Preventive Veterinary Medicine 114 (2014) 174–187

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Viral hemorrhagic septicemia IVb status in the United States: Inferences from surveillance activities and regional context

L.L. Gustafson[a,*], M.D. Remmenga[a], I.A. Gardner[b], K.H. Hartman[c], L.H. Creekmore[d], A.E. Goodwin[e], J.E. Whaley[f], J.V. Warg[g], S.L. Gardner[a], A.E. Scott[a]

[a] National Surveillance Unit, Centers for Epidemiology and Animal Health, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, 2150 Centre Avenue, Fort Collins, CO, USA
[b] Department of Health Management, Atlantic Veterinary College, Charlottetown, Prince Edward Island C1A 4P3, Canada
[c] Eastern Regional Office, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, 1408 24th Street SE, Ruskin, FL, USA
[d] Western Regional Office, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, 2150 Centre Avenue, Fort Collins, CO, USA
[e] United States Fish and Wildlife Service, Pacific Region, 911 NE 11th Avenue, Portland, OR 97232, USA
[f] Aquaculture Program, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture, 4700 River Road, Riverdale, MD, USA
[g] Diagnostic Virology Laboratory, National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, United States Department of Agriculture, 1920 Dayton Avenue, Ames, IA, USA

ARTICLE INFO

Article history:
Received 26 August 2013
Received in revised form 20 February 2014
Accepted 26 February 2014

Keywords:
Disease freedom
Aquatic animal health
Risk-based surveillance
Context-based surveillance
Viral hemorrhagic septicemia

ABSTRACT

The United States (U.S.) response to viral hemorrhagic septicemia virus (VHSV) IVb emergence in the Laurentian Great Lakes (GL) included risk-based surveillance for cost-effective decision support regarding the health of fish populations in open systems. All U.S. VHSV IVb isolations to date derive from free-ranging fish from GL States. Most originate in the region designated by US Geological Survey hydrologic unit code (HUC) 04, with the exception of two detections in neighboring Upper Mississippi (HUC 05) and Ohio (HUC 07) regions. For States outside the GL system, disease probability was assessed using multiple evidence sources. None substantiated VHSV IVb absence using surveillance alone, in part due to the limited temporal relevance of data in open systems. However, Bayesian odds risk-based analysis of surveillance and population context, coupled with exclusions where water temperatures likely preclude viral replication, achieved VHSV IVb freedom assurance for 14 non-GL States by the end of 2012, with partial evidence obtained for another 17 States. The non-GL region (defined as the aggregate of 4-digit HUCs located outside of GL States) met disease freedom targets for 2012 and is projected to maintain this status through 2016 without additional active surveillance. Projections hinge on continued basic biosecurity conditions such as movement restrictions and passive surveillance. Areas

* Corresponding author at: 2150 Centre Avenue, Bldg B, Mail Stop 2E6, Fort Collins, CO 80526-8117, USA. Tel.: +1 970 494 7297; fax: +1 970 494 7174.
E-mail address: lori.l.gustafson@aphis.usda.gov (L.L. Gustafson).

http://dx.doi.org/10.1016/j.prevetmed.2014.02.011
0167-5877/Published by Elsevier B.V.

with navigable waterway connections to VHSV IVb-affected HUCs (and conducive water temperatures) should receive priority for resources in future surveillance or capacity building efforts. However, 6 years of absence of detections in non-GL States suggests that existing controls limit pathogen spread, and that even spread via natural pathways (e.g., water movement or migratory fish) appears contained to the Great Lakes system. This report exemplifies the cost-effective use of risk-based surveillance in decision support to assess and manage aquatic animal population health in open systems.

Published by Elsevier B.V.

1. Introduction

The emergence of viral hemorrhagic septicemia virus IVb (VHSV IVb) in the Laurentian Great Lakes (GL) was of consequence both to fish populations and the aquatic animal health systems designed for their support (Elsayed et al., 2006; Lumsden et al., 2007; Groocock et al., 2007). VHSV IVb causes highly visible mortality events in many species of wild fish, with impacts extending to ecosystems and their related industries. Twenty-eight freshwater species are considered susceptible to natural infection or disease caused by VHSV IVb (USDA APHIS VS, 2008a), including species harvested, cultured, and stocked for bait, sport, or food. Thus, the VHSV IVb emergence spurred aquatic animal health initiatives and debate about best methods to enhance the prevention and control of this and future aquatic health events (Faisal et al., 2012). In 2006, the United States (U.S.) enacted a Federal Order (FO), amended in 2008, to reduce spread of VHSV IVb by restricting the movement of susceptible species from GL States (Illinois, Indiana, Michigan, Minnesota, New York, Ohio, Pennsylvania, Wisconsin) and Canadian provinces (Ontario and Quebec) (USDA APHIS VS, 2008b). Concurrently, U.S. Federal and State governments and local stakeholders enhanced systems for VHSV IVb recognition, investigation, and response, as well as mitigation of introduction risks.

Enhanced systems included surveillance to better document the distribution and spread of the virus. The initial scope of VHSV IVb concern was extremely broad, reflecting interest to distinguish the status of farmed versus wild populations in 50 States and multiple Provinces, among a myriad of susceptible freshwater species. The broad scope coupled with a predominance of open systems raised the need for many and frequent (i.e., costly) surveillance initiatives. Further, VHSV IVb shows varied clinical expression, prevalence, and detection capacity across species, life stages, seasons, and habitats (Kane-Sutton et al., 2010; Kim and Faisal, 2010a, 2010b, 2010c; Frattini et al., 2011; Cornwell et al., 2012a, 2012b; Faisal et al., 2012; Groocock et al., 2012); and testing modalities differ in their sensitivity (Bain et al., 2010; Cornwell et al., 2012a, 2012b). These complexities magnify surveillance expense and limit its value.

Consequently, also in 2006, USDA Animal and Plant Health Inspection Service Veterinary Services (APHIS VS), U.S. Fish and Wildlife Service (USFWS), Great Lakes Fish Health Committee (GLFHC) and the Canadian Food Inspection Agency (CFIA) established a bilateral working group to set guidance to harmonize surveillance efforts (Bilateral VHSV Surveillance Working Group, 2007). Given limited surveillance resources, and acknowledging the value of mitigations to reduce pathogen transmission or promote its recognition, the working group developed a methodology to prioritize or offset surveillance with available knowledge on risk in a regional context (Gustafson et al., 2010; VHSV Expert Panel and Working Group, 2010). The resulting framework credits multiple evidence sources indicative of pathogen status including active surveillance by virus isolation, passive surveillance structured to investigate clinical signs, and regional risk factors or mitigations.

This paper summarizes results from multiple evidence streams to infer VHSV IVb status for individual States in the U.S. For the 8 GL States, we describe the spatial extent of known VHSV IVb occurrence as evidenced by virus isolation detections from APHIS, USGS, and USFWS databases. For the 42 States outside of the GL systems (non-GL States), we use a Bayesian odds method of assessment relatively new to veterinary surveillance to estimate State disease freedom credibility through time from Federal, Tribal, and State data on active surveillance and historical context. Similarly, we estimate disease freedom credibility for the non-GL region as a whole (defined as the aggregate of 4-digit HUCs located outside the GL States). Results facilitate resource allocation and regulatory decisions by identifying regions that meet disease freedom targets, regions with the greatest need for renewed surveillance, or mitigations that limit introduction risk and dependence on recurrent testing. Results also highlight the long-standing value of enhancements to aquatic health infrastructure.

2. Methods

2.1. Data sources

Surveillance data available to USDA-APHIS-VS for this report include those funded by (1) Cooperative Agreement between USDA-APHIS-VS and State or Tribal Agencies, (2) USFWS as a component of the National Wild Fish Health Survey database (U.S. Fish and Wildlife Service, (http://www.fws.gov/wildfishsurvey/), or (3) independent State or local initiatives as captured by the U.S. Geological Survey (USGS) VHSV database (http://gis.nacse.org/vhsv/). Additionally, the GLFHC, many States and one Territory shared historical data and risk evaluations voluntarily.

2.2. Test protocols

Samples were handled and processed following protocols described by American Fisheries Society Blue Book

(AFS-FHS, 2010). Whole fish (e.g., fry) or spleen and kidney tissues from up to five fish of the same species, collection date, and site were pooled for virus isolation, chilled, and shipped overnight to participating State, Federal, or private laboratories. Virus isolation used cell culture lines recognized by the Blue Book or the World Organisation for Animal Health (OIE) Manual of Diagnostic Tests for Aquatic Animals (OIE, 2013b) for VHSV investigation and included epithelioma papulosum cyprini (EPC), fathead minnow (FHM), Chinook salmon embryo (CHSE) and bluegill fry (BF-2). Sensitivity of virus isolation was originally estimated at 85% (Bilateral VHSV Surveillance Working Group, 2007), though disease probability analyses were also run at 25% sensitivity for comparison.

2.3. Decision criteria

2.3.1. Targeted surveillance

OIE guidelines (OIE, 2013a, 2013b) describe several pathways to demonstrate freedom from disease. The first pathway, targeted surveillance, compiles active surveillance data sufficient to reach target confidence (typically 95%) of disease absence, as defined by the target design prevalence (dp). OIE guidelines for dp are not VHSV-specific, deferring instead to general targets in the surveillance chapter (chapter 1.4). These targets, 2% at the fish level and 2% at the facility level, however, apply best to farms or operations with distinct separation between units (e.g., ponds) and may not pertain to open systems. For example, if a pathogen occurred in a single farm or pond, it is reasonable to believe it could remain isolated without spread to neighboring facilities. To ensure isolated events are not missed, sampling must be extensive, covering many sites in a region. In contrast, VHSV IVb occurs in free-ranging wild or feral populations with less distinct boundaries. Natural routes of connectivity between areas make highly isolated occurrences of disease less likely. Thus, to assess regional status of VHSV IVb in natural waterways we selected the default value of 2% dp at the fish level, but elected a higher (10%) dp for geographic areas within a region. Targets were set bilaterally in surveillance working group discussions with CFIA and described in the bilateral VHSV surveillance plan (Bilateral VHSV Surveillance Working Group, 2007). The working group also agreed to a 95% confidence (or credibility) level where single values from probability distributions were required.

2.3.2. Absence of host species or temperatures

Absence of susceptible species is the second, and simplest, pathway for demonstration of disease freedom per OIE guidelines (OIE, 2013a, 2013b). Where host species susceptible to virus replication are absent, disease freedom status is obtained without surveillance. VHSV IVb is also temperature dependent, with cessation of virus replication at temperatures above 18–20 °C (Goodwin and Merry, 2011; Goodwin et al., 2012). Consequently, we extended this definition to include absence of conducive water temperatures. States satisfying either criterion were considered VHSV IVb free based on environmental conditions prohibitive to viral replication.

The VHSV expert panel defined VHSV IVb-conducive water temperatures as the presence of cool to cold water environments (VHSV Expert Panel and Working Group, 2010). Most States have local environments classified cool to cold water; however, in some southern States these environments are few in number and exceed 18 °C on a regular or seasonal basis. Brook trout (*Salvelinus fontinalis*) do not tolerate temperatures exceeding 20 °C for long duration. Thus, we selected the range of brook trout as a proxy for conducive conditions (http://www.natureserve.org/explorer/servlet/NatureServe?searchSciOrCommonName=brook+trout, data accessed July 2012). States lacking listed, self-sustaining brook trout populations were classified VHSV IVb free on the supposition that appropriate temperatures are not maintained year-round. Though absence of susceptible species or environments is a qualitative assessment, we infer that its endpoint confidence approximates that derived through targeted surveillance (95% credibility, given 2% and 10% dp for fish and sites, respectively).

2.3.3. Historic absence

Historic absence is the third approach to substantiating disease freedom per OIE guidelines (OIE, 2013a, 2013b). This designation requires 10 years of disease absence supported by basic biosecurity conditions for disease prevention, investigation, and reporting. This designation applies to countries or to zones, where a zone could be defined by a water catchment (OIE, 2013a, 2013b). Though historic absence is also qualitative, we again infer that its endpoint confidence approximates that derived through targeted surveillance.

Basic biosecurity conditions include systems to support early detection and compulsory notification, as well as import requirements to prevent disease introductions (OIE, 2013a, 2013b). VHS is notifiable for OIE member countries. Disease reporting in the U.S. is supported through State-Federal reporting systems (e.g., National Animal Health Reporting System) and mandated through legal requirements of the APHIS-accredited veterinarians constituting the vast majority of veterinary work force in the U.S. The appearance of VHSV IVb in the GL prompted government outreach and education to enhance public recognition and veterinary response. Initial detections also spurred enhancements in aquatic laboratory diagnostic capacity, such as groundwork to build a National Aquatic Animal Laboratory Network and diagnostic testing standards for select pathogens such as VHSV. Further, as noted above, in October 2006 the U.S. issued a FO restricting inter-State transfers of live regulated fish species from the GL States and affected Canadian Provinces. Consequently, we deemed U.S. basic biosecurity conditions active for VHSV IVb by the end of 2006 with a few caveats.

The first caveat is a variable State capacity to support aquatic disease detection and response. As part of a risk evaluation (VHSV Expert Panel and Working Group, 2010), State competent authorities for fish health were asked to judge the functionality of their fish health infrastructures. The query asked whether State "oversight (is) sufficient to ensure the timely investigation and reporting of VHSV outbreaks." An example of 'sufficient' described

regional availability of trained fish health professionals, oversight of fisheries and fish facility registration, movement permits and health certification, and a reliable system for the efficient response and reporting of aquatic disease outbreaks. Second, while the FO reduces opportunities for inter-State transfer of pathogens via commerce, it does not prevent natural movement of fish between States. Third, the historic absence argument presumes a pathogen would manifest clinically in observable susceptible species should it occur in a particular facility, zone, or country's environment (OIE, 2013a, 2013b).

Consequently, for this analysis, States began to accrue evidence of historic absence starting the end of October 2006, presuming they met the following conditions: (1) sufficient infrastructure as reported by State competent authorities for fish health, (2) separated from known infected waters by movement restrictions (State or Federal) and natural or man-made barriers preventing migration of free-ranging fish (Supplemental Materials), and (3) environmental conditions (e.g., species and temperatures) that support the clinical manifestation of disease. Thus, States with navigable connections to known affected waters or infrastructures insufficient to meet demands of fish health did not accrue historic absence credit in this analysis. Similarly, States with non-conducive temperatures for clinical expression were excluded from historic absence credit. However, temperatures precluding clinical expression of VHSV also preclude its viral replication. Consequently, States excluded by temperature extremes achieved VHSV IVb freedom status through the previous argument (Section 2.3.2).

2.4. The assessment framework

The odds form of Bayes' Theorem enables assessment of a region's disease probability through combination of surveillance and contextual information about the potential for pathogen introduction or establishment (Gustafson et al., 2010). The theorem can be written as follows:

$$\text{Posterior odds of disease} = \text{Prior odds of disease} \times \text{Likelihood Ratios}$$

Likelihood ratios (LRs), epidemiologic measures of association, here represent the predictive strengths of regional risk factors. The product of LRs applicable to a given region or State is hereafter termed the State's or region's risk score (RS). The prior odds of disease are here informed by surveillance results. Surveillance results include: (1) current surveillance data, (2) prior years' surveillance data devalued by time and introduction risk, and (3) the time elapsed (credibility accrued) toward a historic absence claim after meeting basic biosecurity conditions. Fig. 1 provides an overview of the evidence streams and assessment process.

2.5. Likelihood ratios for regional risk factors

An expert panel, following an Integrative Group Process, developed a set of conditionally independent LRs for VHSV IVb risk factors associated with population context prior to this study (VHSV Expert Panel and Working Group, 2010). Population context was defined as a collection of environmental and management factors characterizing a region. Risk factors deemed predictive of VHSV IVb status by the panel included proximity, trade, fomite, and hydrologic connection to known infected regions (VHSV Expert Panel and Working Group, 2010). Questions on regional status of these factors were posed to representatives from State agencies overseeing aquatic health. Information on waterway connectivity (http://water.usgs.gov/GIS/huc_name.html, http://cfpub.epa.gov/surf/locate/index.cfm, http://geology.com/state-map/), navigable waterways (http://www.accessscience.com/loadBinary.aspx?filename=345100FG0010.GIF), distance (GIS) and temperatures profiles were collected independently for comparison (accessed June 2013).

Where State and independent responses differed, the latter were selected to maintain consistency across regions. Results were used to calculate scores predicting VHSV IVb occurrence (risk score, RS) and introduction risk (IR) for each State or region.

2.6. Risk scores and introduction risk

Three summary measures of the VHSV IVb predictive strength of population context were used in this analysis. RS is the product of LRs for all context risk factors and is used in the Bayesian odds equation to balance surveillance with context. Introduction risk (IR) is the partial product of the subset of LRs representing pathways open to new or ongoing viral exposure. This includes all factors scoring LR > 1, excluding the disease establishment (i.e., non-pathway) factors 'presence of optimum temperature' and 'known susceptible species'. The IR is used to decrease the value of prior surveillance data through time, and stage the need for repeated surveillance (Gustafson et al., 2010). The IR*, in contrast, was here defined as a restricted form of IR that excludes anthropogenic factors and focuses only on natural pathways ('proximity by linear distance' and 'water connectivity').

2.7. Prior odds informed by surveillance

2.7.1. Current surveillance

The prior odds of disease for each State were informed by surveillance results. These were computed as disease probability divided by one minus disease probability, $p(d)/(1-p(d))$. Disease probability, $p(d)$, was formulated as a beta distribution, where beta parameters (a, b) represent the total number of positive and negative surveillance samples respectively, with the addition of a hyperprior (e.g., $a+1$, $b+1$), which is discussed below (Section 2.7.3). The output is a beta distribution for prevalence, and is the basis for statements such as "results provide 95% certainty that disease prevalence is less than 10%". When 95% certainty is the target, the 95th percentile of the beta distribution provides a conservative point estimate for disease probability.

The beta distribution structure can accrue evidence from repeated sampling efforts. For example, if one

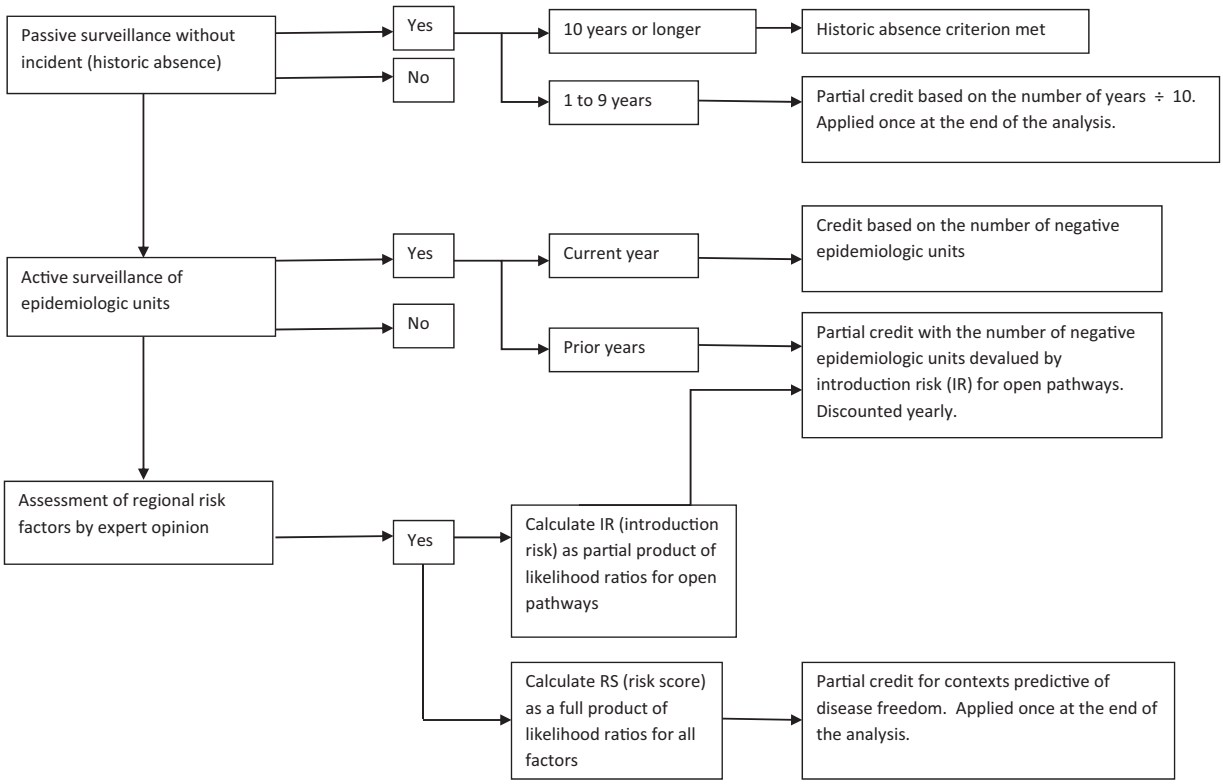

Fig. 1. Flow chart of factors to be considered in evaluating pathogen freedom for VHSV 1b and credit toward that target, assuming that susceptible host species and conducive water temperatures are present in a region.

survey generates *b* negative samples and a second generates c negative samples, their combination is expressed by a beta (1, $b+c+1$). If there is a need to discount historic data due to VHSV introduction risks in the interim, this can follow the same format (Section 2.7.2).

To address multi-stage sampling (e.g., fish within areas, areas within States), we compiled results for fish sampled in each geographic area, then compiled area information to infer State or regional status. Geographic areas were delineated according to USGS HUCs. The HUC system divides the U.S. into 21 hydrologically distinct regions, which are further subdivided into smaller units with increasing levels of hydrologic connectivity (http://water.usgs.gov/wsc/map_index.html; data accessed June 2007). Eight-digit HUCs were the epidemiologic unit for State inferences; 4-digit HUCs were the unit for regional inference. The credibility about dp derived from fish sampled in a given HUC is interpreted as the binomial probability of a HUC being VHSV free. The sum of derived credibility across HUCs ('HUC points') was used as the beta *b* parameter in subsequent State or regional analyses.

Fish populations were considered infinite for sample size and disease probability estimation. Populations of 8-digit HUCs within a given State, or 4-digit HUCs within the non-GL region of the U.S., were also considered infinite. However, 8-digit HUC analyses were conducted in parallel with finite population analyses to check whether conclusions from the simpler (infinite) model were robust. We used @Risk software to build beta distributions for infinite populations, and a hypergeometric approximation (Cannon, 2001) for finite populations. Calculations for finite populations proceeded in two steps: (1) determine a risk-adjusted target sample size given a finite population; and (2) compare this to the sample value obtained from temporally-revised surveillance and historic data. Target sample sizes can be calculated on-line (accessed January 31, 2014), FreeCalc (http://www.ausvet.com.au/content.php?page=software, or BayesFreeCalc http://www.epi.ucdavis.edu/diagnostictests/bayesfreecalc.html).

2.7.2. Time-devalued surveillance

Temporal devaluation adjusts previous surveillance data by VHSV IVb introduction risk, with the shortest data resilience corresponding to areas of greatest risk. The product of LRs for open pathways calculates the State's IR in the form of a Bayesian revision factor. The IR is used to revise posterior disease probabilities from previous assessments (Gustafson et al., 2010). The impact is approximated by IR division of the portion of the beta distribution parameter representing prior surveillance, beta (1, $b/\mathrm{IR}+c+1$). We devalued historic data by IR in each intervening period between surveillance assessments (annually). Current surveillance, '*c*', does not need temporal discounting until its subsequent period.

2.7.3. Historical absence hyperprior

The beta distribution for prevalence requires a hyperprior. This hyperprior represents prior expectation of disease status, either informed or uninformed by existing knowledge. Where prior knowledge is lacking, we assign a uniform (1, 1) distribution, i.e., the source of the 1's in the beta (1, b/IR + c + 1). However, where basic biosecurity conditions are in place and criteria met for historic absence we allowed the hyperprior to be informed instead by the number of years elapsed without incident under passive surveillance. Presuming 10 years of historic absence despite vigilant conditions is comparable to successful completion of active surveillance, we assigned 95% credibility that prevalence is less than 10% among geographic units to both endpoints. We interpret less than 10 years of accrual as partial evidence toward the same goal. For example, by the end of 2012, 6.17 years had elapsed since October 2006 (date of initiation of the FO). For States meeting historic absence conditions, we interpreted 6.17/10 years without incident as providing 61.7% of the needed 95% credibility that disease prevalence is less than dp, here 10% (i.e., 0.10 is the 61.7th percentile on a prevalence distribution). This percentile, coupled with a mode of zero denoting all negative results, can generate a beta distribution (Betabuster, available at http://www.epi.ucdavis.edu/diagnostictests/betabuster.html, accessed January 31, 2014). The b parameter that results, here 9.11, was used as the informed hyperprior (historic) in the final beta distribution, beta (1, b/IR + c + historic), representing disease probability for the region for 2012 calculations. The relationship between years elapsed and sample credit is non-linear and weighted most heavily in later years. It can be adjusted for finite population sizes using hypergeometric approximations (Supplemental Material). States not meeting historic absence requirements were assigned an uninformative beta (1, 1) hyperprior.

Some States that met historic absence conditions did have introduction risks due to non-controlled factors, e.g., movements of frozen fish, fomites or wastes. Others declared fish transfer risks despite the FO, perhaps reflecting a potential for illegal transfers, or questioning whether the FO list of susceptible species was complete. As time elapses without disease, however, confidence in the efficacy of adopted mitigations should improve. Thus, we allowed 10 years of historic absence to effectively neutralize remaining introduction risk.

2.8. Approximations

We used approximations to the Bayesian odds method to facilitate analysis and discussion. All adjust effective sample size and thereby facilitate comparison of each evidence stream's impact on the final inference. This is because the approximations modify the beta distribution parameter b representing negative samples; adjustments to b result in greater or lesser estimates of VHSV probability; and b equals sample size when surveillance is negative.

Using these approximations, whether and when States meet disease freedom standards is determined by comparing their total surveillance value to the sample size target.

$$\text{Sample size target} = \text{Traditional sample size} \times \text{RS}$$

$$\text{Total surveillance value} = \text{Accrued sample credit} + \text{Historic absence credit}$$

Accrued sample credit is estimated as follows:

$$\text{Accrued sample credit} = \text{New sample credit} + \text{Time-devalued sample credit}$$

$$\text{New sample credit} = \text{New surveillance sample size} \times \text{Se}$$

$$\text{Time-devalued sample credit} = \frac{\text{Prior years accrued sample credit}}{\text{IR}}$$

Presuming RS, IR, historic absence credits, and sensitivity estimates are known, approximations allow surveillance needs to be calculated by hand. The impact of RS and IR on sample size values and targets, captured in the above equations, are previously described (Gustafson et al., 2010). Historic absence credit, represented by a sample size of comparable value, is also previously described (Section 2.7.3). The impact of imperfect sensitivity on sample value is derived from standard equations for true (TP) versus apparent (AP) prevalence assuming perfect specificity, namely AP = TP × Se.

$$\text{TP} = \frac{\text{AP}}{\text{Se}} = \frac{\text{Positives}/n}{\text{Se}} = \frac{\text{Positives}}{n \times \text{Se}},$$

where n refers to sample size.

Given all negative results and perfect specificity, this relationship calculates new sample credit (sensitivity-adjusted) from sample size × Se. For example, a sample size of 10 sites, each with 90% sensitivity of detecting disease at or above dp, is credited at 10 × 0.9 = 9 sites ('HUC points') after accounting for imperfect sensitivity. This relationship applies to all stages of surveillance (fish within HUCs, HUCs within States) with imperfect Se.

Hypergeometric approximations were used to determine traditional sample sizes for finite populations (Cannon, 2001). For example, a traditional sample size of 29 sites can achieve 95% credibility that disease is below 10% dp across geographic units (presuming perfectly accurate tests, all-negative results and an infinite population). A protective RS (<1) effectively reduces this target. For a State with an RS of 0.4, the approximation suggests the State need only sample 29 × 0.4 = 11.6 sites to achieve the same target assurance. Rounding and adding one to the result helps to ensure a conservative estimate; here, it is 13 sites.

These approximations overestimate the credibility derived from very small sample sizes (e.g., 1–2 sites) due to the assumption that disease probability fits a beta distribution (with a narrow tail). However, as small sample sizes typically do not support conclusions of disease freedom, we expect decision equivalence in most cases. Approximations also assume point values for LRs. Consequently, when it is necessary to capture uncertainty in the LRs, or when conclusions are near decision thresholds, a full Bayesian simulation is recommended.

2.9. Example assessment

A State conducts VHS IVb surveillance in years 2011 and 2012. In 2011, the State samples 17 HUCs by collecting 100 fish from each. All results are negative. Presuming a test sensitivity of 85%, the adjusted sample (100 × Se) approximates 85 fish. The resulting beta distribution, beta (1, 85 + 1), assigns 82% credibility about disease absence (for 2% dp) among fish within any given HUC. Summing this credit over all tested HUCs (17 × 0.82) gives a sensitivity-adjusted sample size of approximately 14 HUC points for the State.

The State is connected by navigable waterways (LR = 3.16) and there is potential transfer of untested frozen fish (LR = 2.24) from known positive regions. Its IR is the product of the two LRs (equal to 7.08). Thus, after a single year, HUC points for 2011 are reduced by 1/7 to an IR-adjusted (time-devalued) sample size of approximately 2. Therefore, if the State follows the same sampling protocol in 2012, it accrues 14 new HUC points from 2012 and two reduced HUC points from 2011. The total does not meet target sample size (29 sites) for 95% credibility about 10% dp among HUCs. However, the resulting beta distribution, beta (1, 2 + 14 + 1), concludes 83% credibility about disease absence (for 10% dp) among HUCs within the State for 2012. Accrued credit is adjusted each time it is carried into a subsequent year; hence data devalue in accordance with their time since collection.

This particular State scored an RS > 1. However, if it had scored low risk for its general context (RS < 1), we could compare its accrued HUC points to the RS-reduced target to determine when and whether it compiled sufficient evidence to claim freedom.

This particular State is also disqualified from historic absence credit due to its navigable connection to known affected waters. However, if it did qualify, it would have accrued 9 additional points by the end of 2012 for 6.17 years of observation without incident. A 9 would replace the default "1" as a hyperprior in the beta distribution, beta (1, 2 + 14 + 9), to afford 92% credibility for the State. Though this is very close to the target (95%), note that the sample-based portion (2 + 14) of the credibility is transient without continued sampling. Thus, disease freedom claims are only resilient over time in States with negligible risks of introduction (IR = 1), ongoing surveillance, or where criteria are met for claims of historic absence.

3. Results

3.1. Participation

Eighty-two percent (41/50) of States and one Territory participated in active surveillance, risk evaluation, or both. Active surveillance results were available from 29 States; risk evaluations were available from 40 States and one Territory. All but one State sharing active surveillance data completed a risk evaluation. This State, without a risk evaluation (and thus IR and RS metrics), did not meet disease freedom targets. Though voluntary, the participation rate was high, with only 9 States declining any participation. States not contributing data are still obligated to report disease findings in compliance with OIE member country agreements. APHIS-accredited veterinarians are further required to report suspicious or confirmed findings to their APHIS area veterinarian-in-charge. Consequently, lack of State participation does not imply lack of cooperation, but rather competing surveillance priorities.

3.2. Basic biosecurity conditions

Activities were undertaken during 2006–2012 to bolster national or regional biosecurity conditions. Examples include public campaigns to educate recreational boaters and fishermen on about VHSV IVb relevance, mechanisms of spread, associated precautions, and networks for reporting clinical signs (http://www.focusonfishhealth.org/, accessed January 31, 2014). Laboratory capacity and research developments improved diagnostic capacity and availability. Database development was supported to improve compatibility across agencies (http://gis.nacse.org/vhsv/, accessed January 31, 2014). Epidemiologic and pathologic research helped answer questions such as whether shipping was a risk factor in disease spread (Bain et al., 2010) and whether VHSV IVb or other novel pathogens were present in the GL system prior to their original detection. Training of veterinarians in aquatic animal health emergencies was strongly encouraged and supported. Advancements in biosecurity guidance for partially open systems, and efforts to coordinate legislation between States were also supported.

3.3. Evidence sources

3.3.1. Active surveillance

Twenty-nine states (eight GL States, 21 other) provided results from active surveillance: 14 States included only wild fish data, 12 included both wild and farmed fish data, and 3 States included only farmed fish data. There are 21 regions (2-digit HUCs), 221 subregions (4-digit HUCs) and 2264 cataloging units (8-digit HUCs) in the U.S (Supplemental Materials).

3.3.1.1. Wild fish locations. Sampling covered 21 States and 88 4-digit HUCs (Fig. 2). Despite intensive efforts, none of the States compiled sufficient wild fish data to substantiate regional disease freedom status based on active surveillance alone. Some States came close in individual years. However, adjustments for open introduction pathways (IR > 1) caused poor data resilience through time.

3.3.1.2. Farmed fish locations. An average of 74 farms was sampled annually, with 1 to 10 States involved in any given year (Fig. 3, and Supplemental Materials). All test results were negative.

3.3.2. Passive surveillance

States are obligated to report to the Federal Government any VHSV IVb detections occurring in new species or new regions. However, the reporting of negative surveillance results is not similarly required, unless funded by

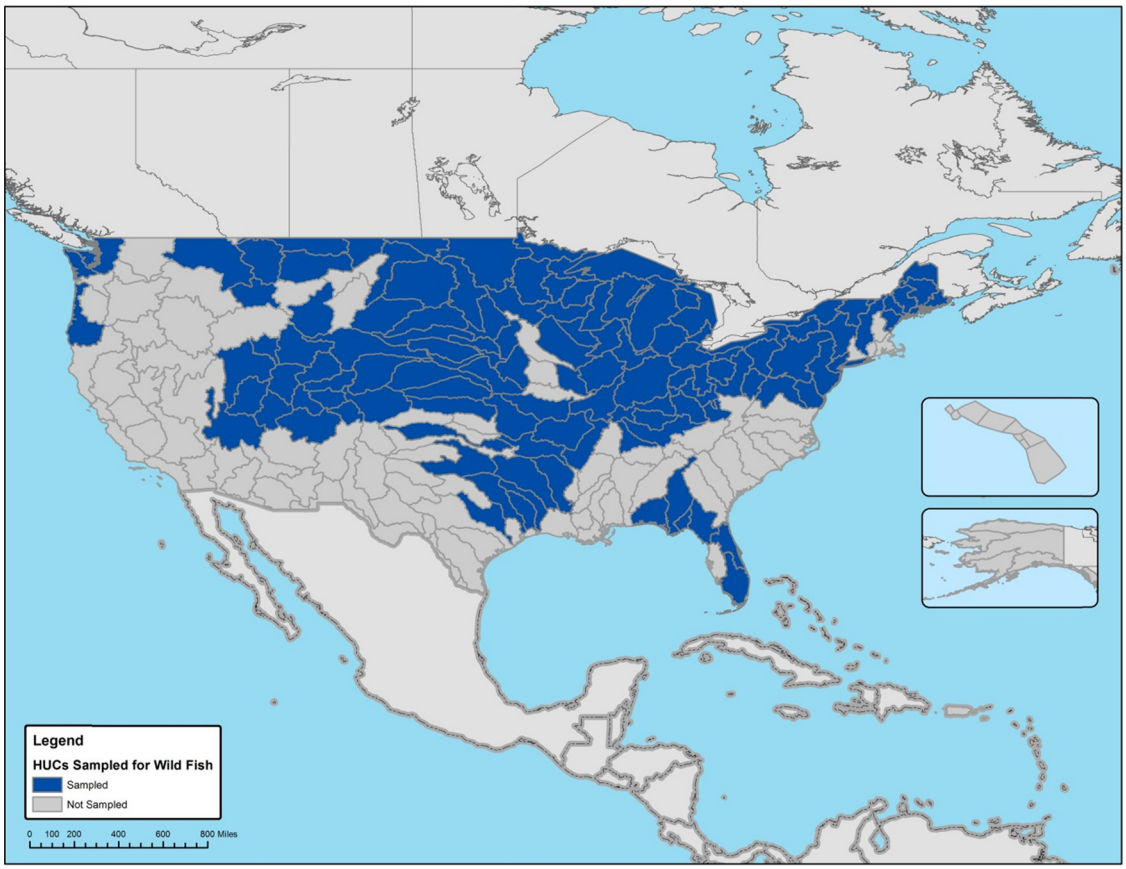

Fig. 2. Locations of VHSV IVb surveillance data from free-ranging fish populations. Boundaries describe 4-digit HUCs. Other locations may also conduct surveillance, but outside of cooperative agreement. Non-participating locations would still be obligated to report positive, but not negative, data to APHIS VS. Consequently, this map provides the minimum distribution of free-ranging fish population sampling.

APHIS cooperative agreement. Consequently, the performance of passive surveillance could not be evaluated with this dataset. However, trends shared by the State of Wisconsin (personal communication, S. Marcquenski) suggest that passive surveillance may be at least as likely as active to identify new cases of VHSV IVb infection.

WI Department of Natural Resources conducts active and passive surveillance (Meyer and Barclay, 1990). Active sampling involves the collection of 60–150 fish, typically of healthy appearance, per location. Passive systems involve testing up to 10 moribund fish to investigate fish kills or lesions noted by creel clerks, tournament biologists, commercial harvest fishermen, etc. During 2007–2011, VHSV IVb was detected in 5 different 8-digit HUCs covered by both active and passive surveillance. Both systems detected VHSV IVb in one of the 5 geographic units. Passive surveillance alone was responsible for detections in another 3, while active surveillance alone was responsible in the remaining one. Randomized selection of water bodies within a surveyed HUC may explain one active surveillance failure (zero fish sampled in that particular lake); inopportune timing or low prevalence may account for the other 2.

3.3.3. *Risk scores and introduction risk*

RS predicts VHSV IVb status from regional context. Eighteen of 40 States completing the risk evaluation, as well as the single Territory, produced scores predicting VHSV IVb absence (RS < 1). The remainder, including all 8 GL States, calculated scores > 1 suggesting a higher risk context. RS > 1 were capped at one to avoid increasing surveillance requirements beyond internationally accepted standards.

IR is the partial product of the subset of LRs representing pathways open to new or ongoing viral exposure. Thirty-five of 40 States, including all GL States, reported IR values > 1. The IR*, in contrast, excludes anthropogenic factors. As it assumes appropriate mitigations for anthropogenic factors (the best possible IR), we could assess IR* for all States regardless of whether they submitted a risk evaluation (Supplemental Materials). Accordingly, of 42 non-GL States, 21 were considered connected to known affected regions either by 'navigable waterways' (20), linear distances of less than 100 km (1), e.g., covering distances a predatory bird or mammal might range and move fish as part of their feeding patterns, or both (1). Remaining States have the potential to mitigate risk of future introductions (IR* < 1), e.g., through

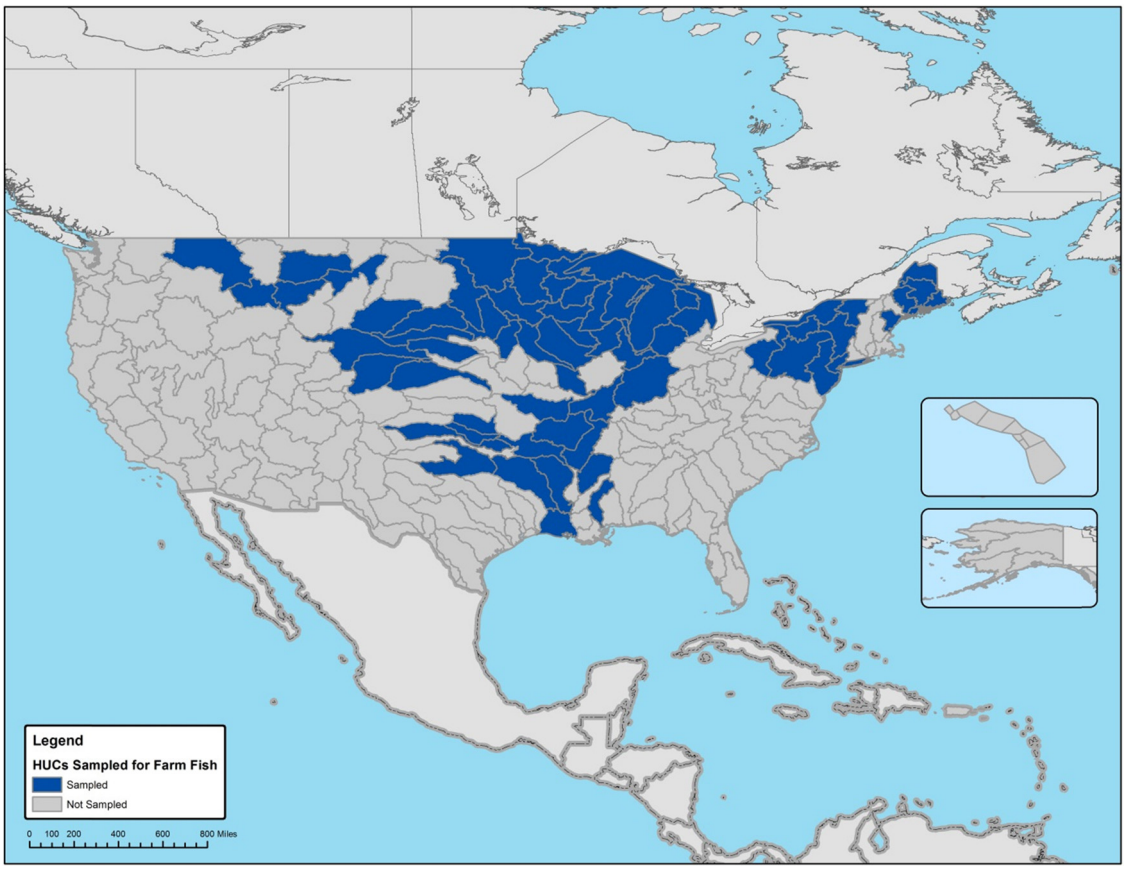

Fig. 3. Locations (4-digit HUC) of VHSV IVb surveillance data from farmed fish facilities. Additional locations may conduct surveillance outside of cooperative agreement and thus not obligated to report negative data to APHIS VS. Non-participating locations are still obligated to report positive findings.

movement restrictions and educational campaigns to reduce un-intentional spread via live fish transfers, frozen fish, boats, or equipment.

The most prevalent of the anthropogenic risk factors (i.e., factors for which mitigations are plausible) were the potential for fomite exposure (20/41 States) and untested import of frozen fish (15/40 States) used as feed or bait (Supplemental Materials). Twenty-nine of 41 respondents claimed infrastructure sufficient to support passive surveillance.

3.4. VHSV IVb status assessment: great lakes states

To date, VHSV IVb has been found only in GL States (Fig. 4 and Supplemental Materials) and only in wild or feral fish. Since its initial discovery, VHSV IVb has been isolated from fish from all major water bodies of the GL system, including Lake Erie, Lake Huron, Lake Michigan, Lake Ontario, Lake St. Clair, Lake Superior, and the St. Lawrence Seaway (Thompson et al., 2011). VHSV IVb has also been isolated in several inland lakes of Michigan, New York, Wisconsin, and Ohio. Since 2007, the National Veterinary Services Laboratories (NVSL) has been asked to confirm detections in new species or regions. However, insufficient sample quality, or the inability to re-sample the same free-ranging population, can hinder confirmation. Consequently, VHSV IVb virus isolations from laboratories involved in cooperative agreement sampling were also considered positive detections for this report.

GL States tested extensively outside of known-affected 4-digit HUCs. Portions of some States achieved substantive credibility in disease absence in select years of sampling; however, due to a relatively high IR associated with proximity via navigable waterways and distance, these data show limited resilience through time. Consequently, disease freedom derived for a portion of a GL State would likely require ongoing surveillance to maintain. Except for Clear Fork Reservoir and Waukegan Harbor, all VHSV IVb affected locations reside within the GL Region, a watershed coded by the HUC classification system as Region 04. Clear Fork Reservoir is an inland lake in the Ohio River watershed (Region 05), just 3.5 miles from Region 04. Waukegan Harbor, associated with the Upper Mississippi watershed (HUC 07), abuts Lake Michigan.

3.5. VHSV IVb status assessment: non-great lakes states

3.5.1. Absence of species or temperatures conducive to viral replication

All participating States acknowledged the presence of VHSV IVb susceptible species, and environments in 41 of 50 States were classified with cool to cold water. The

Fig. 4. Known extent of VHSV IVb in U.S. freshwater systems. HUCs classified as 'abutting' include the shorelines of Great Lakes not otherwise included in the list of positive 4-digit HUCs. Positives denote HUCs with positive virus isolations made by NVSL or participating laboratories.

participating Territory reported lack of both VHSV IVb susceptible species and cool to cold water environments. An absence of self-sustaining brook trout populations led 9 States to be classified too warm to support VHSV IVb viral replication year-round (Alaska, Florida, Hawaii, Kansas, Louisiana, Missouri, Mississippi, Oklahoma, and Texas). These States were categorized VHSV IVb free based on non-conducive temperatures alone. Seven of the 9 high temperature States also compiled risk evaluation or surveillance evidence to further support in full (1 State) or in part (6 States) their disease freedom status. However, surveillance and risk evaluation data from these States are presented as ancillary information only. After excluding the 8 GL States classified as VHSV IVb affected, and the 9 States and single Territory considered free per temperature exclusion, 33 States remained for formal VHSV IVb probability estimation.

3.5.2. Historical absence

Eleven non-GL States were eligible for historic absence credit based on reported functional fish health infrastructures, environments supporting disease expression, and absence of navigable connections to known affected regions. The value of historic absence increases over time (Supplemental Material) until the 10-year mark when it can be used as an independent mechanism for substantiating disease freedom following OIE guidelines. For States meeting historic absence criteria, the number of years elapsed since 2006 was used to generate an informed hyperprior probability of disease. Based on this logic, all 11 States will be considered disease free if they remain without incident through 2015. States that did not meet the described criteria were assigned a uniform (i.e., uninformed) hyperprior.

3.5.3. Risk-based surveillance evidence for disease freedom

Active surveillance evidence alone was insufficient to demonstrate freedom for any of the States. However, regional risk factors and historic absence were used to supplement active surveillance. Risk-based analysis achieved disease freedom targets for 5 of 33 States requiring disease probability assessment. All met historic absence criteria so should retain disease free status in 2016. Another 17 States accrued transient or partial evidence of disease freedom in 2012. Of these, 6 met historic absence criteria so should achieve full status by 2016 (Supplemental Materials). Conclusions were robust to changes in estimated test sensitivity (85% or 25%) and finite versus infinite population assumptions.

3.6. VHSV IVb status assessment: non-great lakes region

Aggregating results by HUC (rather than State) for a regional assessment gives 95% credibility that VHSV IVb would have been detected by 2012 if present in 4.1% or more of the 169 4-digit HUCs located fully in the non-GL region. This level of credibility is projected to be maintained in 2016 (95% credibility for 4.4% dp) and was robust to changes in estimated test sensitivity. Finite population adjustments were not conducted, but would improve confidence.

4. Discussion

Available evidence streams were combined to infer State and regional status for the emerging aquatic animal pathogen VHSV IVb (Gustafson et al., 2010). Assessments were completed at the State and non-GL region levels; however, distinct status HUCs within VHSV IVb affected States may also be identified using similar methods. Evidence streams included (1) active (test-based) surveillance data, (2) historic absence data, and (3) regional risk factors predictive of disease occurrence and introduction.

Surveillance can be costly and challenging in open and changing systems. The Bayesian odds method measures the impact of each evidence stream through its contribution to an effective sample size, and as such helps identify key information sources, knowledge gaps, and the relative efficiency of surveillance strategies and alternatives. The method supports surveillance reductions in regions characterized by historic absence or minimal risk environments, and directs surveillance resources to regions characterized by high disease risk or uncertainty. The method also helps identify alternatives to frequent sampling in high risk regions, such as mitigations to prevent introductions or infrastructure to enhance passive surveillance. By linking impacts to sample size, costs of such investments can be compared to savings achieved through consequent reductions in ongoing sampling.

VHSV IVb analyses highlight the impermanence of surveillance data in open systems. Despite substantive effort, none of the States were able to compile sufficient surveillance data to meet VHSV IVb disease freedom targets alone. This was due, in part, to the temporal devaluation of data from open systems. However, complementary use of alternative evidence streams allowed science-based conclusions to extend to a majority of the U.S. Ultimately, 14 of the non-GL States and one Territory met disease freedom targets in 2012 (8 by temperature exclusion alone, 5 by risk-based analysis alone, and 1 by both), and another 17 achieved partial credibility, despite the impermanence of surveillance data (Fig. 5). Assessed in aggregate, the non-GL region met disease freedom target in 2012, and is projected to maintain this status in 2016 without additional active surveillance.

Disease freedom status depends on the maintenance of basic biosecurity conditions including movement restrictions and passive surveillance. Regions with navigable connections to VHSV IVb-affected HUCs should receive priority for further surveillance or capacity building activities. However, results suggest that even spread via natural pathways may be contained to the GL system. Future research may find, for example, whether key susceptible species exhibit localized ranges or reduced movement during viral shedding. Similarly, fish barriers to dissuade fish passage are present in many waterways and may be efficacious at limiting spread. Regardless, the continued absence of detection in non-GL States suggests that existing controls, natural and applied, are successfully limiting disease spread.

Results facilitate strategic planning for VHSV IVb and other aquatic animal pathogens. The current analysis attributes only transient value to active surveillance in regions with pathways open to new introductions of VHSV IVb. In many non-GL States the value of past years' sampling declined rapidly, emphasizing the long-term importance of resources spent on mitigations to close introduction pathways or operationalize systems for passive surveillance. Similarly, GL States did extensive sampling outside their known affected HUCs. In these States, surveillance data identify regions still free of the VHSV IVb pathogen and help to focus legislation or public education campaigns to limit spread within the State. Combined with basic biosecurity to maintain distinct health status between zones, surveillance results could support reduced risk status for select protected regions in GL States.

VHSV IVb surveillance was conducted in both wild and farmed populations. No positive results were found in farmed populations. However, facility testing was voluntary and, in some cases, anonymous. Without demographic information to categorize sites by facility type or representation, it is difficult to make meaningful inferences about disease freedom. Farms may range, for example, from highly biosecure compartments to systems on untreated surface waters, from single to multiple species and single to multiple age classes. With additional data to characterize these operations, States could potentially use the open system portion (reflecting the disease status of surrounding populations) as further evidence of disease status for their region. Alternatively, individual States could use results from the highly biosecure portion to substantiate a distinct health status for represented farming groups or compartments.

Regardless, the lack of positive findings in any farmed fish facility is noteworthy. Even in GL States with immediate proximity to known VHSV IVb positive waters, surveyed farms maintained a distinct health status from wild populations. Sampled farms likely represent the most secure facilities, as farms participated on a voluntary basis. However, these data suggest that existing farming practices, even in areas deemed relatively high risk (e.g., GL States), have the potential to effectively mitigate risks of exposure to endemic disease.

Published evidence suggest that virus isolation is not as sensitive for VHSV IVb detection as projected (< 85%) (Bain et al., 2010; Hope et al., 2010; Frattini et al., 2011). There is also indication that baseline prevalence of VHSV IVb in infected populations may be higher than projected (Bain et al., 2010; Hope et al., 2010; Frattini et al., 2011;

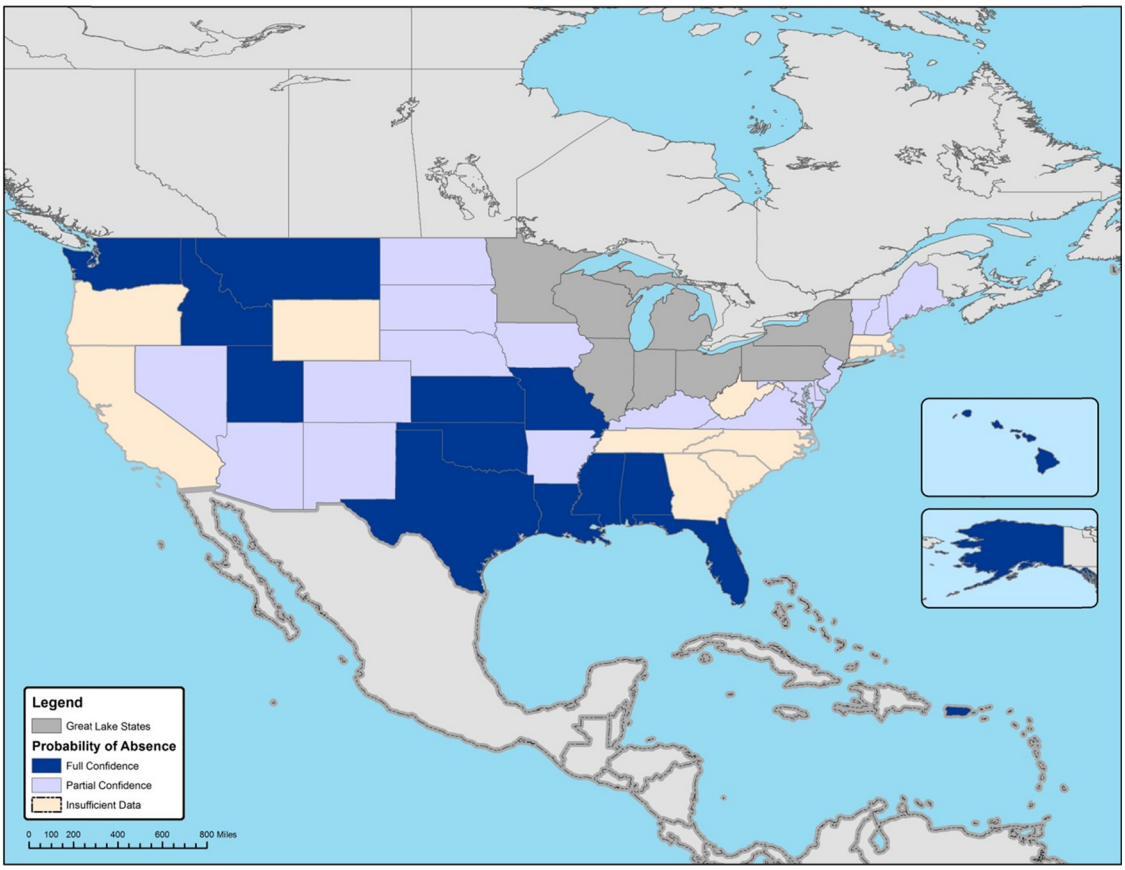

Fig. 5. Strength of conclusions about VHSV IVb absence in 2012, including States achieving disease freedom via temperature constraints along with States achieving disease freedom via surveillance and historic absence. Full confidence indicates 95% credibility that disease is present at less than design prevalence of 10% among geographic units (8 digit HUCs). Partial confidence indicates evidence is available, but provides less than 95% credibility.

Cornwell et al., 2012a), though others suggest that a low dp, such as 2%, is appropriate in some contexts (Cornwell et al., 2012a, 2012b). However, conclusions were invariant to the lowered test sensitivity (25%). Raising dp at the fish level to 5% from 2% would only improve disease status designations.

The minimal impact of test sensitivity in this analysis likely reflects data impermanence modeled for open systems, the high volume of fish sampled at some sites, and the observation that changes in diagnostic test sensitivity cause less substantive impacts to conclusions when aggregated for a higher (State or regional) level of analysis. Consequently, less costly or more sustainable testing strategies for ongoing surveillance in open systems are needed. Strategies may include observational surveillance, RT-PCR screening, or others. The key to incorporating new modalities is that their process is standardized, estimates of test accuracy are available, and confirmatory testing protocols are highly specific. Misclassification by false-negative results is limited by sufficient sample size and repeated sampling, providing suboptimal sensitivity is captured in analyses. However, mistaken conclusions due to false-positive results are not easily rectified and are likely of high and immediate consequence to trade or population enhancement activities. Lost freedom status is difficult to recover without years of routine surveillance.

Test accuracy impacts surveillance conclusions through differing sample sizes required to meet confidence targets. However, decisions on location, species of fish, and time periods to focus structured surveillance also influence conclusions. For example, a dp guided by experiments in one species may be too high if broadly applied to less susceptible species or life stages, or less conducive environments. Similarly, surveillance-based conclusions are subject to waning of confidence as opportunities for new virus introductions arise, especially in open systems. To best guide decisions, it is instructive and efficient to balance surveillance and associated uncertainty with a depiction of the risk (or stability) of the population's context.

Our results are provisional as we have not incorporated a mechanism to validate the accuracy of State risk survey responses or the comparability of passive surveillance systems. Similarly, we used USGS HUC delineations available at the time of FO implementation and revision; changes adopted since have not been considered here. Conclusions depend on the validity of LRs estimated by expert panel (VHSV Expert Panel and Working Group, 2010). Similar to validating sensitivity or dp assumptions, field data

is needed to measure the accuracy of constructed LRs. Empirical validation of RS and IR is constrained by the small number of positive regions available for analysis. All VHSV IVb detections occurred within the GL States (RS > 1); none of the States with RS < 1 detected VHSV IVb during subsequent surveillance. Partnering with a GL State that conducted extensive research in positive waters (using a smaller definition of region) might allow for construction of this type of localized analysis.

Similarly, the value of historic absence was gauged in proportion to OIE benchmarks for disease freedom. While many surveillance programs emphasize active surveillance, open systems may require complementary systems of detection for VHSV IVb or other pathogens. Where clinical signs permit opportunistic selection of moribund fish, passive surveillance may in some situations be more efficient than active surveillance. However, these suppositions, and the credibility of historic absence claims, also require field validation.

Finally, approximations to Bayesian odds analysis based on sample size adjustments simplify calculation and discussions about the relative contributions of the evidence streams. However, development of Bayesian computer code for future analyses would facilitate processing large quantities of data in a structured and simplified format and also facilitate simulations to explore parameter ranges within which approximations are most accurate.

This report evaluates historic absence and risk of regional context alongside surveillance evidence to ascertain VHSV IVb status. Given the use of novel evidence streams, it is prudent to confer with decision makers and trade partners to agree on decision criteria and analytic structure in advance of this type of analysis. U.S. trade of VHS susceptible fish principally occurs between States or Canadian provinces. This allowed us to negotiate disease-specific detection thresholds and adopt new strategies toward crediting partial historic absence and risk through population context (Bilateral VHSV Surveillance Working Group, 2007).

Acknowledgments

This project was successful in large part due to stakeholder advice and support. We thank the many individuals representing State, Tribal Nation, laboratory, academic and industry cooperators who provided data, support and direction for this study. We also thank the affiliated agencies and industries, and the Canada Excellence Research Chairs program for supporting time and funds for these efforts. Finally, we thank the members of the VHSV Expert Panel for their continuing correspondence regarding questions related to VHSV IVb epidemiology and detections.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2014.02.011.

References

AFS-FHS (American Fisheries Society – Fish Health Section), 2010. Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens. AFS-FHS (American Fisheries Society - Fish Health Section), Bethesda, MD http://www.fws.gov/pacific/fisheries/fishhealth/Documents/BlueBook/no_index/group1/toc.pdf (accessed 31.01.14).

Bain, M.B., Cornwell, E.R., Hope, K.M., Eckerlin, G.E., Casey, R.N., Groocock, G.H., Getchell, R.G., Bowser, P.R., Winton, J.R., Batts, W.N., Cangelosi, A., Casey, J.W., 2010. Distribution of an invasive aquatic pathogen (viral hemorrhagic septicemia virus) in the GL and its relationship to shipping. PLOS ONE 5, e10156 http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0010156 (accessed 31.01.14).

Bilateral VHSV Surveillance Working Group, 2007. Surveillance Proposal for Viral Hemorrhagic Septicemia Virus in Freshwater Fish in Canada and the United States, Version 1.0. http://www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/downloads/vhs_surv_plan.pdf

Cannon, R.M., 2001. Sense and sensitivity – designing surveys based on an imperfect test. Preventive Veterinary Medicine 49, 141–163.

Cornwell, E.R., Eckerlin, G.E., Thompson, T.M., Batts, W.N., Getchell, R.G., Groocock, G.H., Kurath, G., Winton, J.R., Casey, R.N., Casey, J.W., Bain, M.B., Bowser, P.R., 2012a. Predictive factors and viral genetic diversity for viral hemorrhagic septicemia virus infection in Lake Ontario and the St. Lawrence River. Journal of Great Lakes Research 38, 278–288.

Cornwell, E.R., Getchell, R.G., Groocock, G.H., Walsh, M.G., Bowser, P.R., 2012b. Low prevalence of VHSV detected in round goby collected in offshore regions of Lake Ontario. Journal of Great Lakes Research 38, 575–579.

Elsayed, E., Faisal, M., Thomas, M., Whelan, G., Batts, W., Winton, J., 2006. Isolation of viral haemorrhagic septicaemia virus from muskellunge, *Esox masquinongy* (Mitchill), in Lake St-Clair, Michigan, USA reveals a new sublineage of the North American genotype. Journal of Fish Diseases 29, 611–619.

Faisal, M., Shavalier, M., Kim, R.K., Millard, E.V., Gunn, M.R., Winters, A.D., Schulz, C.A., Eissa, A., Thomas, M.V., Wolgamood, M., Whelan, G.E., Winton, J., 2012. Spread of the emerging viral hemorrhagic septicemia virus strain, genotype IVb, in Michigan, USA. Viruses 4, 734–760.

Frattini, S.A., Groocock, G.H., Getchell, R.G., Wooster, G.A., Casey, R.N., Casey, J.W., Bowser, P.R., 2011. A 2006 survey of viral hemorrhagic septicemia (VHSV) virus type IVb in New York State waters. Journal of Great Lakes Research 37, 194–198, http://dx.doi.org/10.1016/j.jglr.2010.11.004.

Goodwin, A.E., Merry, G.E., 2011. Mortality and carrier status of bluegills exposed to viral hemorrhagic septicemia virus genotype IVb at different temperatures. Journal of Aquatic Animal Health 23, 85–91.

Goodwin, A.E., Merry, G.E., Noyes, A.D., 2012. Persistence of viral RNA in fish infected with VHSV-IVb at 15 °C and then moved to warmer temperatures after the onset of disease. Journal of Fish Diseases 35, 523–528.

Groocock, G.H., Getchell, R.G., Wooster, G.A., Britt, K.L., Batts, W.N., Winton, J.R., Casey, R.N., Casey, J.W., Bowser, P.R., 2007. Detection of viral hemorrhagic septicemia in round gobies in New York State (USA) waters of Lake Ontario and the St. Lawrence River. Diseases of Aquatic Organisms 76, 187–192.

Groocock, G.H., Frattini, S.A., Cornwell, E.R., Coffee, L.L., Wooster, G.A., Getchell, R.G., Bowser, P.R., 2012. Experimental infection of four aquacultured species with viral hemorrhagic septicemia virus type IVb. Journal of the World Aquaculture Society 43, 459–476.

Gustafson, L., Klotins, K., Tomlinson, S., Karreman, G., Cameron, A., Wagner, B., Remmenga, M., Bruneau, N., Scott, A., 2010. Combining surveillance and expert evidence of viral hemorrhagic septicemia freedom: a decision science approach. Preventive Veterinary Medicine 94, 140–153.

Hope, K.M., Casey, R.N., Groocock, G.H., Getchell, R.G., Bowser, P.R., Casey, J.W., 2010. Comparison of quantitative RT-PCR with cell culture to detect viral hemorrhagic septicemia virus (VHSV) IVb infections in the Great Lakes. Journal of Aquatic Animal Health 22, 50–61.

Kane-Sutton, M., Kinter, B., Dennis, P.M., Coonce, J.F., 2010. Viral hemorrhagic septicaemia virus infection in yellow perch, Perca flavescens, in Lake Erie. Journal of Great Lakes Research 36, 37–43.

Kim, R., Faisal, M., 2010a. Experimental studies confirm the wide host range of the Great Lakes viral haemorrhagic septicaemia virus genotype IVb. Journal of Fish Diseases. 33, 83–88.

Kim, R., Faisal, M., 2010b. The Laurentian Great Lakes strain (MI03) of the viral haemorrhagic septicaemia virus is highly pathogenic for juvenile muskellunge *Esox masquinongy* (Mitchill). Journal of Fish Diseases 33, 513–527.

Kim, R., Faisal, M., 2010c. Comparative susceptibility of representative Great Lakes fish species to the North American Viral Haemorrhagic Septicaemia Virus (VHSV) Sublineage IVb. Diseases of Aquatic Organisms 91, 23–34.

Lumsden, J.S., Morrison, B., Yason, C., Russell, S., Young, K., Yazdanpanah, A., Huber, P., Al-Hussinee, L., Stone, D., Way, K., 2007. Mortality event in freshwater drum Aplodinotus grunniens from Lake Ontario, Canada, associated with viral haemorrhagic septicemia virus, Type IV. Diseases of Aquatic Organisms 76, 90–111.

Meyer, F.P., Barclay, L.A., 1990. Field manual for the investigation of fish kills. In: Department of the Interior, Fish and Wildlife Service, Publication 177.

OIE (World Organisation for Animal Health), 2013a. Aquatic Animal Health Surveillance, Aquatic Animal Health Code. http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_1.1.4.htm (accessed 31.01.14).

OIE (World Organisation for Animal Health), 2013b. Manual of Diagnostic Tests for Aquatic Animals. http://www.oie.int/international-standard-setting/aquatic-manual/access-online/ (accessed 31.01.2014.

Thompson, T.M., Batts, W.N., Faisal, M., Bowser, P., Casey, J.W., Phillips, K., Garver, K.A., Winton, J., Kurath, G., 2011. Emergence of viral hemorrhagic septicemia virus in the North American Great Lakes region is associated with low viral genetic diversity. Diseases of Aquatic Organisms 96, 29–43.

VHSV Expert Panel and Working Group, 2010. Viral hemorrhagic septicemia virus (VHSV IVb) risk factors and association measures derived by expert panel. Preventive Veterinary Medicine 94, 128–139.

USDA APHIS VS, 2008a. Species regulated by title 9 CFR Parts 83.1 through 83.7, 93.900 and 93.910 through 93.916 (the Viral Hemorrhagic Septicemia (VHS) Interim Rule), September 9, 2008. http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/animalhealth (accessed 31.01.14).

USDA APHIS VS, 2008b. Amended Federal Order Viral Hemorrhagic, Septicemia, April 2, 2008. http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/animalhealth (accessed 31.01.2014).