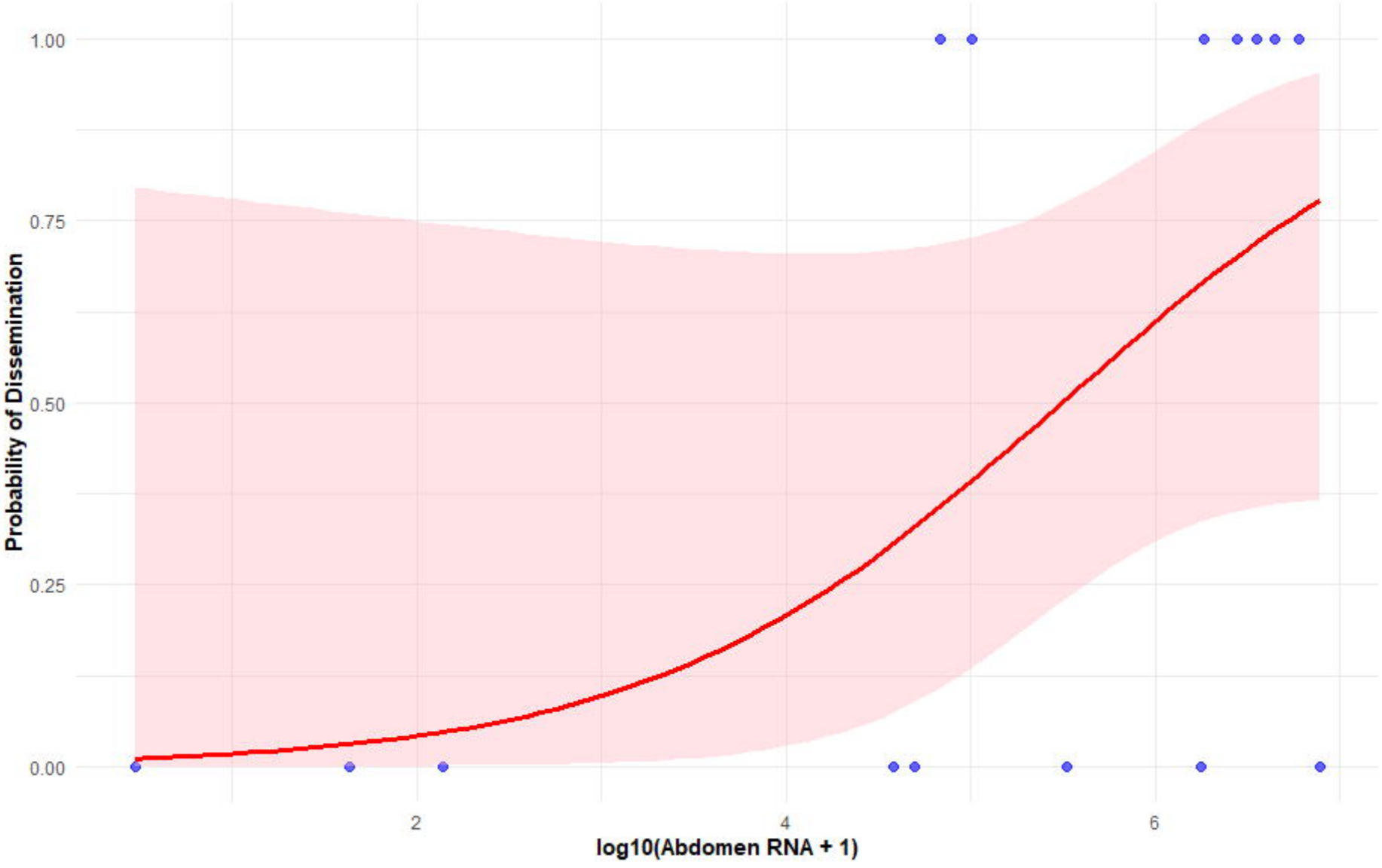

BC
BE
FC
LV
Mean Abundance
Week
Mean VI
Week
2023
Historical

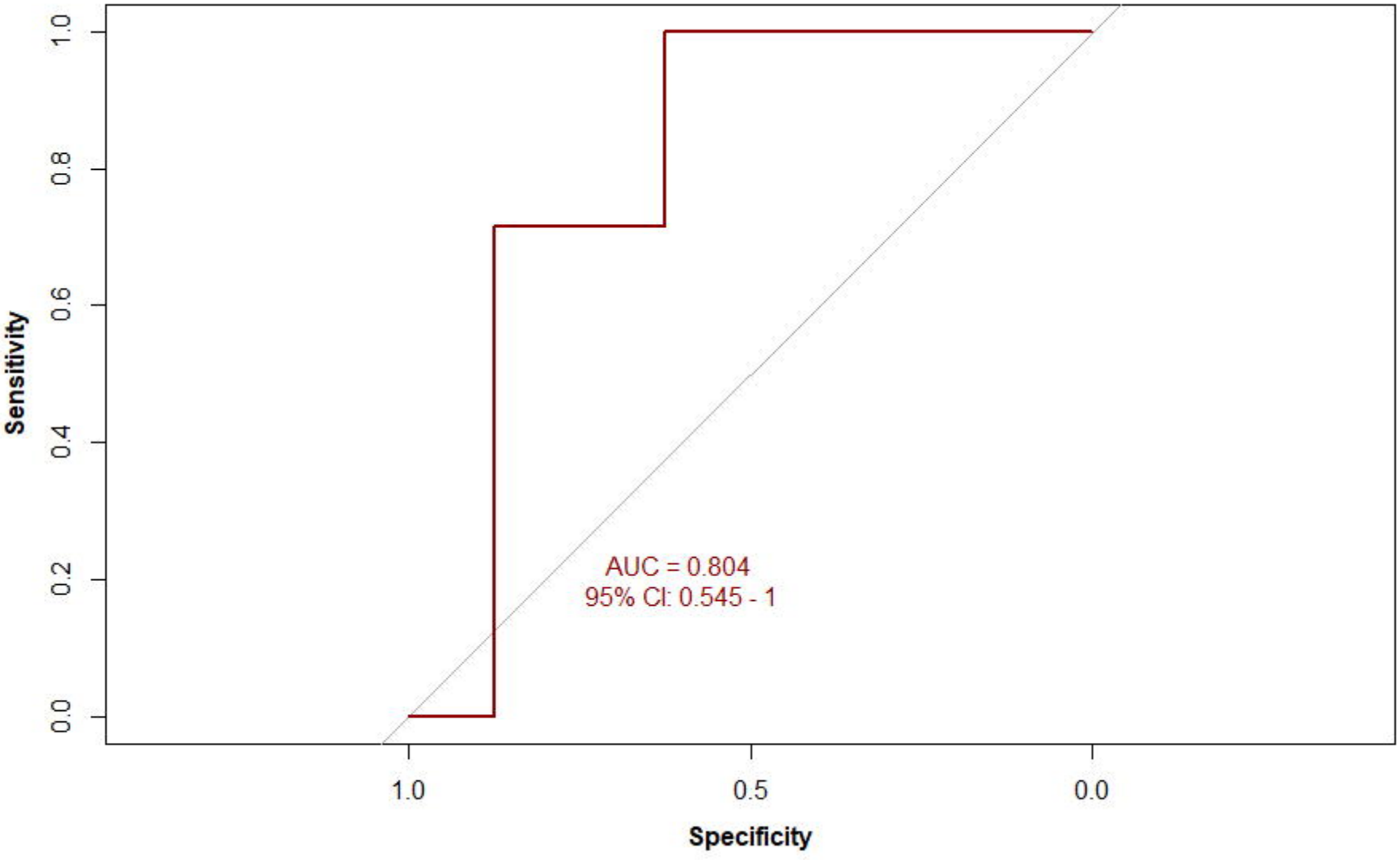

104
100
50
0
Total Human Cases
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
Year


Disseminated
Failed Dissemination
Incomplete Dissemination
Weak Midgut Infection
CT Value
40
35
30
25
20
15
*
**
Abdomen
Thorax
Head
Whole Body (Est)
Tissue
Abdomen
Thorax
Head
Whole Body (Est)
200 RNA copy threshold


Raw CT values
Abdomen
Thorax
Head
Abdomen
Thorax
Head
1.00
0.23
0.49
1.00
0.70
1.00
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1


Log RNA values
Abdomen
Thorax
Head
Abdomen
Thorax
Head
1.00
0.21
0.50
1.00
0.63
1.00
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1


1.00
0.75
0.50
0.25
0.00
Probability of Dissemination
2
4
6
log10(Abdomen RNA + 1)


Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
AUC = 0.804
95% CI: 0.545 - 1
1.0
0.5
0.0
Specificity