Master GIZMAT (M2)

Gestion Intégrée des Zoonoses et Maladies

Animales Tropicales

Rapport de stage

Qualitative risk analysis of the potential introduction of heartwater disease to the united states from the Caribbean

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Abstract:

This study investigates the qualitative risk of introducing heartwater disease, caused by the rickettsial bacterium Ehrlichia ruminantium, into the United States from the Caribbean. Heartwater disease, transmitted primarily by ticks of the genus Amblyomma, poses a significant threat to livestock health and agricultural economies. Endemic to sub-Saharan Africa and certain Caribbean islands, the introduction of this disease to the United States could have severe repercussions for the livestock industry, wildlife, and associated economic sectors.

Utilizing qualitative risk assessment, expert elicitation, and scenario modeling, this research explores potential pathways and influencing factors for heartwater introduction. A diverse panel of 16 experts, including entomologists, epidemiologists, and wildlife specialists, provided insights into the study. Key potential pathways examined include the importation of ticks via migratory birds, domestic animals, and illegal wildlife trade. This study employs advanced statistical methods to evaluate these risks, aiming to inform and enhance biosecurity measures and disease prevention strategies

Key words: Heartwater, Caribbean, Qualitative risk analysis, Expert elicitation, Scenario modeling

Résumé:

Cette étude examine le risque qualitatif d'introduction de la maladie de la cowdriose, causée par la bactérie rickettsiale Ehrlichia ruminantium, aux États-Unis depuis les Caraïbes. La cowdriose, transmise principalement par des tiques du genre Amblyomma, constitue une menace importante pour la santé du bétail et les économies agricoles. Endémique en Afrique subsaharienne et dans certaines îles des Caraïbes, l'introduction de cette maladie aux États-Unis pourrait avoir de graves répercussions sur l'industrie du bétail, la faune et les secteurs économiques associés.

En utilisant une évaluation qualitative des risques, une consultation d'experts et une modélisation de scénarios, cette recherche explore les voies potentielles et les facteurs influençant l'introduction de la cowdriose. Un panel diversifié de 16 experts, comprenant des entomologistes, des épidémiologistes et des spécialistes de la faune, a fourni des informations pour l'étude. Les principales voies potentielles examinées incluent l'importation de tiques par les oiseaux migrateurs, les animaux domestiques et le commerce illégal d'animaux sauvages. Cette étude utilise des méthodes statistiques avancées pour évaluer ces risques, dans le but d'informer et d'améliorer les mesures de biosécurité et les stratégies de prévention des maladies

Mots-clés : Cowdriose, Caraïbes, Analyse qualitative des risques, Consultation d'experts, Modélisation de scénarios

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I. Introduction

Heartwater disease, caused by the bacterium Ehrlichia *ruminantium* and transmitted primarily by ticks of the genus *Amblyomma*, poses a significant threat to livestock health and agricultural economies. Endemic to sub-Saharan Africa and certain Caribbean islands, the introduction of Heartwater disease into the United States could have severe repercussions for the livestock industry, wildlife, and associated economic sectors.

The global movement of animals and goods heightens the risk of transboundary animal diseases. The proximity and trade relationships between the Caribbean and the United States, in particular, emphasize potential pathways for the introduction of Heartwater disease. We aim in this study to conduct a qualitative risk analysis to assess the likelihood of Heartwater disease being introduced to the United States from the Caribbean region.

This study employs expert elicitation and advanced statistical methods to evaluate the risks associated with different introduction scenarios. The scenarios considered include the importation of ticks through various means such as migratory birds, domestic animals, and illegal wildlife trade. By This analysis seeks to inform and enhance biosecurity measures and disease prevention strategies.

II. Context of the study

II.1. Overview of Heartwater Disease

Heartwater is a noncontagious tick-borne disease primarily affecting domestic ruminants and wildlife. It is caused by the rickettsial agent *Ehrlichia ruminantium*, transmitted through the bites of infected ticks, particularly those of the *Amblyomma* genus. The disease was first identified in livestock in South Africa in 1838, when it spilled over from wildlife to domestic sheep. Heartwater is characterized by a range of clinical manifestations, from subclinical infections to severe, acute disease that can lead to death (Deem et al., 1996).

II.2. Clinical Manifestations

The clinical presentation of heartwater varies significantly among affected species. It can range from mild, transient fever in subclinical cases to severe neurological signs and rapid death in acute cases. In domestic cattle, the disease often manifests as profuse, fetid, hemorrhagic diarrhea. The acute form is marked by a rapid onset of symptoms, including fever, tachypnea, and neurological signs such as hyperesthesia and muscle tremors. The incubation period for the disease can vary based on several factors, including the species affected, the route of infection, and the strain of *E. ruminantium* involved (Deem et al., 1996).

II.3. Vector Ecology and Emerging Vectors

Understanding the potential expansion of heartwater in the Caribbean and the risk of its introduction from these islands to the United States requires a One Health perspective due to its multifaceted nature and the complexity of the disease's epidemiology. Similar to other tick-borne diseases, comprehending vector ecology alongside identifying potential emerging vectors is crucial for proper risk assessment. *Amblyomma variegatum* is known to be the vector of heartwater in the Caribbean region (Camus and Barré, 1992). Its capability for complete trans-stadial transmission of the heartwater infectious agent, alongside its wide geographic distribution, makes it the most studied vector for the epidemiology of the disease (Bezuidenhout, 1987).

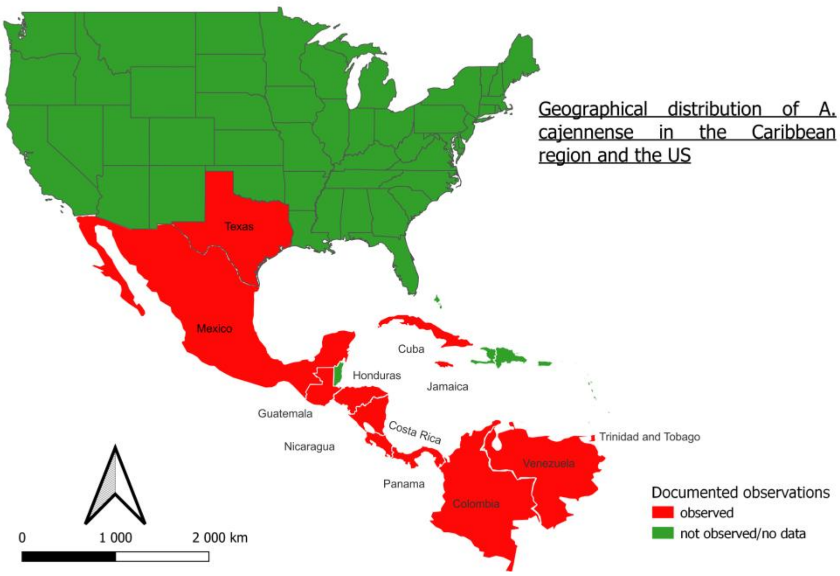

Figure 1: Geographical distribution of *A. cajennense* in the Caribean region

Nevertheless, the study of the vectorial competence of new tick species may reveal new potential vectors present in the region. In fact, *Rhipicephalus microplus* has recently been identified as a vector of *E. ruminantium* in western Africa (Some et al., 2023). It is also the case for *Amblyomma dissimile* and *A. cajennense* (Jongejan, 1992; Walker and Olwage, 1987) (figure 1 & 2). In the absence of research regarding the competence of other non-proven vectors of *E. ruminantium*, their possible ability to transmit heartwater should not be overlooked.

II.4. Prevalence and Transmission in the Caribbean

Heartwater is primarily found in mainland Guadeloupe, Marie Galante, and Antigua, where the prevalence of *A. variegatum* ticks is notably high. Studies indicate that these ticks are present on approximately 45% of farms, with about 15% of these ticks infected with *E. ruminantium* (Vachiéry et al., 2011). The transmission of *E. ruminantium* occurs through the bites of infected *Amblyomma* ticks. The pathogen multiplies in the tick's intestinal cells and salivary glands, allowing it to be transmitted to the host during a blood meal. The disease can be transmitted trans-stadially, meaning that ticks can carry the pathogen into subsequent developmental stages, which complicates control efforts (Vachiéry et al., 2011).

II.5. Role of Wildlife in Transmission

Highlighting the role of wildlife in the transmission of the disease and identifying susceptible and reservoir wild species in the western hemisphere is essential for evaluating the risk associated with the maintenance of a sylvatic cycle. Wildlife can play a significant role in the maintenance and dissemination of tick populations, alongside their potential for being asymptomatic carriers of pet and livestock infectious diseases (Oberem and Bezuidenhout, 1987).

II.6. Risk of Spread Beyond the Caribbean

The potential for heartwater to spread beyond the Caribbean is a significant concern. The introduction of infected cattle from the Caribbean to the American mainland poses a risk of establishing a permanent infection cycle, especially given the presence of susceptible tick vectors and abundant ruminant populations (Vachiéry et al., 2011).

II.7. Eradication Programs and Surveillance Networks

In response to the threat posed by *A. variegatum*, eradication programs have been implemented in some English-speaking Caribbean islands. These programs aim to limit the spread of ticks and, consequently, the incidence of heartwater. However, challenges remain due to the high infestation rates in many islands (Vachiéry et al., 2011). The CaribVET Caribbean Animal Health Network has recommended the development of surveillance schemes for heartwater in islands heavily infested with *A. variegatum*, particularly where tick monitoring is not feasible. Since 2010, a surveillance network for nervous disorders (RESPANG) has been established in Guadeloupe, focusing on heartwater. This initiative aims to better characterize the disease, analyze risk factors, and improve communication with farmers regarding prevention and control measures (Vachiéry et al., 2011).

III. Epidemiology of the disease:

III.1. Tick ecology:

Ticks of the genus *Amblyomma* are of great epidemiological interest because of their trixenous life cycle (i.e. have to feed on three different hosts to successfully accomplish their life cycle) through which there is a potential transmission of pathogens from one host to the next one (Guglielmone and Nava, 2010).

Studies have brought to light the extent of the host range of *Amblyomma* species, showing that most ticks of the genus *Amblyomma* tend to have a large variety of different vertebrate hosts of different species and classes, an interesting example would be that of *A. dissimile* immatures that can be found both on reptiles, amphibians and mammals. The expansion of their host range reaches its optimum during immature stages this not only could promote the infection of the ticks during an early stage of life but also shows the resilience and plasticity of this genus in case of the absence of its preferred host notably in a context of introduction to a new exotic area (McCoy et al., 2013).

The nymphal stage comes across as particularly relevant for the epidemiology of the disease for *Amblyomma* spp., due to the absence of trans-ovarial transmission of the infection in this genus. The infection can only occur after feeding on an infected animal from larval stage onward (Bezuidenhout, 1987), and it is at the nymphal stage that an infected tick has more chances of infesting a non-ruminant host (extended host range) that even if not susceptible to the heartwater might serve as transport for its agent with its own movement.

Infection in *Rhipicephalus microplus* on the other hand follows a different scheme, because it is a monoxene species (i.e. accomplish its full cycle on the same host) infection can only occur at the larval stage by feeding on an infected host or through trans-ovarial transmission from adult females to their offspring (Some et al., 2023), the larval stage is therefore the most relevant to study when assessing the risk of transmission of *E. ruminantium* by this species. Studies have reported the infestation of birds and non ruminant mammals with immature specimens of this species but there is little to no record of adult *R. microplus* infesting hosts other than cattle which might suggest that this species is incapable of accomplishing a successful life cycle on non cattle hosts (Barré, 1997)

III.2. Proven to be competent vectors of heartwater in the Caribbean region:

On the Caribbean islands *A. variegatum* is described as being the most important vector of heartwater therefore making it the most studied and best-known tick species in the region, we must however acknowledge the fact that there are other species experimentally proven to be competent in the Caribbean as well as on the American continent (central and northern). *Amblyomma dissimile* is a herpetophile (i.e. infests reptiles for the most part) tick species that has been proven to be capable of transmitting the heartwater disease agent *E. ruminantium* to goats in the context of an experimentation (Jongejan, 1992) , *A. dissimile* alongside another closely related species *Amblyomma rotundatum*'s presence in the Caribbean, South and

Central American regions is well documented (Morel, 1967) (figure 2), both of these species are also successfully established in Florida and have been recorded on free ranging wild reptiles and amphibians (Oliver et al., 1993; Corn et al., 2011) although these two neotropical ticks are specialized in reptilian and amphibian hosts, studies revealing the extent of *Amblyomma* spp. ticks' host range showed that they have been recorded on a panoply of vertebrate hosts including cattle, goat, sheep and pig (Guglielmone and Nava, 2010; Nava and Guglielmone, 2013). Another competent vector found in Central and South America and the United States is *Amblyomma maculatum* (Walker and Olwage, 1987), a tick known for infesting small mammals and birds and can be found on domestic and wild ruminants (Hixson, 1940). *A. maculatum* proved to be as competent as *A. variegatum* at the transmission of *E. ruminantium* in an experimental environment (Mahan et al., 2000) this highlights its epidemiological relevance.

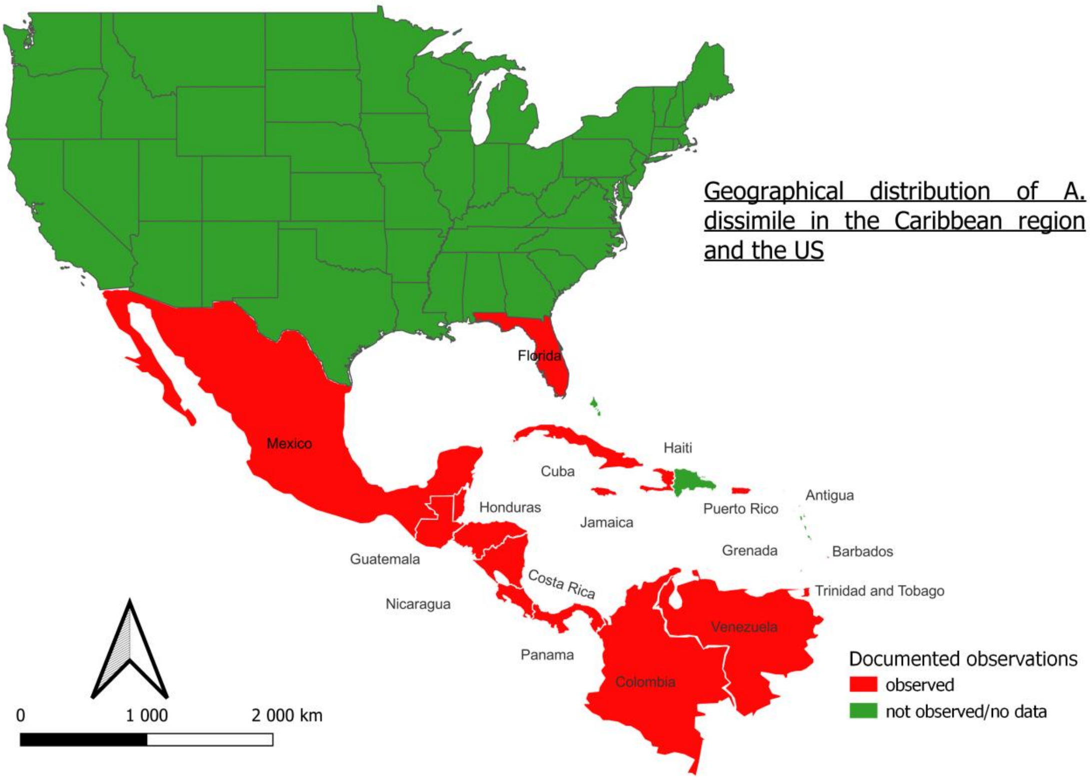

Figure 2: Geographical distribution of *A. dissimile* in the Caribbean region

The study of lesser known vectors could contribute a lot to the understanding of heartwater epidemiology, considering the lack of studies on the susceptibility of wild fauna in the Caribbean and the American continent. Many wild African ruminants demonstrate a clinical infection *e.g.* springbuck *Antidorcas marsupialis* or can be subclinical carriers e.g. Blesbuck *Damaliscus dorcas phillipsi* (Oberem and Bezuidenhout, 1987) although a mention of the white tailed deer *Odocoileus virginianus* being clinically susceptible to heartwater (Logan, Linda L., personal communication, 1986, cited in Oberem and Bezuidenhout, 1987) there is no trial-based evidence that could support this mention. Little to no research has been conducted on reptiles' susceptibility to heartwater, one tortoise species, the leopard tortoise *Geochelone pardalis* have been identified as a subclinical carrier of *E. ruminantium* infection (Peter et al., 2002). Moreover the occurrence of a specific epidemiological event in 2002 and 2003 might suggest more than just subclinical infection in reptiles, clinical manifestations similar to those encountered in *E. ruminantium* infected domestic ruminants and mortality have been documented in 7 different African snake species in Florida. This heartwater-like syndrome has been linked to an emerging *E. ruminantium* relative organism (Kiel et al., 2006). These information leads to the belief that reptiles might be subject to *E. ruminantium* infection while being asymptomatic therefore representing a neglected reservoir susceptible of entertaining a sylvatic cycle of the disease as tick mediated transmission is possible to other domestic and wild animals.

After thorough review of the literature available, we identified four main potential pathways for the introduction of heart water disease to the United States of America namely, through:

- The introduction of infected ticks *via* wild animal legal and illegal transport
- The introduction of infected ticks *via* migratory birds
- The introduction of infected ticks *via* domestic pet dog transport into the U.S.
- The trade and trafficking of domestic ruminants in the Caribbean region and to the U.S.

III.3. Potential Introduction Scenarios

III.3.a. Transport of Wild Live Animals and US Regulation

Wildlife import has always been associated with the risk of introducing exotic diseases and ticks. The American wildlife market represents 80% of the world's live reptile import/export trade. There are no published WOAH (World Organisation for Animal Health) manuals or recommendations regarding reptile and amphibian international trade. Therefore, in 1999, the USDA issued recommendations for controlling tick introduction in reptiles, proposing a tick control protocol that includes proper acaricide treatment for imported reptiles (Burridge and Simmons, 2003). Additionally, the USDA issued a complete ban on the import of three African tortoise species: the Leopard tortoise (*Geochelone pardalis*), African spurred tortoise (*Geochelone sulcata*), and Bell's hingeback tortoise (*Kinixys belliana*), due to the discovery of heartwater-infected exotic Amblyomma spp. ticks on Leopard tortoises imported from Africa to Florida (Burridge et al., 2000). Although acaricide treatment of imported reptiles is highly recommended by the USDA, it remains a non-obligatory requirement for the import of live reptiles to the United States (USDA, 2024a).

III.3.b. Migratory Birds

Bird migration plays a significant role in tick dispersal into the US. It is estimated that 19 million exotic neotropical ticks are imported with bird migrations into the US each spring (Tsao et al., 2021). Birds such as the cattle egret (*Bubulcus ibis*), which frequently infest with ticks like *A. variegatum* and *R. microplus*, are known for their feeding habits in close proximity to cattle. Cattle egrets established themselves on the American continent and the

Caribbean in the mid-20th century and have been implicated in the dispersal of the tropical bont tick (*A. variegatum*) throughout the Caribbean islands (Barré, 1997).

Some populations of this species exhibit long-distance foraging behavior, while others, such as the northern American ones, can be fully migratory. Cattle egrets can travel up to 3,100 km during migration, with an estimated average travel distance of 1,750 km in 32 hours of consecutive flight at an average speed of 50 km/h. This estimation is supported by documented observations of flight speeds of 43 km/h over land (Bridgman et al., 1998; BirdLife International, 2024). Therefore, it is feasible for a cattle egret to travel from Guadeloupe to Florida in 72 hours, a period shorter than the 5-10 days required for the engorgement of *Amblyomma variegatum* nymphs on cattle egrets (Kasari et al., 2010). Outside of migration periods, juvenile males of this species are known to wander away from the colony, traveling up to 5,000 km (Browder, 1973), potentially contributing to the dissemination of ticks and tick-borne diseases, including heartwater, in the Caribbean-American region. Other migratory birds, including several passerine species, can also host multiple *Amblyomma* species, including A. maculatum ticks nymphs and larvae (Cohen et al., 2015).

III.3.c. Pet Transport and US Registration

Domestic dogs, susceptible to infestation by *Amblyomma* spp. and *R. microplus* immatures, might also be subject to *E. ruminantium* infection (Allsopp and Allsopp, 2001). Pet dogs returning to the US after traveling in the Caribbean or Central America might serve as transport hosts for heartwater-infected ticks contracted during their travel. USDA regulations regarding the entry of privately-owned returning dogs into the US do not require inspection for ticks or treatment for external parasites (USDA, 2024b), unlike dogs imported for adoption or resale purposes, which must be parasite-free (USDA, 2024c). Thus, dogs traveling from heartwater-infected areas represent a potential introduction route, especially since air transport can facilitate the rapid return of the animal to US territory before the complete engorgement and detachment of *A. variegatum* nymphs, which typically takes around 6.5 days when feeding on dogs (Yonow, 1995).

III.3.d. Ruminant Trade

US ruminant livestock imports consist of a few million heads of live cattle annually, primarily from Mexico and Canada (USDA, 2024d). Cattle imported from Mexico undergo mandatory anti-tick dip treatment and border inspection for ticks (USDA, 2024e), reducing the chances of introducing infected ticks. However, asymptomatic heartwater carriers could pose a threat, as no heartwater testing is required, and veterinary inspections before export rely only on detecting symptomatic clues of communicable diseases. Chronic asymptomatic infection with *E. ruminantium* has been documented in domestic ruminants for up to 8 months after spontaneous healing (Andrew and Norval, 1989). Thus, asymptomatic carriers on US soil could infect endemic or exotic vector species (e.g., *A. maculatum*), potentially maintaining the infectious cycle.

Illegal cattle trafficking in the region raises concerns about heartwater introduction risks. InSight Crime reports describe networks smuggling cattle bred in Central American countries (Honduras, Guatemala, and Nicaragua) into Mexico. These networks practice illegal cattle ranching in protected natural reserves, causing deforestation and harm to indigenous communities. The smuggled cattle are laundered into Mexico's legal beef production industry (Dittmar and Asmann, 2022), posing a significant risk of introducing infectious cattle diseases and parasites, including heartwater, to the United States if undetected infections reach US farms through legal cattle imports from Mexico. The prevalence of zebu breeds in smuggled cattle, which are more resistant and likely to carry asymptomatic infections, exacerbates this risk.

IV. Material and Methods

IV.1. Heartwater introduction to the US scenario Model

A comprehensive review of the literature on the epidemiology of heartwater disease and vector ecology in the Caribbean region was conducted. This review included previous studies on the potential dissemination of *Ehrlichia ruminantium* and its vectors within the Caribbean and to the United States. Based on this, we developed a scenario tree model that outlines the sequence of events that could lead to the emergence of heartwater disease in continental United states (annexe 1)

IV.2. Expert elicitation

IV.2.a. Questionnaires design

Using this model, we designed three separate questionnaires on LimeSurvey® to target distinct panels of experts, ensuring the questions were tailored to their specific areas of expertise:

- Epidemiology of heartwater in the Caribbean
- Tick ecology and tick-borne diseases
- Wildlife and wildlife diseases

The use of an online survey allowed us to reach a broad pool of experts across different geographical locations and enabled the inclusion of the Spanish translation to accommodate non-English-speaking experts. Additionally, it facilitated efficient data management and reception.

To assist the experts, we provided visual representations of U.S. reptile imports from the Caribbean and the geographical distribution of heartwater vectors in the region (annexes 2 & 3).
Each expert was asked to indicate their estimation of the probability of occurrence of events using the following ordinal scale (Table 1):

Table 1: qualifiers and ordinal scale for probabilities estimation

Qualifier	**Nil**	**Very Low**	**Low**	**Moderate**	**High**	**Very High**
Ordinal Scale	0	1	2	3	4	5

This scale, adapted from the one described by AFSAA (2008), was simplified to facilitate expert estimation during the survey process.

Confidence in answer:

Experts were also asked to evaluate their confidence in each answer on the following scale:

- Not confident
- Somewhat confident
- Confident

- Very confident

IV.2.b. Expert Recruitment

Experts were initially recruited via email through an invitation to participate in our elicitation study, distributed on the "Ticks and Tick-borne Diseases" mailing list of CaribVET. This list includes regional experts in tick-borne diseases, including heartwater. Additionally, we employed a snowball recruitment strategy, requesting participating experts to recommend additional specialists and assist in disseminating information about the study within their professional networks.

IV.3. Data Analysis

In this study, we employed the mathematical approach to aggregate expert opinions, a method that combines individual expert judgments using a structured mathematical formula. This approach was chosen for its ability to eliminate cognitive biases that often arise in group settings, such as groupthink and leadership influence **(Marozzi et al., 2022)**. By collecting and aggregating expert judgments independently, we ensured consistency and objectivity in the data. To address the inherent subjectivity in selecting an aggregation formula, we utilized two weighting methods allowing for the exploration of multiple aggregation methods and a sensitivity analysis to check for the robustness of the results. This methodology provides a reliable framework for synthesizing expert insights.

IV.3.a. Weighting of the experts' estimations

Confidence levels were converted into numeric values using two scales:

- **Linear Scale:**
    - Not confident: 1
    - Somewhat confident: 2
    - Confident: 3
    - Very confident: 4
- **Non-linear Scale:**
    - Not confident: 1
    - Somewhat confident: 3
    - Confident: 4
    - Very confident: 10

Experts' estimations were weighted in favor of the most confident answers using the following methods:

- Linear weighting : estimation * confidence level (linear) / 4 (maximum confidence)
- Non-linear weighting : estimation * confidence level (non-linear) / 10 (maximum confidence)

IV.3.b. Expert Opinion Aggregation

To aggregate the expert opinions, we performed a normality test (Shapiro-Wilk test) on all variables. For normally distributed variables, the mean was used as the representative value.

For non-normally distributed variables, the median was used. In cases where the median equaled zero due to the distribution, a trimmed mean (excluding the top and bottom 10% of values) was applied to minimize the effect of outliers.

IV.3.c. Comparison of Weighting Methods

To compare the outcomes of the two weighting methods, we conducted a Kruskal-Wallis test. This non-parametric test was chosen due to its suitability for comparing the medians of ordinal data across multiple groups without assuming a normal distribution. The test provided a robust comparison of the aggregated opinions, helping identify any statistically significant differences resulting from the choice of weighting method.

IV.3.d. Sensitivity Analysis

A comprehensive sensitivity analysis was conducted to assess the robustness of the aggregated risk estimates. This involved running the aggregation process multiple times (100 runs), each time applying randomly assigned confidence levels to simulate different scenarios. A stability index was calculated for each variable to measure the variability in aggregated results relative to the number of runs, providing a quantitative measure of the sensitivity of the aggregated estimates to changes in input assumptions.

IV.4. Release Risk Calculation

Release risk refers to the likelihood that a pathogen could be introduced into the environment through various pathways, such as the importation of infected animals, vectors like ticks, or contaminated materials (AFSAA, 2008) In this study, the release risk specifically addresses the potential for *Ehrlichia ruminantium* to be introduced into the U.S. through imported livestock, wildlife movement, or infected vectors.

The qualitative release risk for each scenario branch of our event model was calculated using the following multiplication table (table 2) derived from AFSAA (2008):

Table 2: table of multiplication of qualitative expert estimations

Qualifier	**Nil**	**Very Low**	**Low**	**Moderate**	**High**	**Very High**
Nil	Nil	Nil	Nil	Nil	Nil	Nil
Very Low	Nil	Very Low	Very Low	Very Low	Very Low	Very Low
Low	Nil	Very Low	Low	Low	Low	Low
Moderate	Nil	Very Low	Low	Moderate	Moderate	Moderate
High	Nil	Very Low	Low	Moderate	High	High
Very High	Nil	Very Low	Low	Moderate	High	Very High

By multiplying the aggregated expert estimations for each step in the scenario branch, we obtained the risk estimation for specific scenarios.

V. Results

V.1. Expert Panel Description

In total, 16 regional and international experts completed our expert elicitation questionnaires. The distribution by discipline was 6 entomologists, 5 epidemiologists, and 5 wildlife experts.

The majority of our expert panel consisted of veterinarians, some specializing in epidemiology or parasitology. We also included experts in entomology, parasitology, one-health, and wildlife specialists (Figure 3).

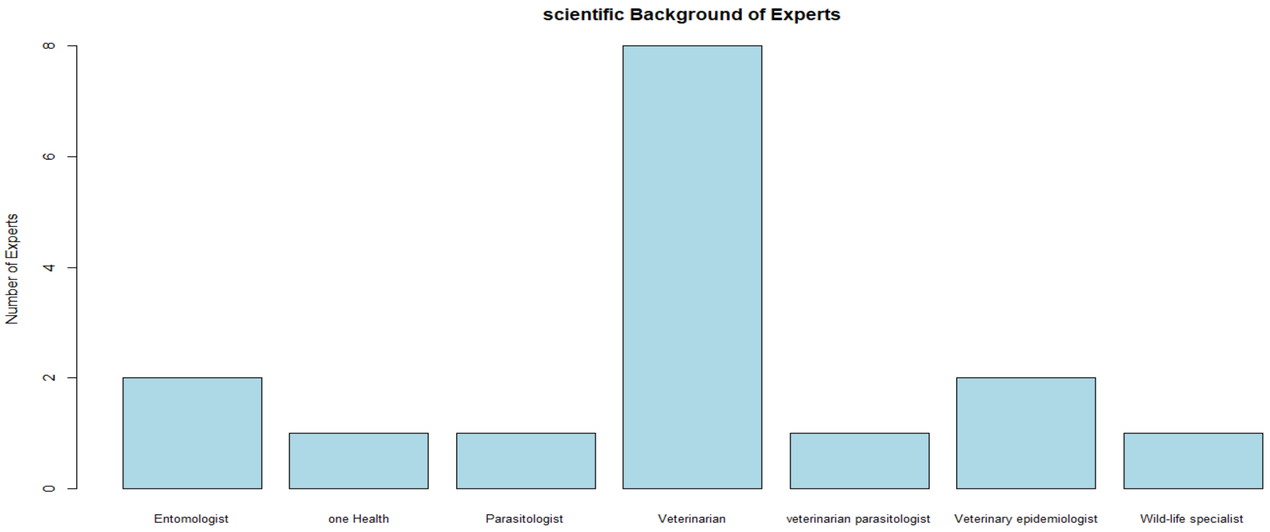

Figure 3: Distribution of expert panel members by discipline

The majority of contributions came from experts with academic backgrounds. We also received valuable input from experts at regional and international governmental and private research institutes or organizations, as well as from one private veterinarian working in the field (Figure 4).

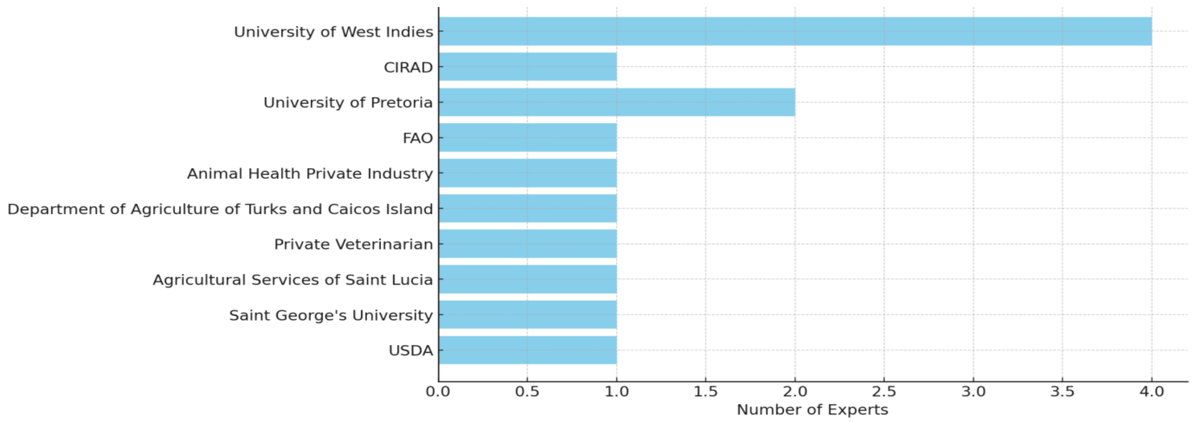

Figure 4: distribution of expert by institute of provenance

Experience working on tick borne diseases (Figure 5):

- **Entomology Expert Panel**: This panel has the highest average years of experience, with a global mean of around 25 years. The green bar significantly towers over the other two, indicating that entomologists involved have extensive experience in tick-borne diseases.
- **Epidemiology Expert Panel**: This panel shows the lowest average years of experience, with the mean slightly under 5 years. The blue bar is relatively short, indicating less experience compared to the other panels.
- **Wild Expert Panel**: The red bar indicates that experts in this panel have a mean experience slightly higher than the epidemiologists, around 5 years. However, this is still much lower than the experience level of the entomologists.

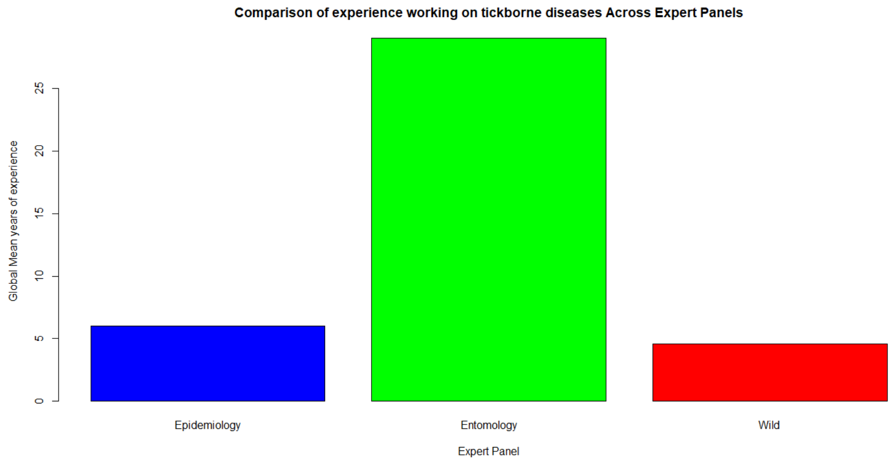

Figure 5: average years of experience working on tick-borne diseases among the three different expert groups

V.2. Reception of Experts' Estimations

Overall, the reported mean confidence level for all three groups falls within the 2 to 3 range. This suggests that, on average, the experts in each group feel either somewhat confident or confident in their answers (Figure 6).

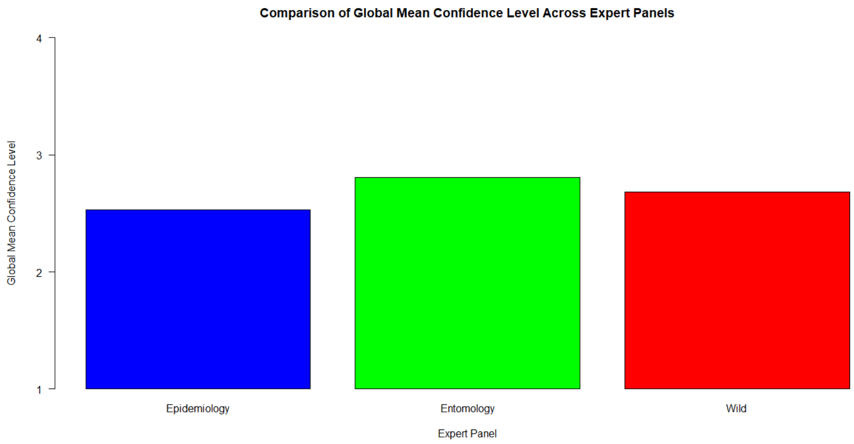

Figure 6: Mean confidence levels of expert groups

V.3. Weighting and Aggregation Methods Sensitivity Analysis

V.3.a. Kruskal-Wallis Test:

The test yielded a p-value of 0.13, which is above the conventional threshold of 0.05. Consequently, the null hypothesis, which states that there is no significant difference between the outcomes of the two weighting methods, cannot be rejected. This suggests that the choice of weighting method does not significantly influence the aggregated experts' estimations in this study.

V.3.b. Sensitivity Analysis:

For each variable, a stability index was calculated, providing a quantitative measure of how sensitive the aggregated estimates were to changes in the input assumptions. A lower stability index indicates greater robustness of the aggregated estimates, while a higher index suggests more variability and sensitivity to changes.

The stability indexes for each expert group were as follows:

- **Entomology Group:** 0.21 for the linear weighting method and 0.26 for the nonlinear method.
- **Epidemiology Group:** 0.20 for the linear method and 0.25 for the nonlinear method.
- **Wildlife Experts:** 0.26 for the linear method and 0.30 for the nonlinear method.

These results indicate that the nonlinear weighting method generally resulted in slightly higher variability in the aggregated results across all groups, suggesting that it may be more sensitive to changes in input assumptions compared to the linear method.

V.4. Heartwater Release Risk Analysis

V.4.a. Cattle Egret Scenario:

The release risk estimates for *Amblyomma variegatum* and *Rhipicephalus microplus* infesting cattle egrets were evaluated across various conditions (Table 3).

- *Amblyomma variegatum:* When originating from endemic regions, the release risk was estimated to be low across all U.S. climate conditions (tropical, continental, dry, temperate) and for both domestic and wild hosts. When originating from non-endemic regions, the release risk was consistently very low under the same conditions.
- *Rhipicephalus microplus:* Regardless of the provenance (endemic or non-endemic regions), the release risk was estimated to be very low across all climate conditions and potential hosts (domestic and wild).

These results suggest that the overall release risk for ticks infesting cattle egrets, particularly *Rhipicephalus microplus,* is generally very low when considering their potential survival and host interactions in U.S. climates.

Table 3: Release risk estimates for cattle egret scenario

tick species infesting cattle egret	provenance of the tick	tick survival in US climate	potential host	Release risk estimate
Amblyomma variegatum	endemic region	tropical	domestic	low
			wild	low
		continental	domestic	low
			wild	low
		dry	domestic	low
			wild	low
		temperate	domestic	low
			wild	low
	non endemic region	tropical	domestic	very low
			wild	very low
		continental	domestic	very low
			wild	very low
		dry	domestic	very low
			wild	very low
		temperate	domestic	very low
			wild	very low
Rhipicephalus microplus	endemic region	tropical	domestic	very low
			wild	very low
		continental	domestic	very low
			wild	very low
		dry	domestic	very low
			wild	very low
		temperate	domestic	very low
			wild	very low
	non endemic region	tropical	domestic	very low
			wild	very low
		continental	domestic	very low
			wild	very low
		dry	domestic	very low
			wild	very low
		temperate	domestic	very low
			wild	very low

V.4.b. Domestic Dogs Travel Scenario:

The release risk for ticks infesting domestic dogs under different travel scenarios was evaluated considering whether the dogs were wearing an anti-tick collar or not (Table 4).

- **Tick Species:** *Amblyomma variegatum* and *Rhipicephalus microplus*
- **Tick Survival in U.S. Climate:** Tropical, Continental, Dry, and Temperate

Wearing an Anti-Tick Collar: The release risk for both *Amblyomma variegatum* and *Rhipicephalus microplus* was estimated to be very low across all climate conditions (tropical, continental, dry, temperate).

Not Wearing an Anti-Tick Collar: Similarly, the release risk remained very low for both tick species across all climate conditions regardless of whether the dogs were protected by an anti-tick collar or not.

These results suggest that the release risk for ticks associated with domestic dogs traveling to the U.S. is consistently very low irrespective of the use of an anti-tick collar and the climate conditions.

Table 4: Release risk estimates for domestic dogs travel scenario

	tick species infesting a dog	tick survival in US climate	Release risk estimate
wearing an anti tick collar	Amblyomma variegatum	tropical	very low
		continental	very low
		dry	very low
		temperate	very low
	Rhipicephalus microplus	tropical	very low
		continental	very low
		dry	very low
		temperate	very low
not wearing an anti-tick collar	Amblyomma variegatum	tropical	very low
		continental	very low
		dry	very low
		temperate	very low
	Rhipicephalus microplus	tropical	very low
		continental	very low
		dry	very low
		temperate	very low

V.4.c. Illegal Ruminant Trade/Trafficking Scenario:

Table 5: Release risk estimates for illegal ruminant trade/trafficking scenario

	Release risk estimate
introduction of undetected clinically infected ruminant through trafficking	low
introduction of subclinically infected ruminant through trafficking	low
introduction of undetected clinically infected ruminant through import from Mexico after laundry	very low
introduction of subclinically infected ruminant through import from Mexico after laundry	low

These results indicate that the risk of introducing infected ruminants through trafficking or illegal importation varies between low and very low depending on the scenario, with laundering reducing the risk slightly for clinically infected ruminants (Table 5).

V.4.d. Wild Amphibians Import from the Caribbean Scenario:

Table 6: Release risk estimates for wild amphibians import from the Caribbean scenario

	Release risk estimate
introduction of an imported subclinically infected Caribbean amphibian released as a pet released in nature	very low
introduction through the release of captive bred amphibians after the introduction if an infected imported amphibian in the breeding facility	very low
introduction in nature of an infected tick imported with Caribbean amphibian	very low

These results indicate that the risk of introducing infections into nature through the import and subsequent release of Caribbean amphibians or associated ticks is consistently very low across all assessed scenarios (Table 6).

VI. Discussion

The findings of this study underscore the significant risks and challenges associated with the potential introduction of heartwater disease into the United States from the Caribbean. Utilizing qualitative risk assessment, expert elicitation, and scenario modeling, this study illuminates the complexity of potential pathways and the factors influencing the introduction of heartwater in the US.

VI.1. Expert Panel Evaluation

The expert panel comprised 16 regional and international specialists, including 6 entomologists, 5 epidemiologists, and 5 wildlife experts. This diverse representation ensured a broad spectrum of insights into the study.

The mean confidence levels reported by experts fell within the range of 2 to 3, indicating that, on average, the experts were somewhat confident to confident in their responses. This level of confidence is crucial for the reliability of the study's conclusions.

VI.2. Weighting and Aggregation Methods

The Kruskal-Wallis test revealed a p-value of 0.13, indicating no significant difference between the outcomes of the two weighting methods (linear and nonlinear). This suggests that the choice of weighting method does not significantly influence the aggregated experts' estimations, allowing for flexibility in the methodological approach without compromising result integrity.

The sensitivity analysis however, revealed higher stability indexes for the non linear weighting method across all three. The slightly higher variability with the nonlinear method suggests it introduces greater variability, this motivated our choice of using the aggregated estimations calculated using the linear weighting method. However the observed variation in aggregated values is approximately **2** units (stability index * number of runs of the analysis - 1) for all three groups.

Considering that the aggregated estimates typically fall within the range of 0 to 5, this variation of approximately 2 units is substantial. It accounts for the third of the entire range of possible values. The high sensitivity implies that the final aggregated results are not as stable as might be desired. Even small changes in the confidence levels lead to large shifts in the aggregated outcomes.

This finding highlights the need of exploring alternative methods for expert opinion aggregation that could yield more stable results such as the Delphi method that seeks consensus from a group of experts through multiple rounds of questionnaires. The method is adaptable for both qualitative and quantitative research. Even though Delphi method is less commonly used in qualitative studies, it might offer a good alternative (Brady, 2015).

VI.3. Pathways and Likelihood of Introduction

The release risk analysis for Heartwater disease across various scenarios, including the cattle egret, domestic dogs, illegal ruminant trade, and wild amphibian importation, provides crucial insights into potential pathways for the disease's introduction into the United States. While the overall risk is low to very low, it is essential to consider additional contextual information and the limitations of the available data to fully understand the implications of these findings.

VI.3.a. Cattle Egret Scenario and Tick Dissemination

Cattle egret has been incriminated in the dissemination of *Amblyomma variegatum* on multiple Caribbean islands, highlighting its role as potential vector in the spread of this tick species (Barré, 1997). The release risk estimates indicated a low risk for *A. variegatum* originating from endemic regions under U.S. climate conditions. This finding is consistent with previous observations that *A. variegatum* infestation in cattle egrets is relatively elevated at 26.2%, as described by Barré (1997). Conversely, the infestation rate of cattle egrets with *Rhipicephalus microplus* is very low, which aligns with the very low release risk associated with this tick species across all evaluated scenarios.

However, while *R. microplus* is known to contribute to the infectious cycle of Heartwater in West Africa (Some et al., 2023), there is currently no data regarding its role or implication in the Caribbean region. This lack of data underscores the need for further research to better understand the potential risks associated with *R. microplus* in the Caribbean context, particularly given the tick's known impact in other regions.

VI.3.b. Domestic Dogs Travel Scenario

The domestic dogs travel scenario consistently showed a very low release risk for both *A. variegatum* and *R. microplus,* irrespective of the dogs wearing anti-tick collars. This suggests that the likelihood of these ticks establishing themselves in the U.S. via domestic dogs is minimal. However, a significant gap in the data is the absence of information on the extent of dog travel from the Caribbean to the U.S. Without data on the frequency and conditions of such travel, it is difficult to fully assess the potential risks. This gap highlights an area where further investigation is needed to ensure that potential pathways for tick introduction are adequately understood and managed.

VI.3.c. Illegal Ruminant Trade and Cultural Practices

The release risk analysis for illegal ruminant trade and trafficking indicated a low to very low risk, depending on the specific scenario. However, there is a notable lack of data on the illegal trade of ruminants within the Caribbean region and a poor understanding of cultural practices that might involve animal movement between islands, such as the “Boeufs Tirants” tradition in Guadeloupe. These gaps in knowledge pose a challenge for accurately assessing the risk of Heartwater disease introduction via these routes. Given that the U.S. only imports cattle from Mexico and Canada, and no Caribbean country other than Mexico (USDA, 2024d) , the primary risk may lie in informal or illegal trade, which is less easily monitored and controlled.

VI.3.d. Wild Amphibian Import and the Role of Amblyomma dissimile

The importation of wild amphibians from the Caribbean was assessed as having a very low release risk across all evaluated scenarios. This low risk is significant considering that the total U.S. import of amphibians over a 14-year period (2000-2014) was 8 million individuals. A key consideration in this scenario is the potential role of *Amblyomma dissimile,* a herpetophile tick with a large host spectrum (McCoy et al., 2013). While *A. dissimile* has not been proven to be a natural vector of Heartwater, it is an experimentally proven vector (Jongejan, 1992). The potential for this tick to contribute to the introduction of Heartwater in the U.S. remains theoretical, but it warrants ongoing monitoring given the large volume of amphibian imports and the tick's broad host range.

VI.4. Consideration of Previous Pathways and Unexplored Routes

Previous researches have explored several potential pathways for the introduction of Heartwater into the U.S., including the cattle egret theory, ruminant transport and movement, and the importation of reptiles from Africa (Burridge et al., 2000, 2002; Kasari et al., 2010). However, our current study introduces new considerations by highlighting domestic dog travel as a potential route, which has not been previously discussed as a vector for Heartwater introduction. Given the very low release risk found in this study, domestic dog travel might not be a significant pathway, but its inclusion in risk assessments is essential for a comprehensive understanding of all potential routes.

VI.5. Gaps in Surveillance and Biosecurity Measures

This study indicates that while biosecurity measures are established, there are notable gaps that could be exploited by the identified pathways. This highlights the need for stricter regulatory measures, particularly concerning wildlife trade and pet transport from the Caribbean. The lack of mandatory tick inspections for pets returning to the US, coupled with limited enforcement of anti-tick treatments in exotic pet trade, represents significant vulnerabilities in the current biosecurity framework. Strengthening these measures could be crucial in mitigating the risk of heartwater introduction.

VI.6. Implications for Policy and Future Research

The findings have several important implications for policymakers and researchers involved in exotic disease prevention. Further research is needed to investigate the vector competence of emerging tick species, the role of wildlife in *E. ruminantium* transmission, and the impact of climate change on heartwater spread. Addressing these gaps will aid in developing more targeted and effective control strategies for preventing disease introduction.

VII. Conclusion

The qualitative risk analysis conducted in this thesis highlights the significant threat posed by the potential introduction of Heartwater disease to the United States from the Caribbean. While the likelihood of introduction is difficult to quantify, the potential consequences for the U.S. agricultural sector could be severe. The analysis suggests that enhanced biosecurity measures, increased surveillance of potential vector species, and further research into disease prevention strategies are critical steps in mitigating this risk. It is essential that policymakers and stakeholders in the livestock industry prioritize these actions to prevent the emergence of heartwater in the united states.

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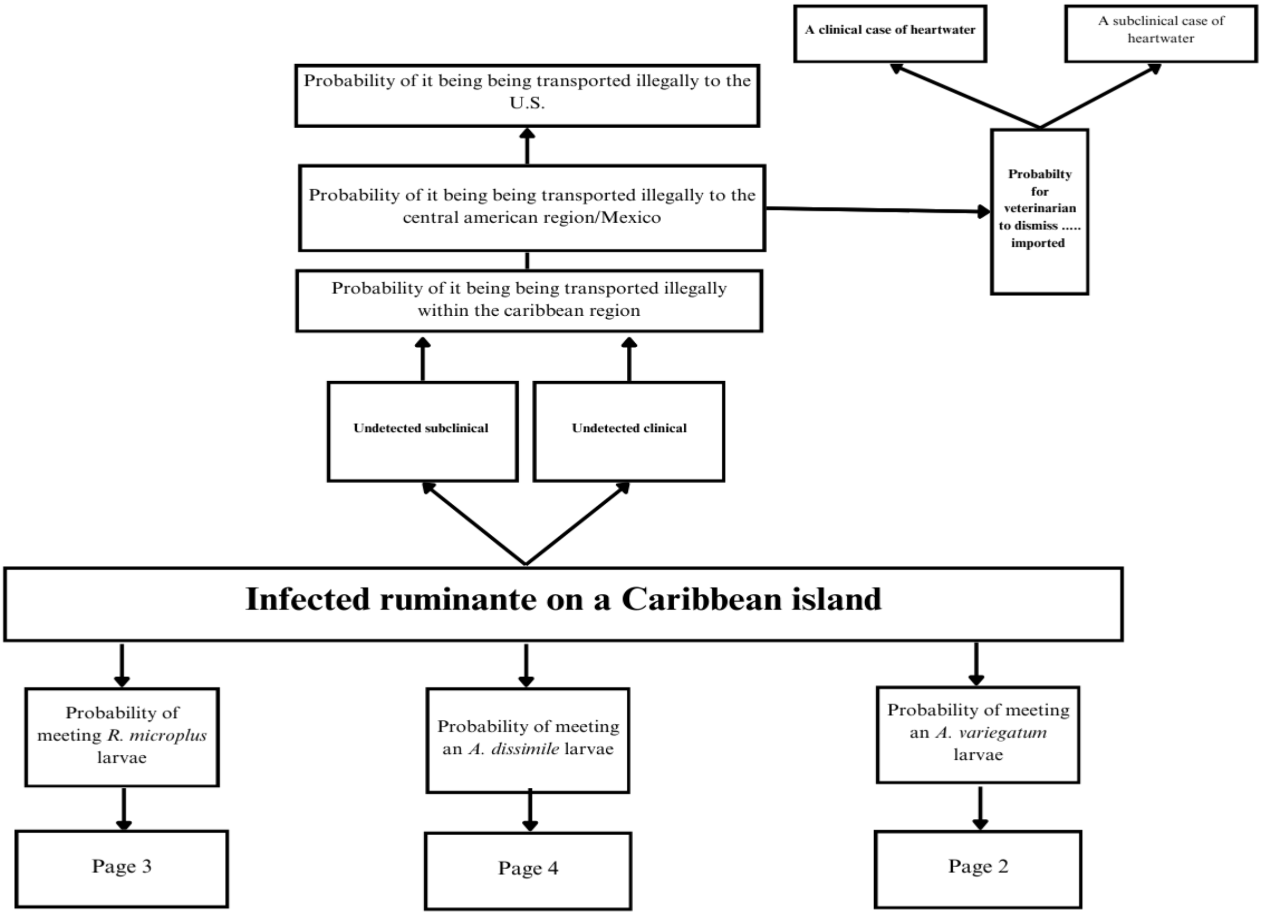
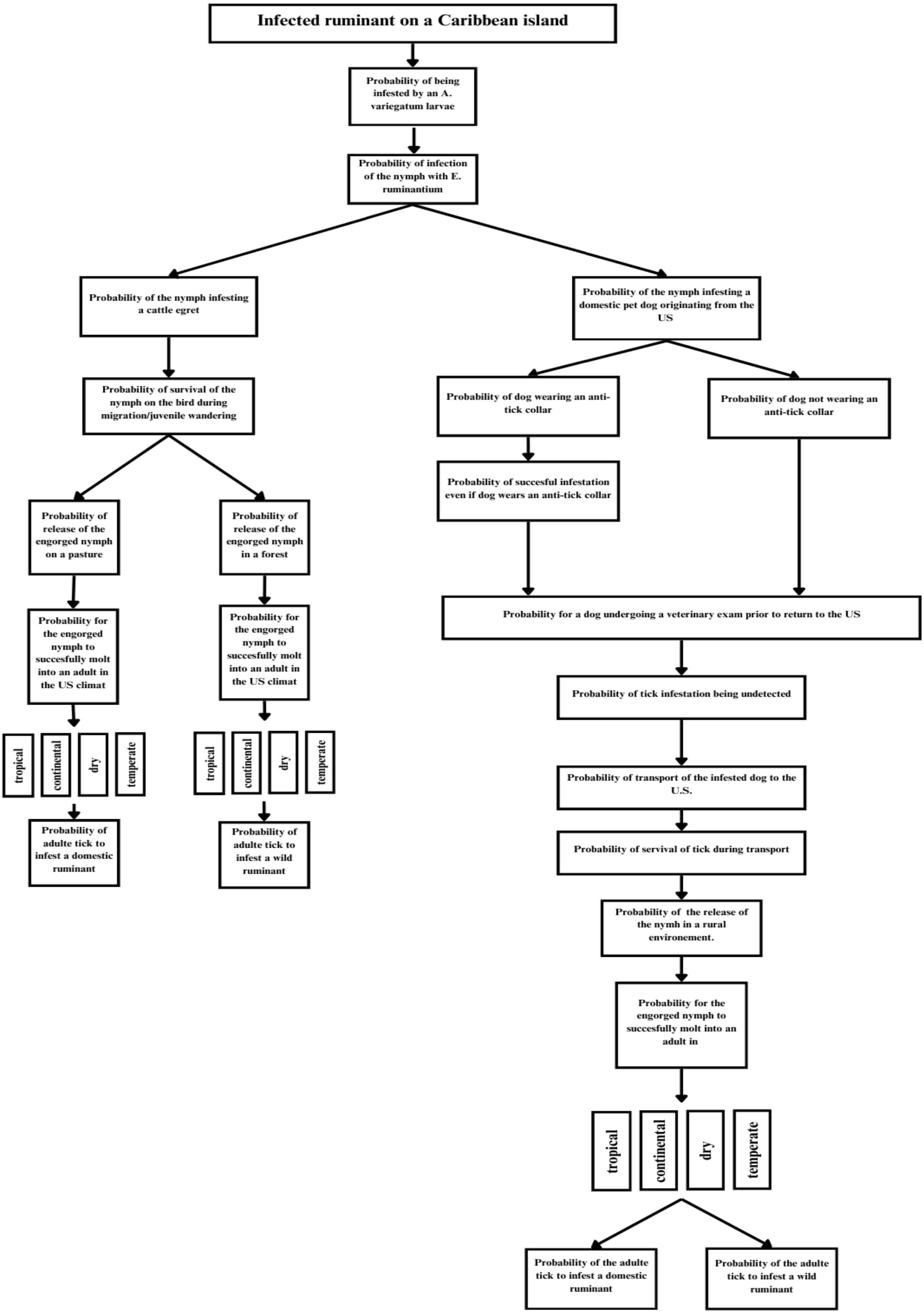
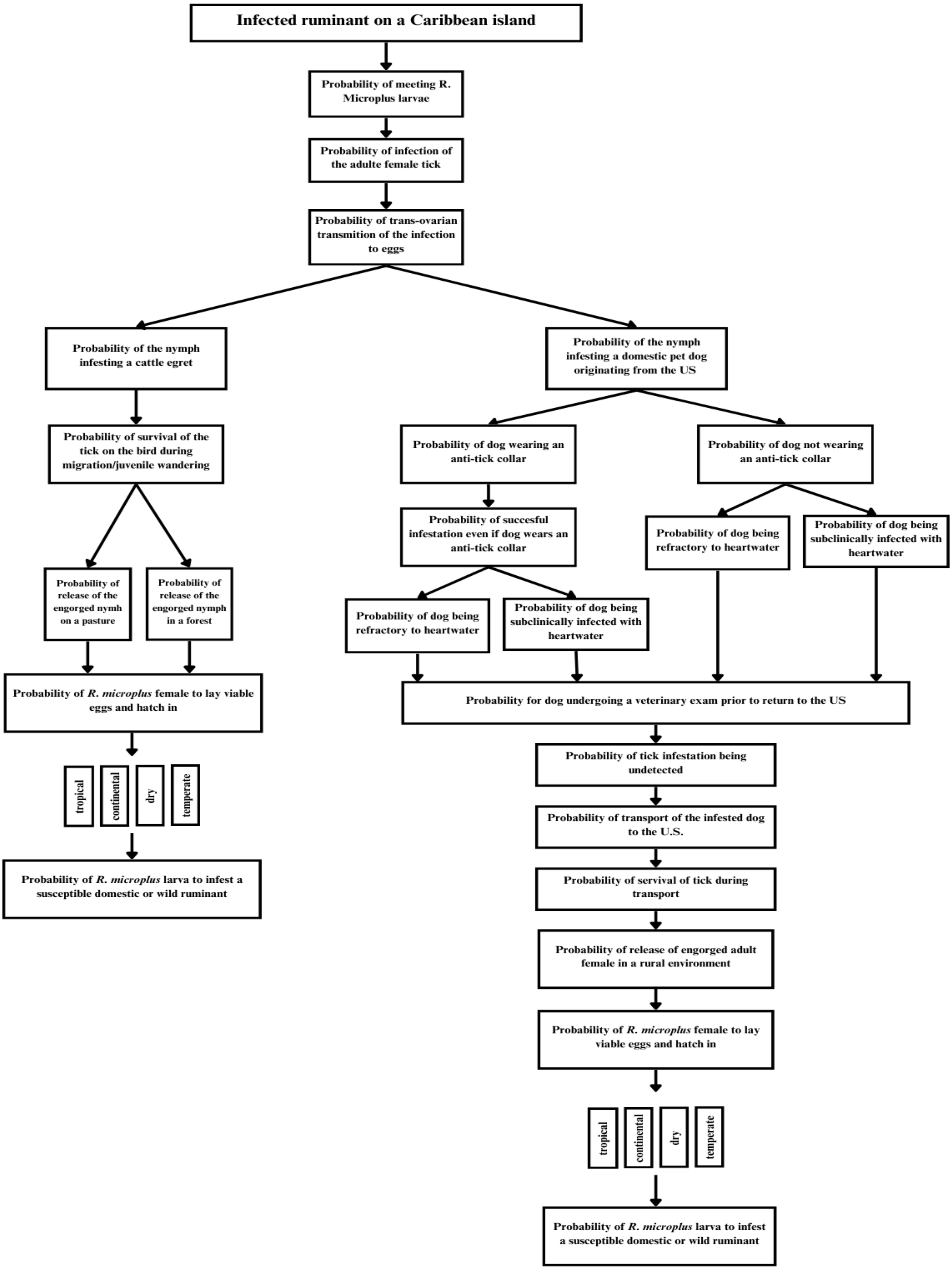
Annex 1



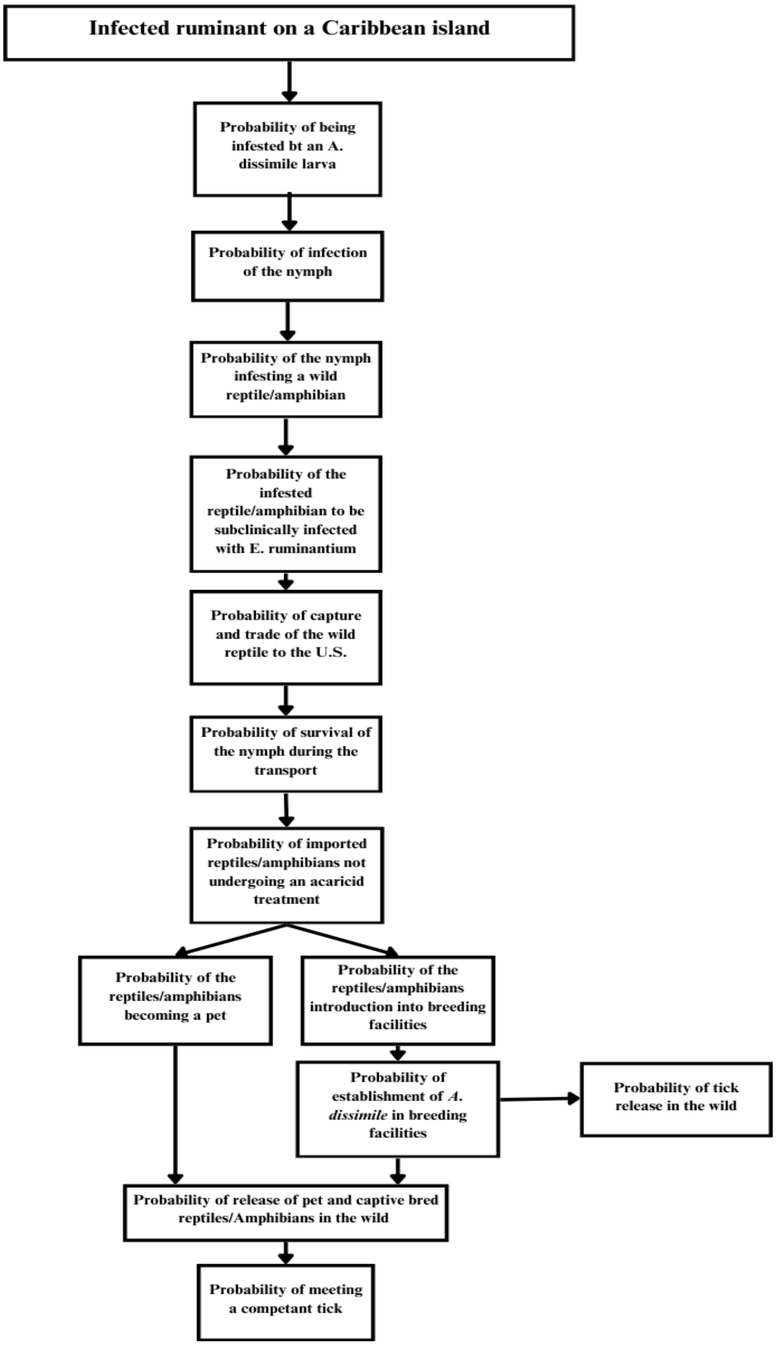
A clinical case of heartwater
A subclinical case of heartwater
Probability of it being being transported illegally to the U.S.
Probability of it being being transported illegally to the central american region/Mexico
Probabilty for veterinarian to dismiss ..... imported
Probability of it being being transported illegally within the caribbean region
Undetected subclinical
Undetected clinical
Infected ruminante on a Caribbean island
Probability of meeting *R. microplus* larvae
Probability of meeting an *A. dissimile* larvae
Probability of meeting an *A. variegatum* larvae
Page 3
Page 4
Page 2


Infected ruminant on a Caribbean island
Probability of being infested by an A. variegatum larvae
Probability of infection of the nymph with E. ruminantium
Probability of the nymph infesting a cattle egret
Probability of survival of the nymph on the bird during migration/juvenile wandering
Probability of release of the engorged nymph on a pasture
Probability for the engorged nymph to succesfully molt into an adult in the US climat
tropical
continental
dry
temperate
Probability of adulte tick to infest a domestic ruminant
Probability of release of the engorged nymph in a forest
Probability for the engorged nymph to succesfully molt into an adult in the US climat
tropical
continental
dry
temperate
Probability of adulte tick to infest a wild ruminant
Probability of the nymph infesting a domestic pet dog originating from the US
Probability of dog wearing an anti-tick collar
Probability of succesful infestation even if dog wears an anti-tick collar
Probability of dog not wearing an anti-tick collar
Probability for a dog undergoing a veterinary exam prior to return to the US
Probability of tick infestation being undetected
Probability of transport of the infested dog to the U.S.
Probability of servival of tick during transport
Probability of the release of the nymh in a rural environement.
Probability for the engorged nymph to succesfully molt into an adult in
tropical
continental
dry
temperate
Probability of the adulte tick to infest a domestic ruminant
Probability of the adulte tick to infest a wild ruminant

2

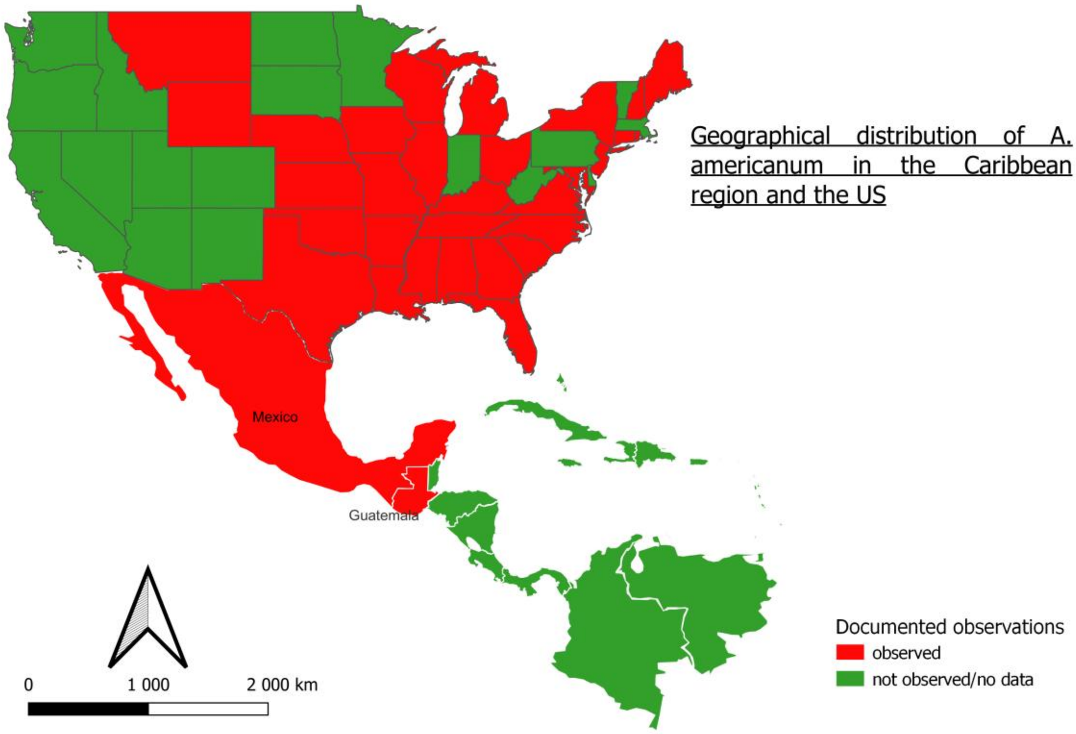

Infected ruminant on a Caribbean island
Probability of meeting R. Microplus larvae
Probability of infection of the adulte female tick
Probability of trans-ovarian transmition of the infection to eggs
Probability of the nymph infesting a cattle egret
Probability of survival of the tick on the bird during migration/juvenile wandering
Probability of release of the engorged nymh on a pasture
Probability of release of the engorged nymph in a forest
Probability of R. microplus female to lay viable eggs and hatch in
tropical
continental
dry
temperate
Probability of R. microplus larva to infest a susceptible domestic or wild ruminant
Probability of the nymph infesting a domestic pet dog originating from the US
Probability of dog wearing an anti-tick collar
Probability of dog not wearing an anti-tick collar
Probability of succesful infestation even if dog wears an anti-tick collar
Probability of dog being refractory to heartwater
Probability of dog being subclinically infected with heartwater
Probability of dog being refractory to heartwater
Probability of dog being subclinically infected with heartwater
Probability for dog undergoing a veterinary exam prior to return to the US
Probability of tick infestation being undetected
Probability of transport of the infested dog to the U.S.
Probability of servival of tick during transport
Probability of release of engorged adult female in a rural environment
Probability of R. microplus female to lay viable eggs and hatch in
tropical
continental
dry
temperate
Probability of R. microplus larva to infest a susceptible domestic or wild ruminant

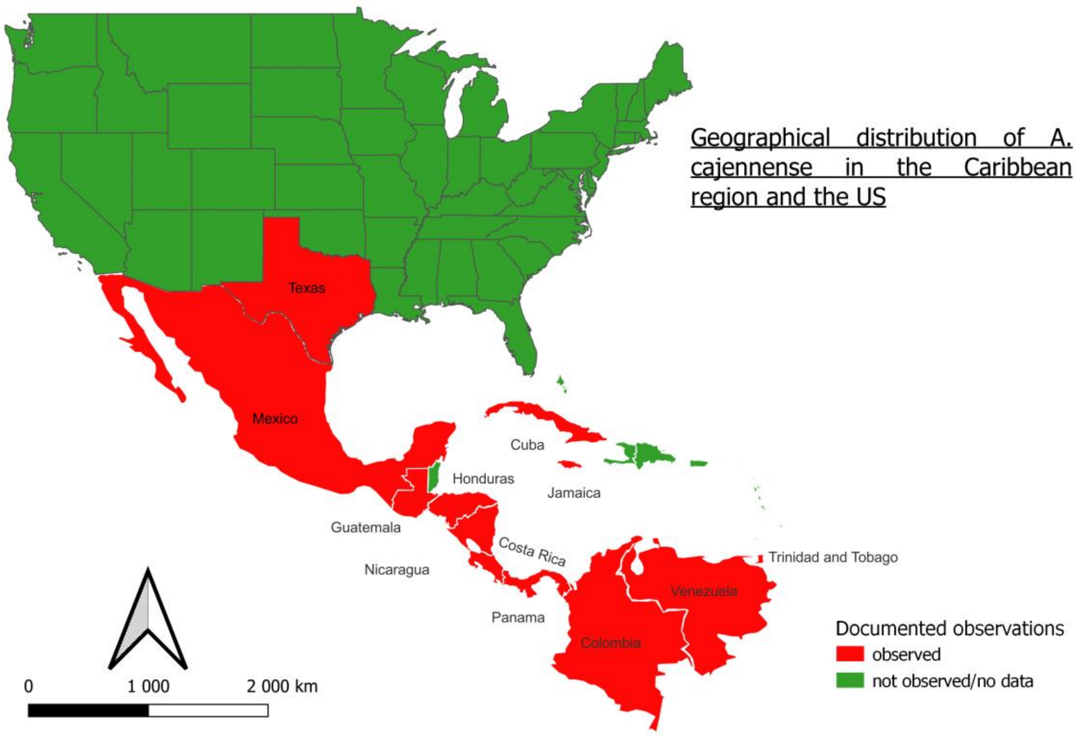
3

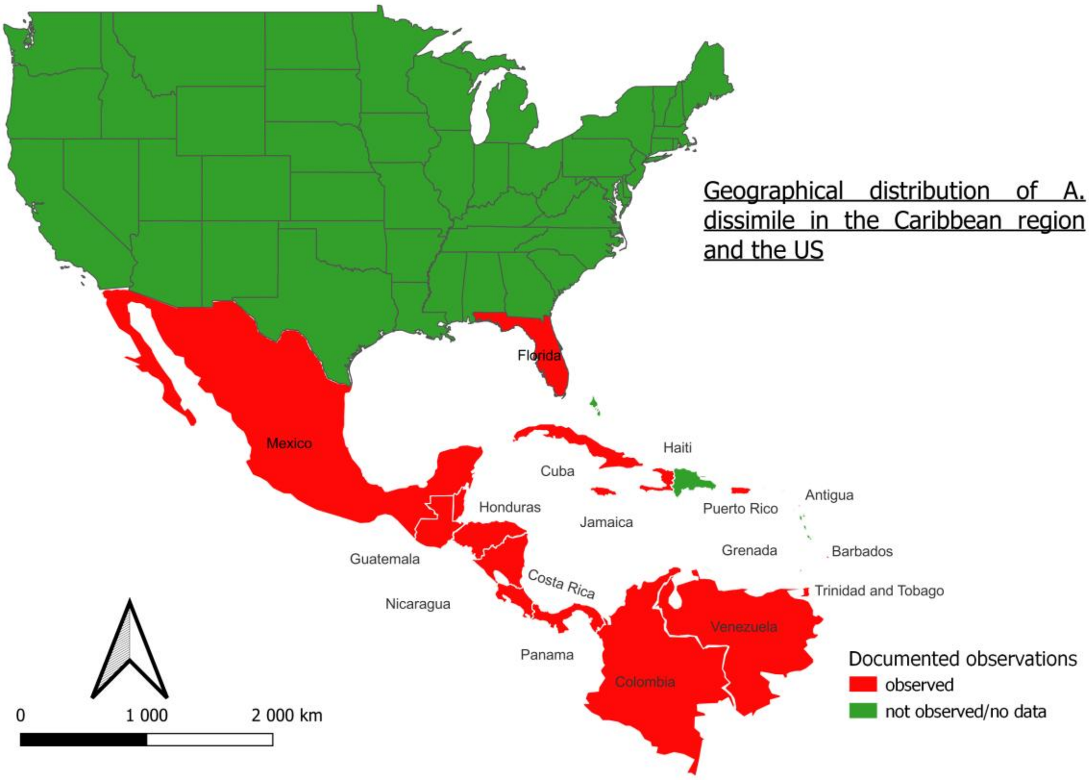

Infected ruminant on a Caribbean island
Probability of being infested bt an A. dissimile larva
Probability of infection of the nymph
Probability of the nymph infesting a wild reptile/amphibian
Probability of the infested reptile/amphibian to be subclinically infected with E. ruminantium
Probability of capture and trade of the wild reptile to the U.S.
Probability of survival of the nymph during the transport
Probability of imported reptiles/amphibians not undergoing an acaricid treatment
Probability of the reptiles/amphibians becoming a pet
Probability of the reptiles/amphibians introduction into breeding facilities
Probability of establishment of A. dissimile in breeding facilities
Probability of tick release in the wild
Probability of release of pet and captive bred reptiles/Amphibians in the wild
Probability of meeting a competant tick

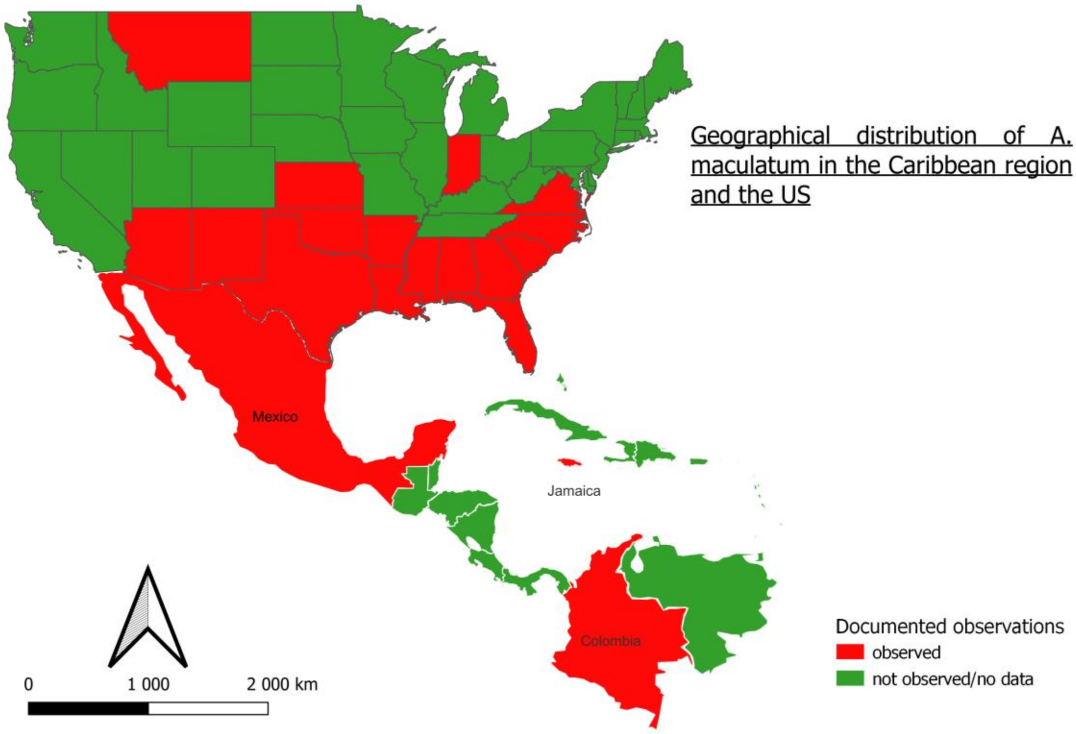
4

Annex 2 : Geographical distribution of four Amblyomma spp vectors of heartwater in the Caribbean region






Geographical distribution of A. dissimile in the Caribbean region and the US
Florida
Mexico
Haiti
Cuba
Antigua
Honduras
Puerto Rico
Jamaica
Grenada
Barbados
Guatemala
Costa Rica
Trinidad and Tobago
Nicaragua
Venezuela
Panama
Colombia
Documented observations
observed
not observed/no data
0
1 000
2 000 km


Geographical distribution of A. maculatum in the Caribbean region and the US
Mexico
Jamaica
Colombia
Documented observations
observed
not observed/no data
0
1 000
2 000 km

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Walter Reed Biosystematics Unit, 2024b. Amblyomma maculatum species page [WWW Document]. URL https://wrbu.si.edu/node/2482 (accessed 6.10.24).

Annex 3: Supplementary data on US reptile and amphibian import

The Following graphics are a representation of the United States reptilian and amphibian import over 15 years (from year 2000 to 2014) originating from the Caribbean region (Caribbean islands + south and central America). We produced these graphics after treatment of United states LEMIS wildlife trade data curated by EcoHealth alliance (Eskew et al., 2019).

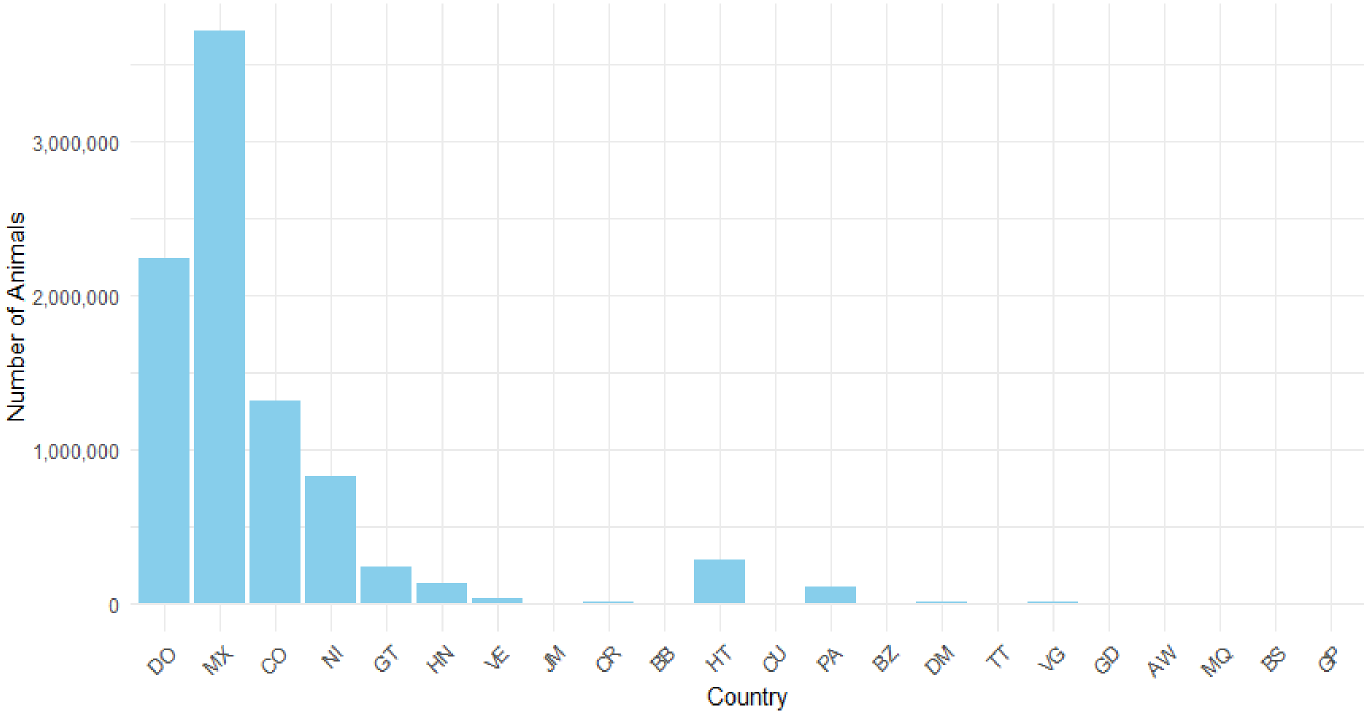


Fig 1: Total quantity of reptile and amphibian import to the US 2000-2014

AI = Anguilla, AG = Antigua and Barbuda, AW = Aruba, BS = Bahamas, BB = Barbados, VG = British Virgin Islands, CU = Cuba, DM = Dominica, DO = Dominican Republic, GD = Grenada, GP = Guadeloupe, HT = Haiti, JM = Jamaica, MQ = Martinique, MS = Montserrat, TT = Trinidad and Tobago, MX = Mexico, GT = Guatemala, BZ = Belize, HN = Honduras, NI = Nicaragua, CR = Costa Rica, PA = Panama, VE = Venezuela, CO = Colombia

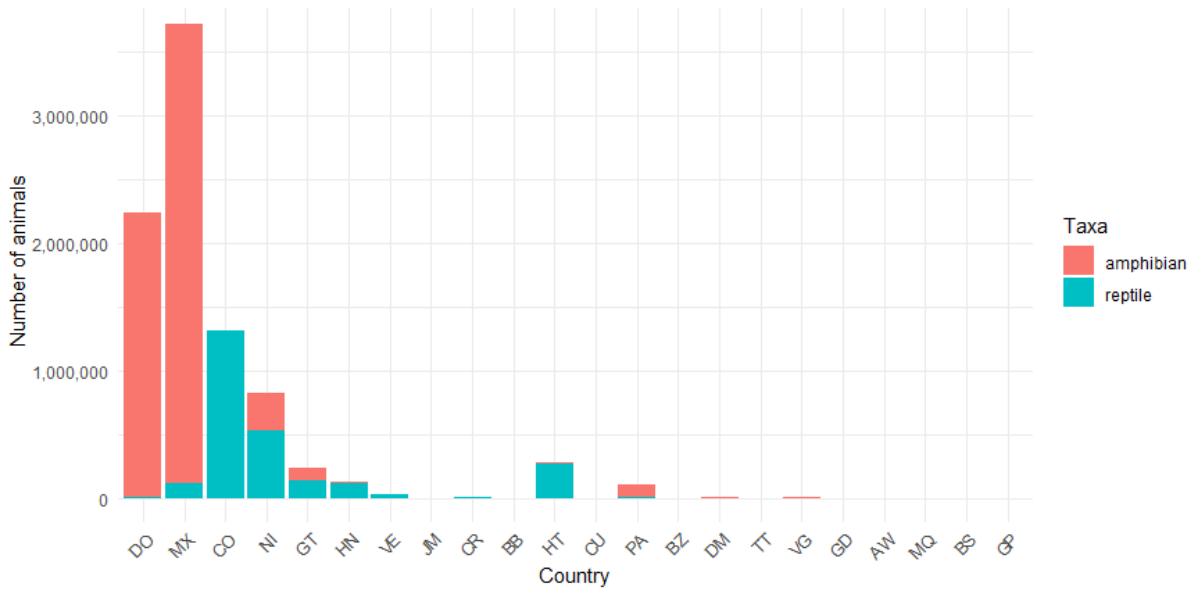


Fig 2: Total quantity of reptiles and amphibians imported to the US represented by taxonomic group 2000-2014

AI = Anguilla, AG = Antigua and Barbuda, AW = Aruba, BS = Bahamas, BB = Barbados, VG = British Virgin Islands, CU = Cuba, DM = Dominica, DO = Dominican Republic, GD = Grenada, GP = Guadeloupe, HT = Haiti, JM = Jamaica, MQ = Martinique, MS = Montserrat, TT = Trinidad and Tobago, MX = Mexico, GT = Guatemala, BZ = Belize, HN = Honduras, NI = Nicaragua, CR = Costa Rica, PA = Panama, VE = Venezuela, CO = Colombia

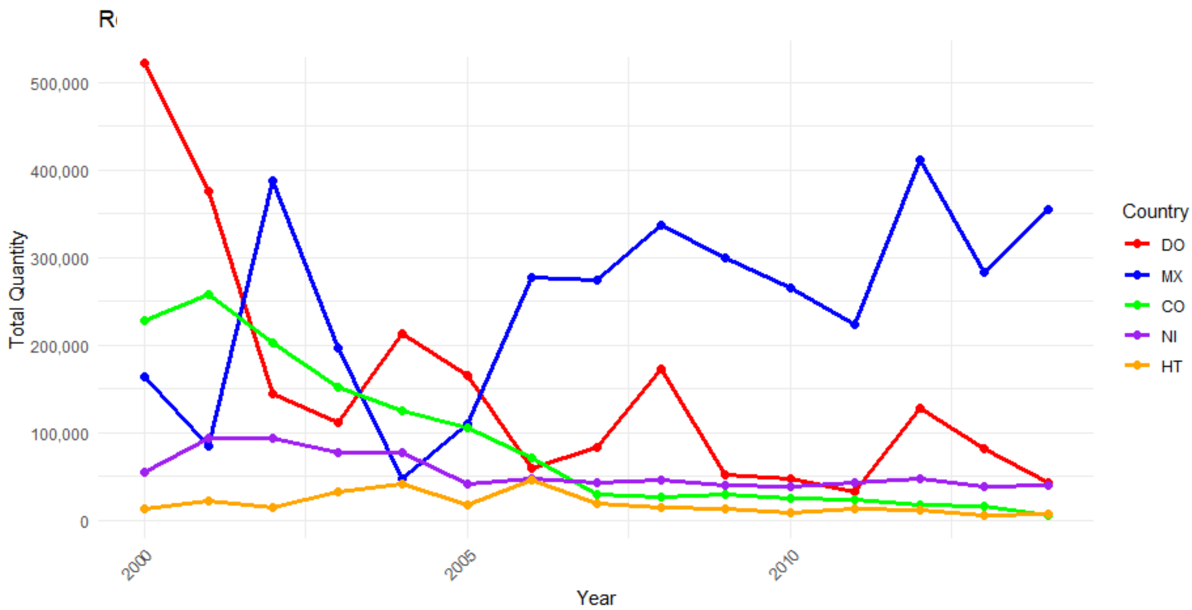


Fig 3: US reptile and amphibian yearly import from 5 most important import countries of the Caribbean 2000-2014

AI = Anguilla, AG = Antigua and Barbuda, AW = Aruba, BS = Bahamas, BB = Barbados, VG = British Virgin Islands, CU = Cuba, DM = Dominica, DO = Dominican Republic, GD = Grenada, GP = Guadeloupe, HT = Haiti, JM = Jamaica, MQ = Martinique, MS = Montserrat, TT = Trinidad and Tobago, MX = Mexico, GT = Guatemala, BZ = Belize, HN = Honduras, NI = Nicaragua, CR = Costa Rica, PA = Panama, VE = Venezuela, CO = Colombia

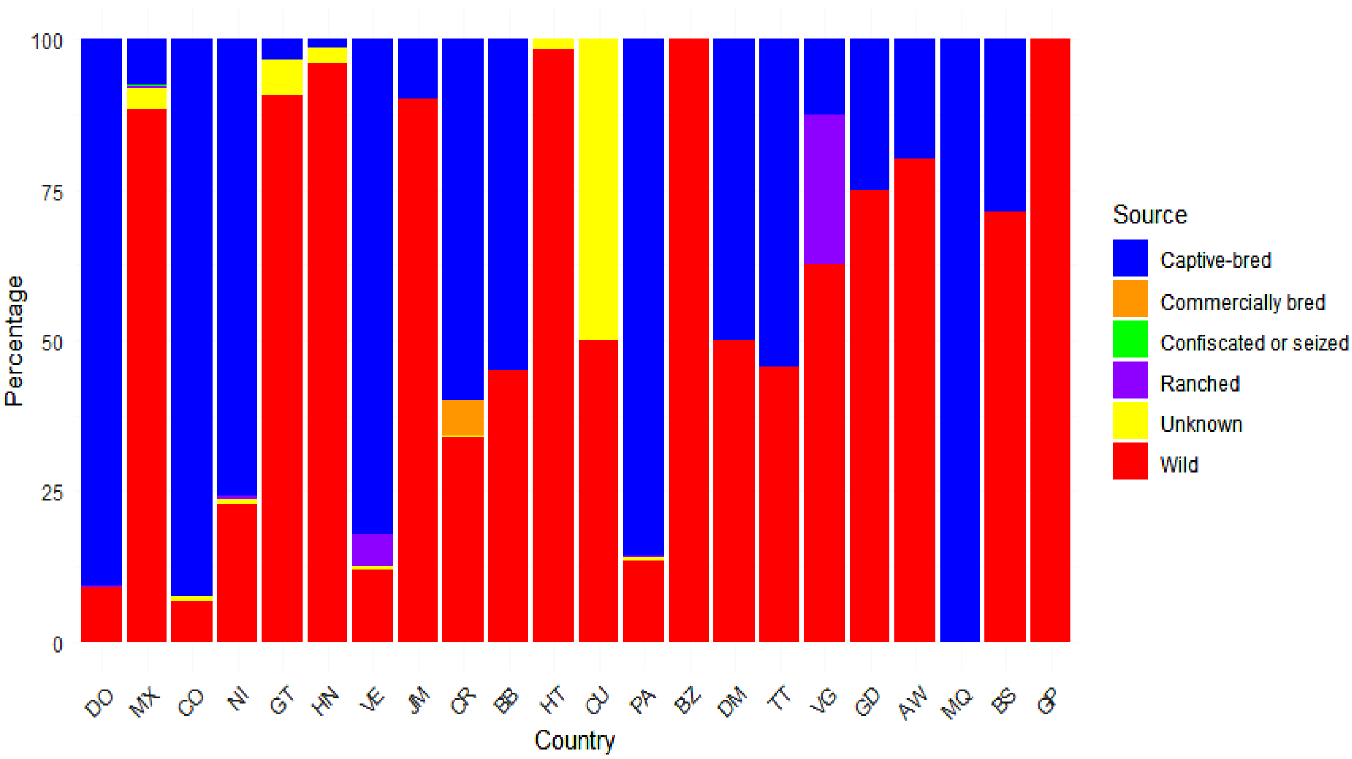


Fig 4: Provenance of reptiles and amphibians imported to the US from the Caribbean region 2000-2014

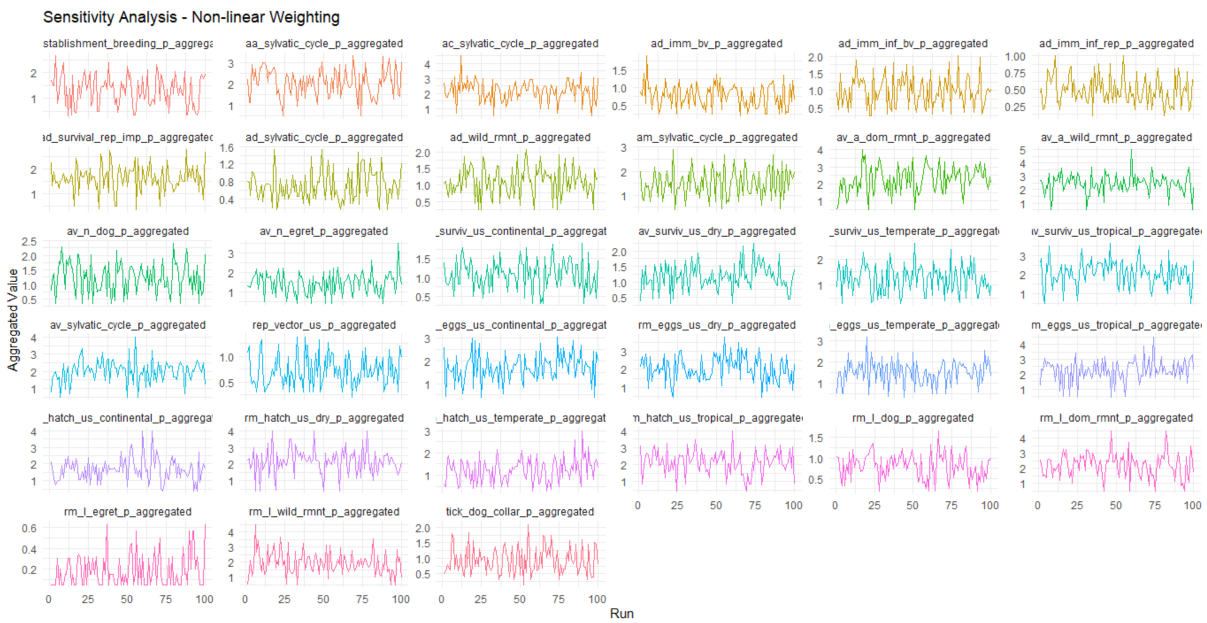
AI = Anguilla, AG = Antigua and Barbuda, AW = Aruba, BS = Bahamas, BB = Barbados, VG = British Virgin Islands, CU = Cuba, DM = Dominica, DO = Dominican Republic, GD = Grenada, GP = Guadeloupe, HT = Haiti, JM = Jamaica, MQ = Martinique, MS = Montserrat, TT = Trinidad and Tobago, MX = Mexico, GT = Guatemala, BZ = Belize, HN = Honduras, NI = Nicaragua, CR = Costa Rica, PA = Panama, VE = Venezuela, CO = Colombia

<u>Data:</u>

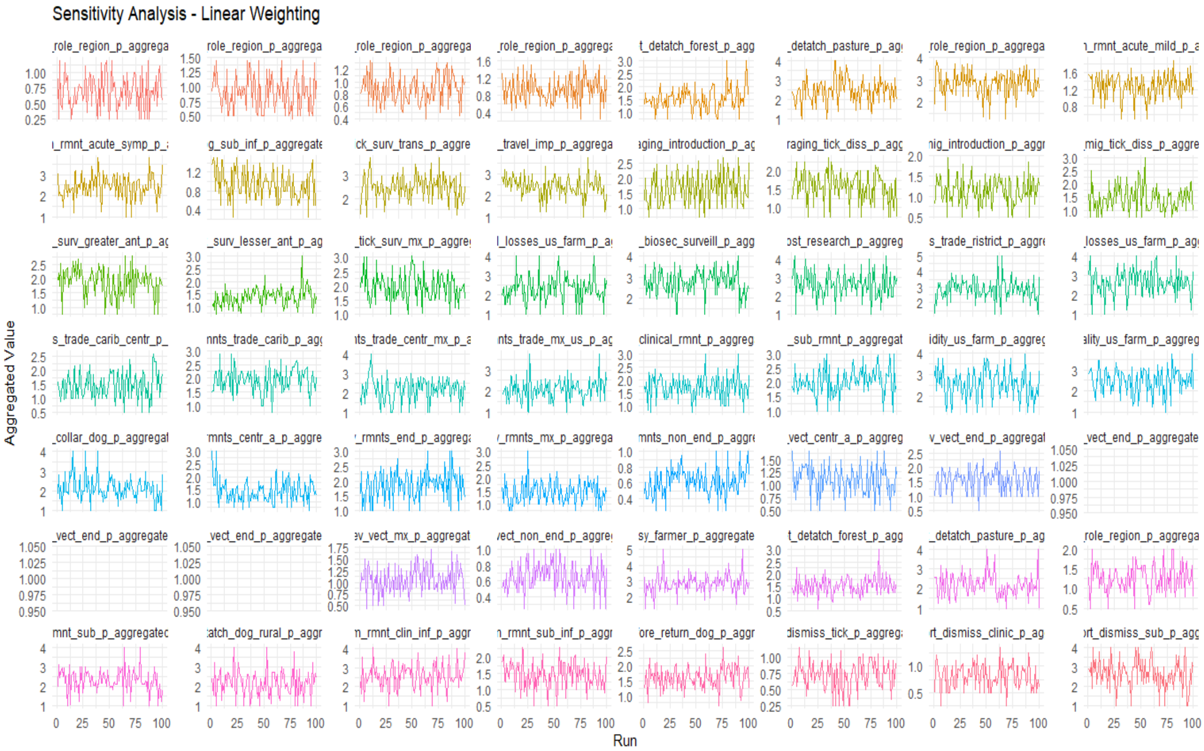
Eskew, E.A., White, A.M., Ross, N., Smith, K.M., Smith, K.F., Rodríguez, J.P., Zambrana-Torrelio, C., Karesh, W.B., Daszak, P., 2019. United states LEMIS wildlife trade data curated by EcoHealth alliance. https://doi.org/10.5281/zenodo.3565869

Annex 4: Sensitivity analysis output

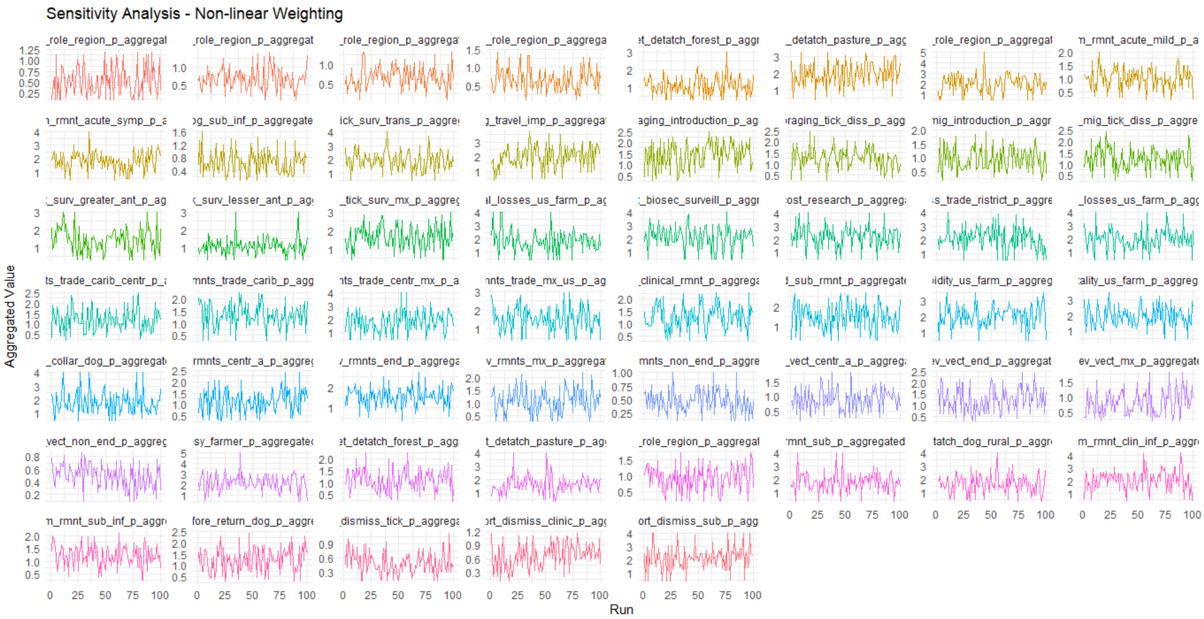
Entomology group linear weighting



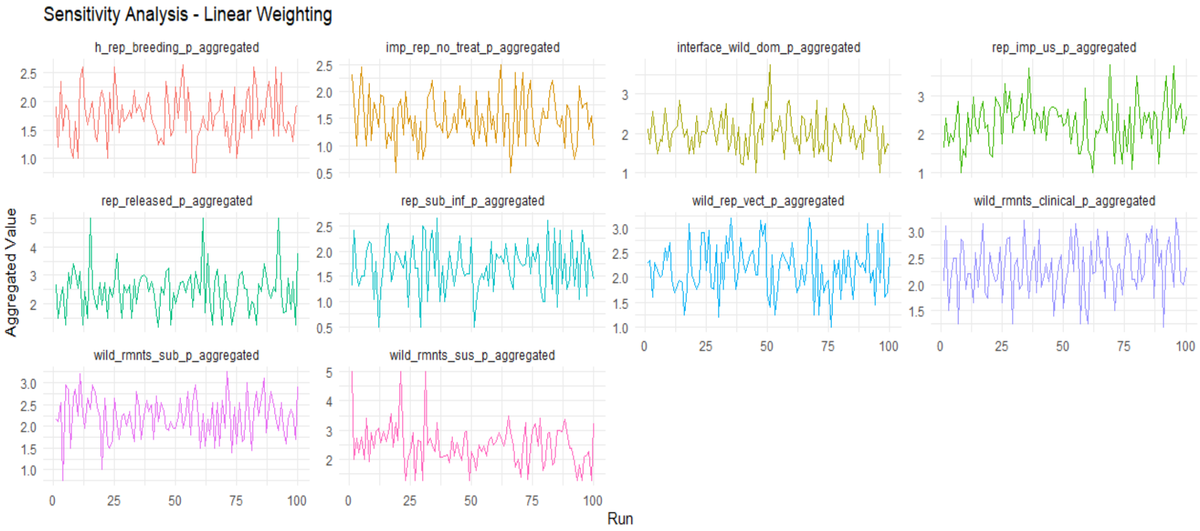
Entomology group non linear weighting



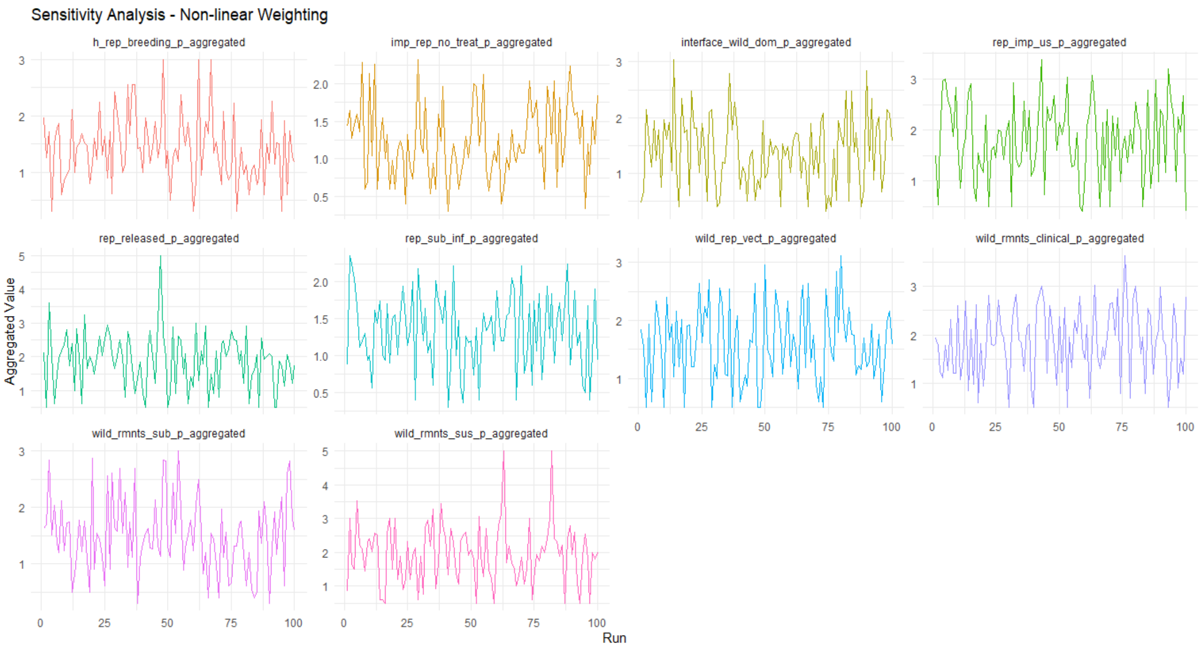
Epidemiology group linear weighting



Epidemiology group non linear weighting



Wildlife group linear weighting



wildlife group non linear weighting