Reviewer 1:

The objectives and reasons for research plan are laid out in a plausible manner. Some sections (e.g., flavivirus drug development, vector-microbe interactions) seem somewhat exploratory, but perhaps that is the point of this type of project (unclear to this reviewer). The challenge will be in applying some of these findings to real-world (user-friendly) tools that are affordable to vector control programs. The research plan states that the proposed work focuses on "mosquitoes, sandflies, kissing bugs, ticks, and other vectors of human pathogens."...but kissing bugs were not mentioned in the resarch plan...seemed focused primarily on mosquitoes (some tick work). With the descriptive context that "This proposal has purposefully been written to define the project in broad research terms." the proposal seems appropriate and could be successful if adequate collaboration between academic, state/federal, and professional vector control programs is achieved. Multi-state collaboration between different types of "experts" to benefit public health is a plus if the leadership team has a high level of organization (reviewer cannot see composition of team, but understand this is for the northeast region of the United States).

We thank the reviewer for their feedback on the proposal. The reviewer is correct that the project aims intentionally were written broadly to encompass the scope of work of the project members' labs and to ease new members' participation in the project. Part of our proposed outreach efforts involve engaging with vector control programs to facilitate the uptake of our applied research findings. In the revised proposal, we clarified the management structure and the species addressed as part of the research plan.

Reviewer 2:

Overall, an established team with a track record of meaningful work/productivity. The framework for collaboration is well defined and they have procedures for shared ownership and responsibility. The scientific goals/objectives are important and this team is well suited to achieve them. I would have liked to have seen clear milestones. However, the objectives are clear and valuable (needed). This project should continue and in many respects they provide a role model for other multistate collaborations related to vector-borne diseases. The team may consider leveraging/coordination with the CDC's Centers of Excellence that reach into their states/region.

We thank the reviewer for their consideration of the proposal. We agree that coordination with the CDC's Centers of Excellence will improve the reach of the project, and we included language to this effect in the revised proposal.

Reviewer 3:

The rationale for a multi-state project is sound and justifiable. Each sub-objective has strengths and weaknesses.

We thank the reviewer for their detailed feedback, and have responded to comments on individual aims below.

(1.1) Predict and model the distribution and density of the vectors of public and veterinary health importance

Strengths – A robust network of sampling sites and diverse vectors will be studied.

Weaknesses – Details of the prediction / modelling methods are lacking. Are these ecological niche models? Details of trapping are not provided. If these are light traps then they will be biased toward certain species.

We omitted some details of the modeling and trapping efforts because multiple labs are engaged in this research aim, which may have slightly different protocols depending on the species of interest. We agree with the reviewer that different forms of trapping and surveillance have their own sampling biases and we will consider this in carrying out the proposed research.

(1.2) Improve the efficiency of surveillance programs vis-a-vis vector trapping and diagnostics.
Strengths – This objective applies modern techniques for surveillance.
Weaknesses - next generation sequencing will likely be prohibitively expensive for pathogen surveillance. The results will yield every potential pathogen, which may cause undue panic if used as an actual public alert surveillance system.

We agree that the next generation sequencing is expensive for pathogen surveillance, although the cost of the technology is declining and we feel this is an important tool that can be used by researchers. As noted by the reviewer, it is important to consider how the findings are communicated to the public to prevent unnecessary anxiety about pathogen exposure risk. This is encompassed in our proposed outreach plan.

(2.1) Determine the phenology, habitat, and climatic requirements of historical, extant, and emerging vectors, hosts, and pathogens using historical and contemporary datasets. Strengths – An ambitious objective to understand how the pathosystems currently function and how key interactions may be changing.

Weaknesses – No details are provided on how the hosts or vectors will be quantified. Given that diverse wildlife (birds and mammals) are likely important as hosts for diverse pathogens (Flaviviruses, Alphaviruses, Orthobunyaviruses) the potential targets are very broad. Details of the modelling are not provided.

Again, we omitted some details of the modeling efforts and the quantification of hosts and vectors because multiple labs are engaged in this research aim, which may have slightly different approaches depending on the species of interest.

(2.2) Define physiological, ecological, and behavioral drivers of transmission of vector-borne pathogens of public health importance.

Strengths – An ambitious objective to manipulate key environmental factors and measure response in vector-borne disease ecology.

Weaknesses – A very large number of factors are proposed that will have confounding impacts on the biological traits listed. Investigating impacts of any one of these would be challenging to manage adequately. High risk.

This aim intentionally was written broadly to encompass the interests of the multiple labs involved in the proposed project. Each of these factors will be studied individually across different labs, using observational and experimental approaches to disentangle the impacts of the environmental factors on biological traits.

(2.3) Evaluate the evolutionary relationships and genetic diversity of vectors and pathogens they transmit.

Strengths – An interesting empirical assessment of genetic drivers of virulence. Weaknesses – The application of this work is unclear. Apart from genetic modification of pathogens (which will not be permitted for release) how will this work impact vector-borne disease management?

We agree with the reviewer that pathogens with genetic modifications that enhance pathogenicity will not be permitted for release. However, the identification of genes or loci potentially associated with pathogenicity can lead to the identification of targets for attenuation of these pathogens. Likewise, the identification of differentially methylated regions (DMRs) that are potentially associated with vector capacity can be targeted with newly developed CRISPR/Cas-9DM to define their role in pathogen transmission and for the creation of vector populations with reduced vectorial capacity. We have revised the aim to add this information.

(3.1) Screening of natural products for new chemical control tools with novel modes of action (insecticides/acaricides, repellents).

Strengths – Potentially achievable goals of identifying new active compounds for control of medically important arthropods.

Weaknesses - None identified.

Thank you for these comments.

(3.2) Discovery of novel control targets by investigating vector-microbe interactions and molecular/physiological mechanisms.

Strengths – May provide novel insights in vector physiology.

Weaknesses - it is unclear how the "molecular, biochemical, and physiological techniques" will explicitly lead to a better understanding of the processes associated with pathogen transmission and vector survival, as stated. The investigators have not demonstrated how measuring electrical signals during probing (when an arthropod vector takes a blood meal), even if correlated with specific feeding behaviors, are helpful toward reducing or blocking transmission.

We revised this paragraph in the proposal to clarify the point raised by the reviewer.

(3.3) Develop countermeasures against flavivirus infection and transmission. Strengths - Drugs that can be effective against flaviviruses are needed. The comprehensive approach, terminating in preclinical studies should determine whether the compounds have anti-flaviviral activity.

Weaknesses - May or may not lead to effective treatment.

Yes, it is a challenging task, but is essential for developing some means to combat these viral epidemics. This proposal has selected a very conserved target in the flavivirus species. Using cutting-edge technologies such as Structure-based and combined with AI based design, it will greatly increase the chances of success. We are confident that we can achieve our goals.

(3.4) Develop high throughput methods to detect insecticide resistance in Culex vectors of West Nile virus that can be combined with virus surveillance.

Strengths – Integrating insecticide resistance monitoring into ongoing vector and pathogen surveillance is a worthy goal.

Weaknesses – The methods provided are more about differentiating tow species of Culex than about high throughput assays for determining insecticide resistance.

We believe the reviewer's comments correspond to objective 3.5 rather than objective 3.4.