Viral Hemorrhagic Septicemia Virus IVb U.S. Surveillance Report 2009

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Executive Summary

Viral hemorrhagic septicemia (VHS) is a World Organization for Animal Health (OIE)-listed disease. In 2005, an emergent genotype of VHSV IV (referred to as VHSV IVb) was detected in freshwater fish associated with fish kills in the Great Lakes, an extensive watershed shared by the United States (U.S.) and Canada. Currently, 28 freshwater species are considered susceptible to natural infection or disease caused by VHSV IVb, including species harvested, cultured, and/or stocked for bait, sport, or food. A Federal Order currently regulates the anthropogenic movement of these susceptible species from the Great Lakes States and provinces.

Since fiscal year 2007, the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) has offered cooperative agreements for VHSV IVb surveillance in freshwater fish populations of the U.S. Stated objectives of the first 2 years of study included establishing an understanding of VHSV IVb distribution in freshwaters of the United States, supporting decisions on the delineation of VHSV IVb control zone boundaries (2007-2008), and investigating potential VHSV IVb occurrence in aquatic animal production systems (2008).

This report compiles surveillance results available to the National Surveillance Unit (NSU) through August 2009, and generally includes the first 2 years of surveillance sampling. Cooperative agreement surveillance results were available from 18 States, including 6 of the 8 Great Lakes States (Illinois, Michigan, Minnesota, New York, Ohio, and Wisconsin) and 12 of the 42 remaining States (Arkansas, Colorado, Iowa, Maine, Maryland, Mississippi, Montana, Nebraska, Oklahoma, South Dakota, Texas, and Vermont). Risk evaluations were completed in 13 States (Arkansas, Colorado, Hawaii, Iowa, Michigan, Minnesota, Missouri, North Dakota, New York, Tennessee, Vermont, Washington, and Wisconsin). Surveillance results were either not yet available, or were missing key fields (e.g., HUC location or test results), from cooperative agreements with five States (Indiana, Kansas, New Jersey, Pennsylvania, and West Virginia).

An expert panel, convened prior to surveillance implementation to evaluate risk of VHSV IVb outside the known-positive regions, produced a list of nine weighted risk factors. Relationships with known VHSV IVb affected regions, through hydrologic connectivity and proximity, history of untested shipments of live or frozen fish, and shared equipment or fish wastes, scored highest as perceived predictors of VHSV occurrence in a natural watershed. Presence of known VHSV IVb susceptible species, conducive (cool to cold) water temperatures, and an insufficient regulatory framework for aquatic animal health were also considered risks for VHSV infection. These factors were weighted for contribution to baseline disease status assessment, and also to prioritize efforts for ongoing surveillance.

To date, VHSV IVb has been found in all of the Great Lakes except Lake Superior and in several inland waters in neighboring watersheds. These findings involve watersheds from two distinct U.S. Geological Survey (USGS) hydrologic unit code (HUC) Regions

(Great Lakes Region and Ohio River Region). However, though involving two HUC regions, all VHSV IVb positive findings have occurred in 4-digit HUCs contained within the jurisdictional boundaries of the eight currently regulated States (Figure 5).

Though VHSV IVb has not yet been identified outside the regulated area, surveillance coverage in non-regulated regions has, to date, been clustered and relatively sparse. Consequently, surveillance results can only substantiate assertions of disease freedom outside the regulated area on an individual State basis. Multiple evidence streams were included in individual State VHSV IVb disease status evaluation, including historical absence, surveillance data and risk evaluation. A baseline surveillance confidence in VHSV IVb disease absence, at a 10 percent site-level detection threshold, was established for Arkansas and Minnesota. Colorado and Hawaii also met target confidence when additional evidence streams (risk evaluation and passive surveillance) were considered. Nebraska met target levels when farmed fish were included in the analysis. Illinois, Maine, South Dakota, and Vermont accumulated substantive, though not target, levels of baseline evidence of disease absence. In conclusion, though VHSV IVb appears currently localized to Great Lake States, baseline surveillance outside the Great Lakes region is, to date, only partially complete. Furthermore, introduction risks in States associated with known positive waters may necessitate ongoing surveillance to maintain baseline confidence in disease freedom.

Selected States were predicted to be at risk of disease introduction through natural movements of fish and/or water from known VHSV IVb affected regions. However, varying State capacities to mitigate risks of anthropogenic transmission could also contribute to spread of the disease outside the Great Lakes States. Consequently, both to demonstrate as well as to maintain confidence in disease absence, all States are encouraged to support any or all of the following surveillance and control activities: (1) regulatory and public education actions to reduce the potential for anthropogenic introductions; (2) fish health infrastructure development to support passive surveillance and early detection; and (3) structured active surveillance in regions where baseline assessment is incomplete or where introduction risks, e.g., associated with location, are difficult or impossible to mitigate.

Introduction

Purpose of Report

This report summarizes cooperative agreement surveillance data for VHS from 2006 to the present. The core products of this analysis include a description of the apparent distribution of VHS virus (VHSV) IVb in U.S. freshwaters, and an estimate of the probability of VHSV IVb absence in non-affected States and/or regions. We identify VHSV data gaps and introduction risks, and, targeting these areas of uncertainty, recommend future surveillance strategies to provide continuing support for zonation and other disease control decisions.

Disease Description

VHS is an OIE-listed disease. Four major genogroups of VHSV have been identified (Einer-Jensen et al., 2004; Snow et al., 2004). Genogroup VHSV IV is considered endemic in certain marine populations of fish along the Pacific and northern Atlantic coasts of North America. However, an emergent genotype of VHSV IV, referred to as VHSV IVb, was detected in freshwater fish associated with fish kills in the Great Lakes, an extensive watershed shared by the United States and Canada. First reported in 2005, VHSV IVb was later found in archived fish collections, also of Great Lakes origin, dating back to 2003 (Elsayed et al., 2006). Parallel investigations of fish collections from the Great Lakes and associated tidal rivers of Canada identified the emergence of VHSV IVb over a similar period (Lumsden et al., 2007; Gagné et al., 2007).

Since its initial detection in North America, VHSV IVb has been identified in all major water bodies of the Great Lakes system except Lake Superior. The virus has been detected in Lake St. Clair, Lake Ontario, the St. Lawrence River, Lake Erie, Lake Huron, and Lake Michigan. VHSV IVb has also been detected in fish from several inland lakes of Michigan, New York, Wisconsin, and Ohio. Many VHSV IVb detections have been associated with mortality events (http://www.focusonfishhealth.org/vhs-timeline.php). All positive locations, except for one (Clear Fork Reservoir), fall within the Great Lakes Region, a watershed named by the hydrologic unit code (HUC) classification system as Region 04. The HUC system (http://water.usgs.gov/wsc/map_index.html) divides the U.S. into 21 hydrologically distinct Regions (Figure 1), which are further subdivided into smaller units representing increasing levels of hydrologic connectivity. Clear Fork Reservoir is an inland lake in the Ohio River watershed (Region 05), a few miles from Region 04.

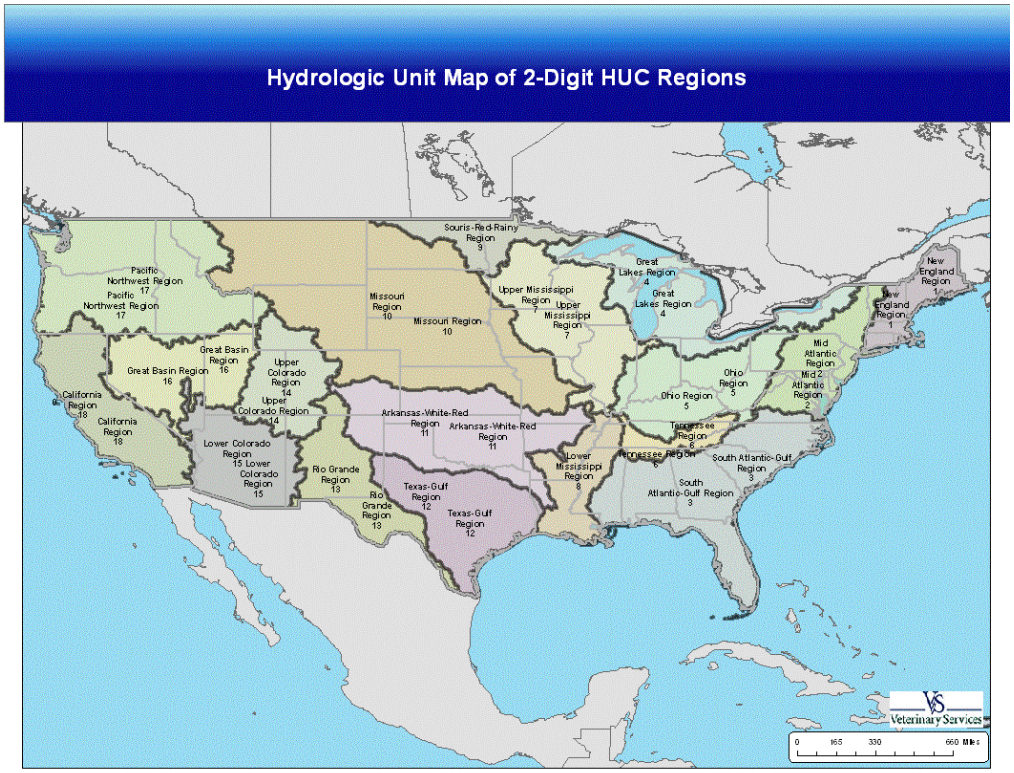

Figure 1: Hydrologic Unit Map of U.S. Regions (based on USGS classification system, http://water.usgs.gov/GIS/regions.html). Region 04 is the Great Lakes Region; region 05 is the Ohio River Region.

Control Measures/Federal Order

Currently, 28 freshwater species are considered susceptible to natural infection or disease caused by VHSV IVb (USDA VHSV IVb-Susceptible Species List, http://www.focusonfishhealth.org/species-affected.php, September 2009), including species harvested, cultured, and/or stocked for bait, sport, or food. A Federal Order instituted to reduce the risk of VHSV IVb spread from the Great Lakes to other regions regulates the movement of susceptible species from Great Lake States and provinces (http://www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/).

Effective zonation for disease control depends on accurate delineation of diseased and disease-free regions. The working premise behind the Federal Order is that VHSV IVb is not present in freshwater populations outside of the Great Lakes States and provinces. This presumption of disease freedom outside the regulated area was based on the observation that initial positive findings were located within the Great Lakes watershed (Region 04), the U.S. portion of which is contained entirely by Great Lakes States.

A disease-free status based on historical absence of disease in non-regulated States typically requires assurances that basic systems are in place to prevent, observe, and report potential outbreaks of clinical disease. However, because VHSV IVb has the potential to affect a broad range of species, populations, and production systems, these

basic biosecurity conditions are not consistent, or necessarily sufficient, across industries and States. Furthermore, fish movements and/or water passage from known-affected water bodies into Lake Superior, Lake Champlain and the Mississippi drainage provide natural pathways for virus spread via waterways; the recent finding of VHSV IVb in fish from Clear Fork Reservoir located outside the Great Lakes watershed; and anthropogenic movements of fish and fomites (e.g., by anglers, ballast, stocking practices, or commercial trade) extend the potential distribution of VHSV IVb to much of the U.S. Consequently, in 2007, cooperative funds were identified to evaluate the freshwater distribution of VHSV and ensure that the emergent disease in wild freshwater fish populations had not spread outside of the regulated area and/or into aquatic animal production systems.

Surveillance Overview

USDA-APHIS-VS offered cooperative agreements to States and Tribal Nations to conduct surveillance for VHSV IVb in freshwater fish populations, beginning in FY 2007 to present. Stated objectives include establishing an understanding of VHSV IVb distribution in freshwaters of the United States, supporting decisions on the delineation of VHSV IVb control zone boundaries (2007-2008), and investigating potential VHSV IVb occurrence in aquatic animal production systems (2008).

To facilitate surveillance planning, NSU, in cooperation with the Canadian Food Inspection Agency (CFIA) Aquatic Animal Division, conducted an expert panel evaluation of VHSV IVb risk (VHSV Expert Panel and Working Group, in press). The expert panel identified and weighted risk factors for VHSV IVb. The strongest perceived risks of spread were associated with natural and anthropogenic movements of untested fish from known-affected waters. Prior to the Federal Order, movement testing was a fairly common practice for fish raised for food, such as salmonids, but relatively uncommon to production and stocking practices for many other freshwater species. Consequently, though perceived risks of disease are significant for States hydrographically connected and proximal to affected waters, the probability of VHSV IVb is also non-negligible for distant States with a history of potential movements of untested live or frozen fish, or fish wastes, water or fomites from the Great Lakes Region.

To address the extensive geographic and taxonomic scope of this initiative, NSU used an epidemiological method that accommodates multiple lines of evidence to target limited surveillance resources to regions with the greatest disease risk and uncertainty (Gustafson et al., in press). Using this method, NSU assessed three distinct lines of information to make inferences about VHSV distribution. These evidence streams included (1) diagnostic test-based surveillance data; (2) historic or alternative test data; and (3) an expert panel-derived risk evaluation of the predicted impact of regulatory, resource use, and location factors on the occurrence (risk score, RS) and introduction risk (IR) of disease. We evaluated the confidence derived from evidence streams separately and then combined to describe current knowledge of disease probability for each State and region of interest. For surveillance planning purposes, provisional estimates of RS and IR (RS* and IR*) were made based on location, and presuming sufficient control of anthropogenic risk factors. Surveillance and analysis methods are further described in Appendix 3.

Results

State Participation

This report compiles surveillance results that participating States made available to NSU through August 2009, and generally includes the first 2 years of surveillance sampling. FY 2007 and FY 2008 Cooperative Agreement surveillance results were available from 18 States, including 6 of the 8 Great Lakes States (Illinois, Michigan, Minnesota, New York, Ohio, and Wisconsin) and 12 of the 42 remaining States (Arkansas, Colorado, Iowa, Maine, Maryland, Mississippi, Montana, Nebraska, Oklahoma, South Dakota, Texas, and Vermont). Cooperative Agreement funded surveillance data were also provided by the Great Lakes Indian Fish and Wildlife Commission (GLIFWC), an inter-tribal, co-management agency. Surveillance data from a single site in Washington State were also available, though not through Cooperative Agreement.

Eight States (Colorado, Illinois, Maryland, Montana, New York, South Dakota, Texas, and Vermont) report results from wild populations only. Mississippi and Oklahoma conducted surveillance on farmed facilities only. Eight States (Arkansas, Iowa, Maine, Michigan, Minnesota, Nebraska, Ohio, and Wisconsin) report results from wild and farmed populations. Surveillance results were either not yet available, or were missing key fields (e.g., HUC location or test results), from cooperative agreements with five States (Indiana, Kansas, New Jersey, Pennsylvania, and West Virginia). In total, results represent 63,514 fish sampled from 267 eight-digit HUCs, from 83 four-digit HUCs, and in thirteen (of 21) two-digit HUCs in the U.S. Of these, 50,570 fish were collected from the wild and 12,944 were collected from farm facilities.

Risk evaluations were completed in 13 States: Arkansas, Colorado, Hawaii, Iowa, Michigan, Minnesota, Missouri, North Dakota, New York, Tennessee, Vermont, Washington, and Wisconsin (Figure 2). Nine of the States completing risk evaluation also conducted cooperative agreement surveillance; four non-Great Lake States completing risk evaluation did not conduct cooperative agreement surveillance. RS* and IR* were estimated for all 50 States.

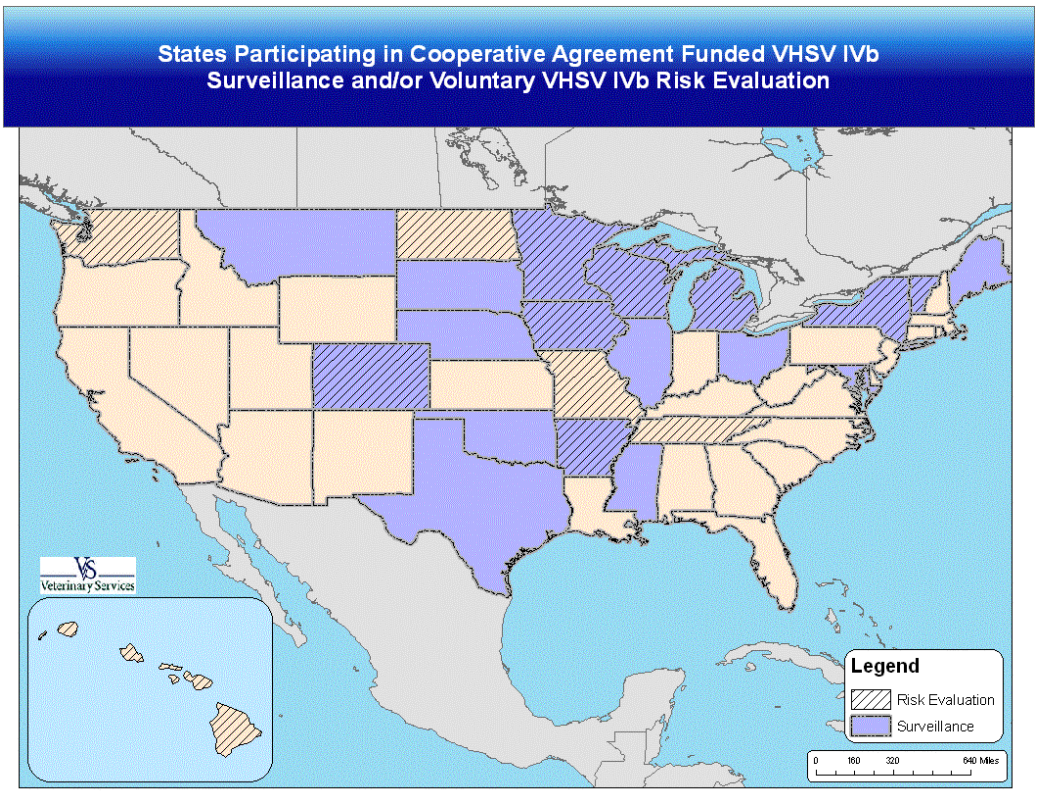

Figure 2: States participating in VHSV IVb cooperative agreement surveillance and/or risk evaluation.

Expert Panel-Derived Risk Factors

An expert panel was convened to evaluate risk of VHSV IVb outside the known positive regions. The panel produced a list of nine weighted risk factors for initial watershed assessment (VHSV Expert Panel and Working Group, in press). The list includes factors reflecting natural and anthropogenic pathways for virus exchange, as well as environmental conditions facilitating disease establishment (Appendix 1). Relationships with known VHSV IVb affected regions, through hydrologic connectivity and proximity, history of untested shipments of live or frozen fish, and shared equipment or fish wastes, scored highest as perceived predictors of VHSV occurrence in a natural watershed (Table 1). Presence of known VHSV IVb susceptible species, conducive (cool to cold) water temperatures, and an insufficient regulatory framework for aquatic animal health were also perceived predictors of VHSV infection. Likelihood ratios (LRs), estimating a factor's predicted strength of association with disease, were estimated by the panel for each factor and response (Table 1).

Table 1: Expert panel likelihood ratios for watershed VHSV IVb risk factors. Connectivity, distance, fomites, fish transfer, and regulatory factors relate to association with known positive regions, i.e., transfer of fish from known positive regions, hydrologic connectivity with known positive regions, etc. LR, Q1 and Q3 were the median, first and third quartiles derived from the full distribution of expert likelihood ratio estimates: n = 24 responses for linear distance, 25 for all others.

Risk Factor	Response Category	LR	Q1	Q3
Hydrologic connectivity	Connected, with fish movement	3.16	2.45	3.46
	Downstream, no fish movement	1.41	1.00	1.79
	Upstream, no fish movement	0.71	0.63	1.00
	No connection	0.32	0.27	0.48
Linear distance	< 100 km	2.50	2.22	3.77
	100 - 500 km	1.00	1.00	1.15
	> 500 km	0.39	0.25	0.45
Known susceptible species	Yes, with known congregation areas	2.00	1.46	3.87
	Yes, but no known congregation areas	1.22	1.12	1.73
	No	0.24	0.12	0.32
Conducive water temperatures	Yes, cool to cold water	1.50	1.18	2.45
	No	0.47	0.34	0.77
Fomite exposure	Yes, shared traffic or wastes	2.24	1.41	3.16
	Yes, but limited by education or regulation	1.00	1.00	1.26
	No	0.39	0.25	0.67
Live fish transfer, bait	Yes, without testing	2.65	2.12	3.87
	Yes, with testing	1.00	0.63	1.10
	No: transfers prevented	0.34	0.28	0.48
Live fish transfer, culture/stock	Yes, without testing	2.45	1.79	3.61
	Yes, with testing	1.00	0.71	1.15
	No: transfers prevented	0.39	0.29	0.71
Frozen fish transfer	Yes, without testing	2.45	1.15	3.00
	Yes, with testing	1.00	0.71	1.00
	No: transfers prevented	0.58	0.32	0.87
Regulatory framework	Sufficient	0.80	0.58	1.00
	Insufficient	1.34	1.00	1.53

VHSV IVb-Affected Locations

Collection locations of confirmed VHSV IVb positive fish involved 9 of U.S. 4-digit HUC watersheds and 2 of the 21 U.S. 2-digit HUC Regions (Table 2, Figure 3). Cooperative agreement surveillance results report VHSV IVb positives from 5 of these 4-digit HUC watersheds (0403, 0406, 0408, 0412, and 0504); NVSL records (including alternative funding sources) expand the list by 4 additional 4-digit HUC watersheds (0409, 0413, 0414 and 0415). Watersheds abutting the Great Lakes are termed 'possible affected watersheds', regardless of negative surveillance (Figure 4).

VHSV IVb detections originated from fish populations residing in the Great Lakes themselves, as well as a collection of inland lakes of New York, Michigan, Wisconsin and Ohio (Figure 3). All but one of these locations is contained within the Great Lakes Region (Region 04); only one occurred in the Ohio River Region (Region 05). All VHSV IVb positive fish to date were collected or originated from wild populations.

Rivers and streams in the Great Lakes Region (04) predominantly drain into the Great Lakes. The Great Lakes drain into the St. Lawrence River, which empties into the Atlantic Ocean. However, portions of the Great Lakes watershed also connect by locks or canals to rivers that flow south toward the Mississippi or east to Lake Champlain.

The single positive location (Clear Fork Reservoir) in the Ohio River Region (Region 05) is situated just a few miles south of the Great Lakes Region boundary. This particular watershed (HUC 0504) drains into the Ohio River bordering Ohio and Kentucky, traversing south and ultimately joining the Mississippi River.

The currently regulated Great Lakes States include Michigan, Wisconsin, New York, Ohio, Illinois, Indiana, Pennsylvania, and Minnesota. All of these States, except Minnesota, either abut or contain waters that have identified VHSV IVb positive fish. Minnesota, in contrast, has been regulated due to Lake Superior's connection to the known-affected Lake Huron. However, to date, insufficient surveillance has been conducted to claim disease freedom for Lake Superior. Consequently, 4-digit HUCs abutting Lake Superior are considered high locational risk.

Table 2: Designations for positive vs. abutting 4-digit HUCs, including associated known positive waterbodies. 4-digit HUCs classified as 'abutting' include the shorelines of positive Great Lakes that are not otherwise included in the positive 4-digit HUC Lake watershed. 0401 and 0402 abutting Lake Superior are included though confirmed detections have not yet occurred in Lake Superior.

4-digit HUC	designation	Includes
401	*abutting*	*(abuts Lake Superior)*
402	*abutting*	*(abuts Lake Superior)*
403	positive	Lake Winnebago, Little Lake Butte des Morts
404	*abutting*	*(abuts Lake Michigan)*
405	*abutting*	*(abuts Lake Michigan)*
406	positive	Lake Michigan
407	*abutting*	*(abuts Lake Huron)*
408	positive	Lake Huron, Budd Lake
409	positive	Lake St Clair, Base Line Lake
410	*abutting*	*(abuts Lake Erie)*
411	*abutting*	*(abuts Lake Erie)*
412	positive	Lake Erie
413	positive	Conesus Lake
414	positive	Skaneateles Lake
415	positive	St Lawrence River
504	positive	Clear Forks Reservoir
712	*abutting*	*(abuts Lake Michigan)*

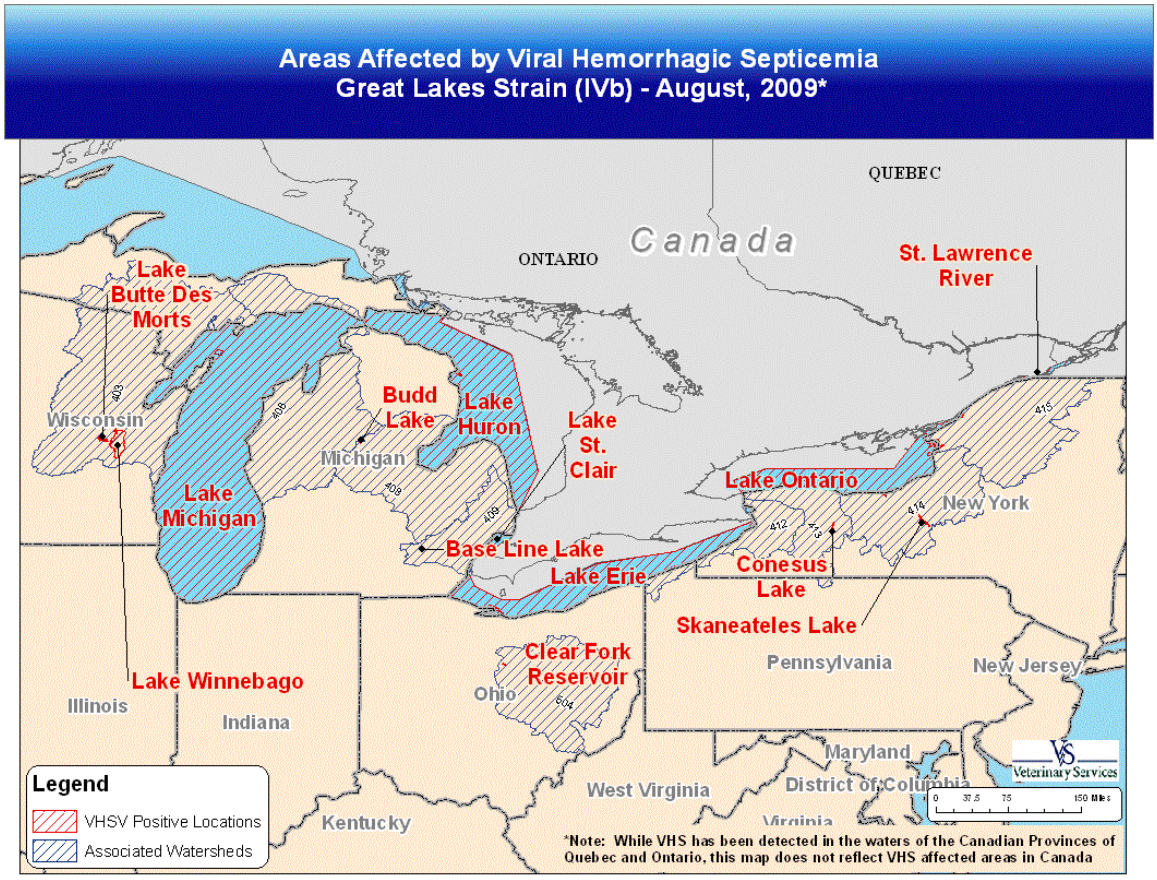

Figure 3: Location of known VHSV IVb affected locations and associated 4-digit HUC watersheds.

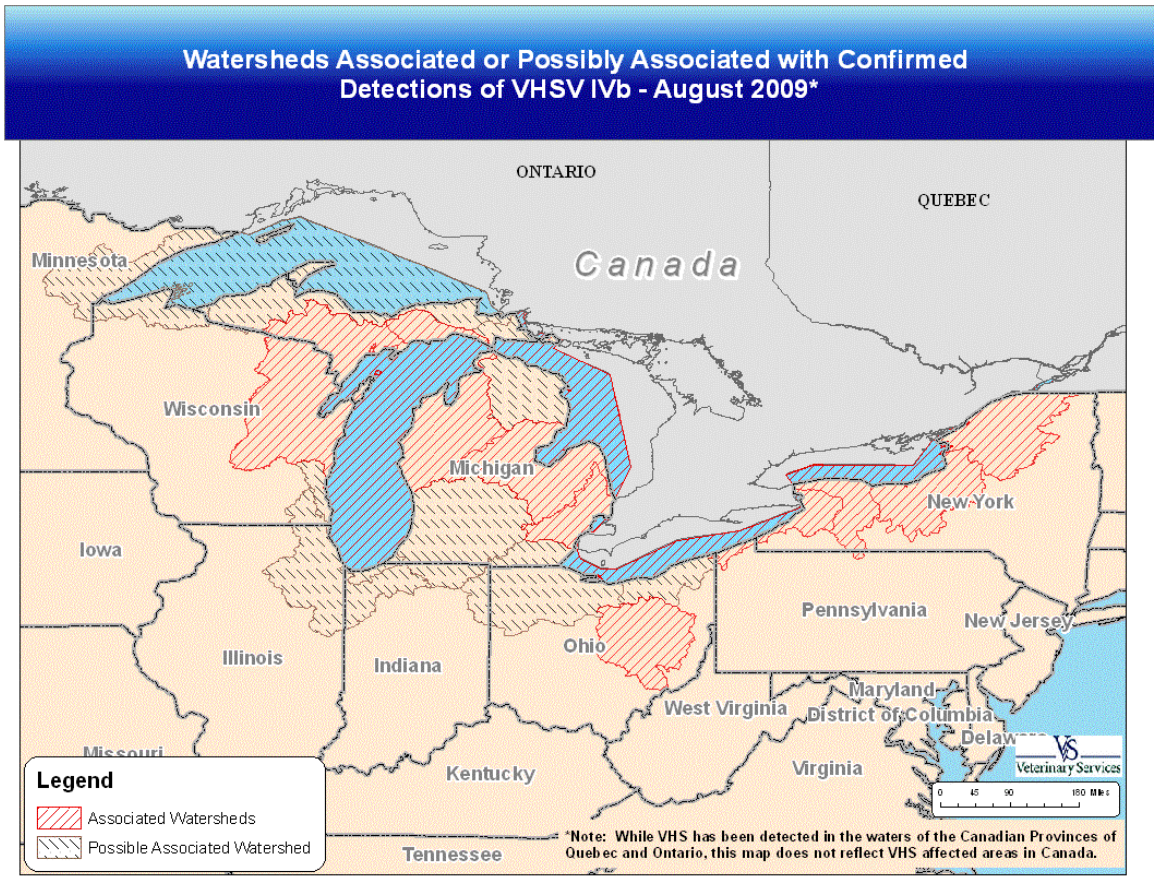

Figure 4: Four-digit HUC watersheds containing confirmed detections of VHSV IVb ('associated waterhseds') or abutting the Great Lakes ('possible associated watershed').

Evaluation of Disease Freedom

Summary. Multiple evidence streams were included in State VHSV IVb disease status evaluation. Arkansas, Minnesota, and Colorado achieved target levels of baseline confidence in disease freedom through surveillance. Vermont, Nebraska, South Dakota, and Maine achieved moderate levels through surveillance; completion of a risk score could boost confidence to target levels for some of these States. Similarly, Hawaii achieved target levels of baseline confidence through risk evaluation. Missouri and Washington also achieved moderate confidence from completion of a risk evaluation; minimal surveillance effort in these States could boost acquired confidence to target levels. Furthermore, farmed fish facilities may be considered appropriate samples for watershed disease status when and if these facilities are on open water sources or routinely draw brood, feed, or other fish from surrounding populations. If farmed fish are an acceptable proxy for wild, Nebraska also has enough data to achieve target levels of confidence about their constituent watersheds, and Maine would be close. Results for each evidence stream are provided below. Consequently, baseline surveillance to confirm that VHSV IVb is currently localized to Great Lake States is incomplete. Furthermore, introduction risks in States associated with known positive waters may necessitate ongoing surveillance to maintain baseline confidence in disease freedom.

Surveillance Evidence for Non-Regulated States. Although VHSV IVb has been detected in watersheds of two HUC Regions (Great Lakes Region and Ohio River Region), all VHSV IVb confirmed positive fish originated from four-digit HUC watersheds incorporated within the eight currently regulated States (Figure 3). Evidence of disease freedom outside the regulated States would support the location of the current boundary separating VHSV regulated and non-regulated regions. However, surveillance coverage outside of the Great Lakes States was clustered and relatively sparse. Only 12 of 42 non-regulated States conducted cooperative agreement surveillance, and only four additional States participated in voluntary evaluation of VHSV IVb risk factors. Consequently, negative results from this sample of 12 States provide only 19 percent confidence (with a calculated (from submission totals) average 65 percent State-level sensitivity) that VHSV IVb would have been detected if it were present in one or more of the 42 non-regulated States. Negative results from 15 States, if we include the 3 additional non-Great Lakes States reporting protective risk scores, provide at most 23 percent confidence that VHSV IVb would have been detected if it were present in 1 or more of the 42 currently non-regulated States. Consequently, surveillance results cannot yet substantiate assertions of disease freedom outside the regulated zone other than on an individual State basis.

Surveillance-derived confidence in VHSV IVb disease absence, at 5 and 10 percent site-level detection thresholds, was determined for individual States in two ways: first, using wild fish survey data (Table 3, Figure 5), and second, using all fish survey data irrespective of wild or farmed type (Appendix 2).

Table 3: Wild fish surveillance confidence in disease freedom for non-regulated States. The epidemiologic unit of analysis is the 8 digit HUC (HUC8). Average HUC8 sensitivity is the average confidence achieved at a single site, and relates to the number of fish sampled and reported.

State	Number of HUC8s sampled	Average HUC8 Sensitivity	Number of HUC8's per State	Confidence of 5% Prevalence Threshold	Confidence of 10% Prevalence Threshold
AR	26	0.95	59	0.81	0.97
CO	25	0.96	94	0.78	0.94
VT	12	1.00	18	0.67	0.90
NE	16	0.99	71	0.64	0.84
SD	13	1.00	56	0.55	0.81
ME	12	0.92	22	0.50	0.76
MT	6	0.91	114	0.26	0.43
IA	5	0.98	57	0.24	0.43
TX	4	1.00	210	0.19	0.34
MD	4	0.84	24	0.14	0.26

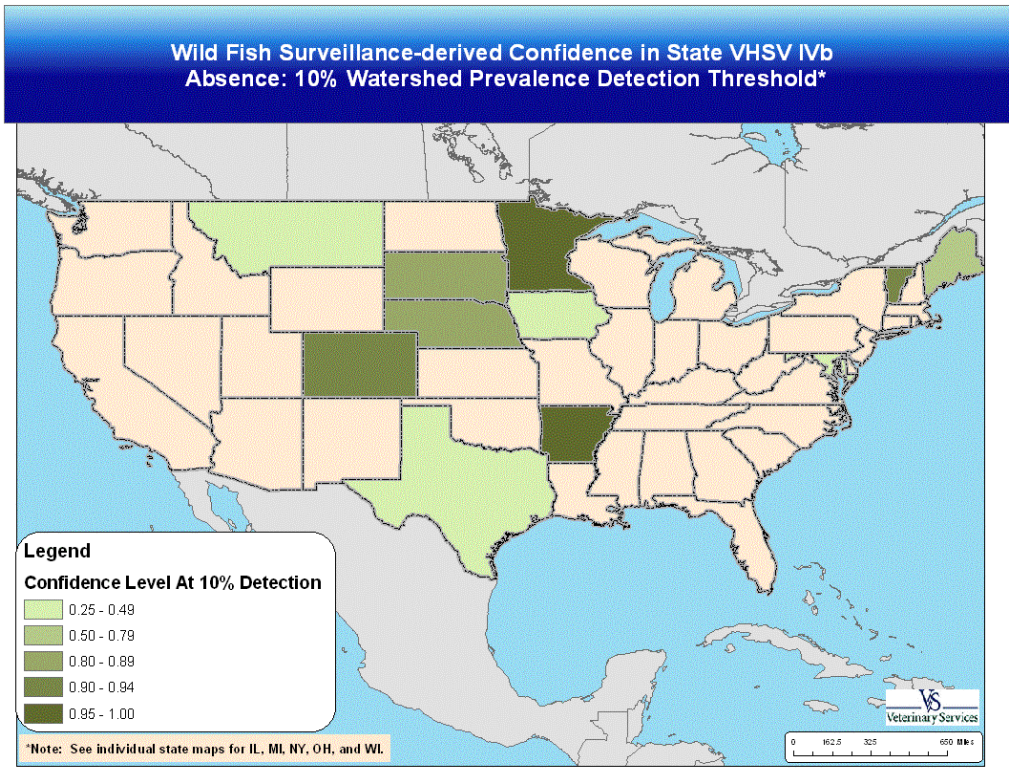

Figure 5: Wild fish surveillance baseline confidence in VHSV IVb absence for non-affected States. A 10% prevalence detection threshold was assumed using 8-digit HUC watersheds as the epidemiologic unit of analysis. Minnesota, a regulated State, was included in this map as does not currently contain or abut VHSV IVb known-affected 4-digit HUCs. Confidence described for Minnesota represents waters excluding Lake Superior.

Surveillance Evidence for Non-affected Portions of Regulated States. Wild fish surveillance confidence in VHSV IVb disease absence, at 5 and 10 percent site-level detection thresholds (Tables 4 and 5), was determined for presumed non-affected portions of regulated States. Because the four-digit HUCs classifying the Great Lakes waters do not always include the adjoining shorelines, this was done using two different boundary divisions. For example, HUCs 0405, 0407 and 0712 abutting VHSV positive Lake Michigan, and 0410 and 0411 abutting VHSV positive Lake Erie, are not classified VHSV affected at the four-digit HUC level. Similarly, 0401 abutting the non-affected Lake Superior was not classified VHSV affected at the four-digit HUC level. Consequently, confidence was evaluated first for the non-affected portion of the State, and second (if applicable) for the non-affected portion that also excluded any remaining four-digit HUCs abutting a Great Lake (Tables 4 and 5).

Some States may be able to show, through independent evaluation of intricacies in their sampling design, greater confidence than ascribed that VHSV has not yet spread beyond the known-affected region. NY, e.g., focused sampling on highest-risk HUCs, a design decision that was not accounted for in the current analysis. Consequently, figures shown below should be considered a conservative estimate of baseline confidence derived.

Table 4: Wild fish surveillance confidence in disease freedom for non-affected portions of VHSV IVb regulated States. This analysis *includes* Ohio samples from 0410 and 0411 abutting Lake Erie, Illinois samples from 0712 abutting Lake Michigan, Michigan samples from 0405 abutting Lake Michigan, and Minnesota, Wisconsin and Michigan samples from 0401 and 0402 abutting Lake Superior. The epidemiologic unit is the 8 digit HUC (HUC8). Average HUC8 sensitivity is the average confidence achieved at a single site, and relates to number of fish sampled.

State	Number of HUC8s sampled	Average HUC8 Sensitivity	Number of HUC8's per State	Confidence of 5% Prevalence Threshold	Confidence of 10% Prevalence Threshold
MN	37	1.00	85	0.90	1.00
IL	16	0.98	56	0.64	0.88
MI	17	1.00	65	0.60	0.89
OH	11	1.00	45	0.43	0.77
NY	9	1.00	57	0.41	0.66
WI	8	0.73	55	0.29	0.50

Table 5: Wild fish surveillance confidence in disease freedom for non-affected and non-abutting portions of VHSV IVb regulated States. This analysis *excludes* samples from 4-digit HUCs abutting the Great Lakes. The epidemiologic unit is the 8 digit HUC (HUC8). Average HUC8 sensitivity is the average confidence at a single site, and relates to the number of fish sampled.

State	Number of HUC8s sampled	Average HUC8 Sensitivity	Number of HUC8's per State	Confidence of 5% Prevalence Threshold	Confidence of 10% Prevalence Threshold
MN	34	1.00	85	0.88	0.99
IL	11	0.97	56	0.48	0.74
NY	9	1.00	57	0.41	0.66
MI	0	0	65	0	0
OH	6	1.00	45	0.25	0.53
WI	5	0.79	55	0.20	0.37

Surveillance Evidence for Farmed Fish Farmed fish data were also analyzed by State (Table 6). Minnesota and Nebraska met target levels of confidence for the farmed fish analysis. However, population data were not available for most States; consequently, we estimated 100 represented farmed fish facilities per State. This could lead to over- or under-estimation of confidence, depending on farming intensity and State criteria for facility selection (maintained in State registry, willingness to participate, etc.). The fish reported from Washington State were positive for VHSV IVa, but not VHSV IVb, so were included as negatives in this analysis.

Table 6: Farmed fish surveillance confidence in disease freedom for farmed facilities by State. Inferences assume that the samples are representative of a total population of 100 facilities per State.

State	Number of sites sampled	Average within-farm surveillance sensitivity	Assumed number of farms per State	Confidence of 5% prevalence threshold	Confidence of 10% prevalence threshold
MN	26	1	100	0.79	0.96
NE	25	0.97	100	0.76	0.95
IA	12	0.97	100	0.47	0.73
ME	11	0.94	100	0.43	0.68
OK	7	0.86	100	0.27	0.48
AR	5	1	100	0.23	0.42
MI	5	0.95	100	0.22	0.4
MS	3	0.99	100	0.14	0.27
WI	4	0.51	100	0.1	0.19
OH	1	1	100	0.05	0.1
WA	1	0.08	100	0.00	0.01

Risk Evidence. State confidence in disease freedom can be improved for low-risk States (RS < 1) by incorporating risk scores. States participating in voluntary risk evaluation scored the full set of expert-derived risk factors (Appendix 1, Table 1) for each of the 4-digit HUCs in their State. The full set of factors address anthropogenic, environmental and locational characteristics of watersheds that can influence the probability of disease. In years following the initial findings of VHSV IVb in the Great Lakes, Federal and State infrastructures supporting and regulating fish health have improved. However, since VHSV IVb could potentially have been introduced in advance of these protective efforts, States calculated their risks for both the current environment (current) as well as 5 years prior (initial). Four States calculated risk scores < 1 for initial conditions; eight States calculated risk scores < 1 for current conditions (Table 7).

Table 7: Risk scores (RS) for states participating in voluntary risk evaluation. RS < 1 suggest a protective context, and may reduce surveillance requirements. States estimated RS for each 4-digit HUC in their State for (initial RS) conditions pre-dating VHSV emergence in the Great Lakes and (current RS) conditions reflecting changes resulting from State and/or Federal efforts to control disease spread. The RS of the highest risk HUC is the reported RS for the State. Survey target is the number of surveillance sites to establish confidence in disease freedom at a 10% site detection threshold, for baseline and repeated assessment. Note, this table does not consider IR; consequently, target sample sizes for repeated surveys may decrease, or reduce to zero, where pathways are closed to new introduction.

State	Initial RS	Current RS	Baseline Survey Target	Repeated Survey Target
AR	0.98	0.02	27	1
CO	> 1	0.08	27	3
HI	0.01	0.01	1	0
IA	> 1	0.56	27	16
MI	> 1	> 1	27	27
MN	> 1	> 1	27	27
MO	0.26	0.26	7	7
ND	> 1	0.22	27	6
NY	> 1	> 1	27	27
TN	> 1	0.10	27	3
VT	> 1	> 1	27	27
WA	0.25	0.25	7	7
WI	> 1	> 1	27	27

Passive Surveillance Evidence. Over time, a historical absence of disease can solidify assertions of freedom in non-regulated areas. Using an OIE benchmark of 25 years to achieve claims of disease freedom through historical absence, each year without incident accrues approximately 1/25 of evidence considered sufficient for disease absence confidence. However, historical absence claims are only as strong as the underlying system for disease recognition, reporting and response.

States with a functional fish health infrastructure are presumed to have the capacity to suspect, investigate and report clinical VHSV IVb observed in their fish population. The National Animal Health Reporting System (NAHRS) for passive surveillance reporting, annual training for aquaculture liaisons, Federal Order movement restrictions and associated testing and reporting requirements, and heightened public awareness justify an expectation that VHSV IVb would eventually be reported if it were to occur in a clinical form outside the Great Lakes States. Previous experience with other aquatic diseases (VHSV IVa, SVC, KHV), and the recent adaptation of the NAHRS to collect and report passive surveillance data for aquaculture species, lend further credibility to this system.

The risk evaluation considers the State's fish health infrastructure (Appendix 1, factor 9), questioning whether State "oversight (is) sufficient to ensure the timely investigation and reporting of VHSV outbreaks". States reporting functional fish health infrastructures for the preceding 5 years include Arkansas, Colorado, Hawaii, Missouri, North Dakota, Michigan, Washington, and Wisconsin. For non-regulated States on this list, the prior probability of disease was adjusted to reflect confidence accrued through 4 years of negative passive surveillance.

Conclusions from Combined Evidence. The combined confidence in disease freedom (Table 8 and Figure 6) draws on contributions from all available evidence streams: active surveillance, passive surveillance and risk evaluation. Without considering alternative evidence, Minnesota and Arkansas met target confidence through baseline surveillance. Inclusion of alternative evidence streams brought Colorado and Hawaii up to target confidence levels. Nebraska also met target confidence levels if farmed fish facilities are considered proxies for wild populations in the watershed analysis (Appendix 2). Risk evaluations were only available for a subset of States; completion of risk evaluations for other States could improve confidence accordingly. Introduction risks may necessitate continued surveillance despite baseline confidence in some States (see 'Resilience of Disease Freedom' section below).

Table 8: Combined confidence in disease freedom for non-regulated States. The probability ascribed to active surveillance is the 95th percentile of a beta distribution approximation of prevalence, determined from the reported surveillance confidence about 10%, given a mode of zero and a uniform prior. The probability ascribed to passive surveillance is a proportional adjustment from a uniform prior, crediting 4/25 years without observed incident, equivalent to a beta (1, 1.6).. The revised surveillance probability replaces the beta (1,1) uniform prior with a beta (1, 1.6) passive surveillance informed prior. Posterior probabilities incorporating risk information were derived through Bayes theorem. The final combined confidence in disease absence, using all available evidence streams, is then reported at the 10% prevalence detection threshold.

State	Confidence of 10% Prevalence Threshold	Active Surveillance, 95th percentile prevalence	Passive Surveillance, 95th percentile prevalence	RS < 1	Posterior Probability of Disease	Combined Confidence of 10% Prevalence Threshold
HI			0.84	0.01	0.05	99
AR	97	0.09	0.84	0.98	0.08	98
CO	94	0.11	0.84		0.09	97
VT	90	0.13			0.13	90
NE	84	0.16			0.16	84
SD	81	0.17			0.17	81
ME	76	0.20			0.20	76
MT	43	0.43			0.43	43
IA	43	0.43			0.43	43
TX	34	0.54			0.54	34
MO			0.84	0.26	0.58	31
WA	1		0.84	0.25	0.57	31
MD	26	0.64			0.64	26
ND			0.84		0.84	16
States without data			0.95			10

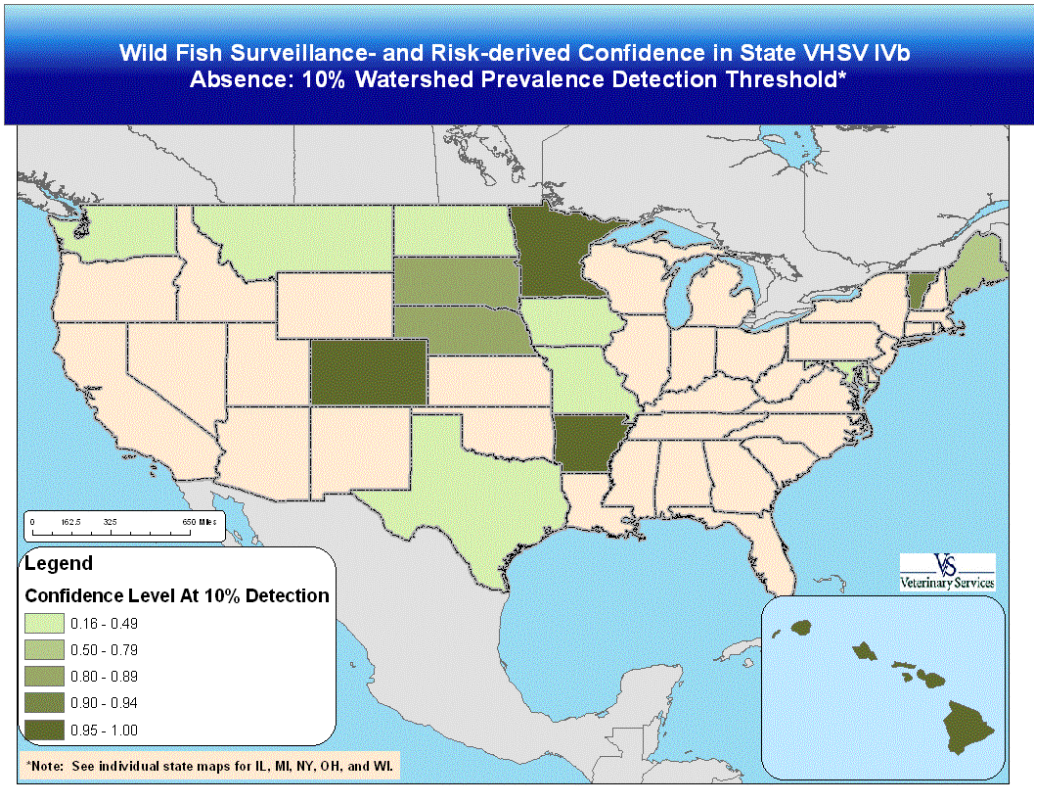

Figure 6: Combined confidence in VHSV IVb disease absence given all available wild fish evidence streams, including active surveillance, passive surveillance and risk evaluation. Analysis was conducted at a 10% prevalence detection threshold using 8-digit HUCs as the sampling unit. Minnesota, a regulated State, is included in this map as it neither contains nor abuts VHSV IVb known-affected 4-digit HUCs.

Evaluation of Future Surveillance Needs

Baseline Surveillance Gaps. States that have not yet achieved baseline confidence in disease status were identified for continued assessment. To gauge necessary sampling effort, NSU predicted location risk for all 50 States (Figure 7) based on a subset of risk factors describing hydrology, fish barriers and distance from current known-affected regions (Appendix 1, questions 1 and 2). Scores ranged from a low of 0.12 for States > 500 km distant and not connected by waterway to known affected waters, to a high of 7.9 for States less than 100 km distant and connected without barrier to fish movement to known affected waters. This location risk provided a basis for sample size estimation, presuming adequate control of anthropogenic factors, infrastructure for disease awareness and reporting and conducive temperature and species environments. Note that locations scores may change if new regions are identified VHSV affected.

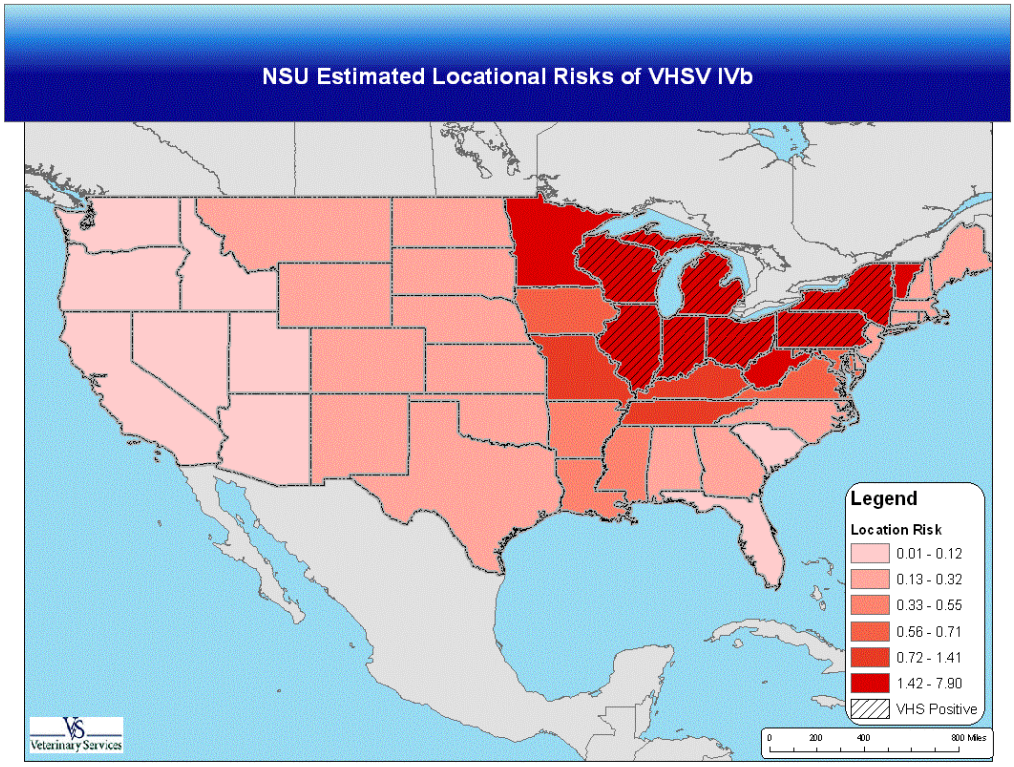

Figure 7: NSU estimated locational risk. Hatchmarks denote States abutting or containing VHSV IVb known positive water bodies.

Recommended surveillance effort for baseline assessment was estimated from location risk scores and is shown in Table 9. Sample sizes presume adequate control of anthropogenic risks, so require concurrent development of State capacity to prevent, recognize and report notifiable fish diseases. States that started baseline surveillance in a previous year may apply these prior samples toward the number described in Table 9, when and if mitigations of anthropogenic risks are also documented. States that exceed these benchmark mitigations (e.g., achieving an even lower RS) may be able to further

reduce sample size requirements. An example case is Missouri, which completed a risk evaluation with an RS score of 0.26. The State-assessed RS score for Missouri suggests a need for only eight sites: down from 27 recommended from the NSU-estimated RS*.

Table 9: This table provides default sample sizes for State or region (Split-State) baseline assessment of disease status. RS* is estimated from location risks, presuming adequate control of anthropogenic risks. Target survey sizes are based on a 10% detection threshold and 95% confidence, or 27 sites, adjusted by RS where scores are protective (RS < 1). States unable to address anthropogenic risks may require greater sampling, though not to exceed 27 sites, to achieve stated confidence. 'Connect' reports likelihood ratios (LR) for fish passage and water connectivity to known affected waters. 'Distance' reports LR for linear separation from known affected waters. RS* is the product of LRs for location risk, adequate control of anthropogenic risks (LR = 1), a functioning system for disease awareness and reporting (LR = 0.8) and a conducive temperature/species environment (LR = 3). States in bold have met target confidence levels for baseline assessment of wild populations. Nebraska met target confidence if farmed fish are considered proxies for wild populations (Table 10).

connect	distance	location risk	RS*	Target # Survey Sites	States
0.32	0.39	0.12	0.29	8	AK, AZ, CA, FL, **HI**, ID, NV, OR, SC, UT, WA
0.32	1	0.32	0.77	21	CT, DE, MA, ME, NC, NH, NJ, RI
0.71	0.39	0.28	0.67	18	AL, **CO**, GA, KS, MT, ND, NE, NM, OK, SD, TX, WY
0.71	1	0.71	> 1.00	27	IA, MD, VA
1.41	0.39	0.55	> 1.00	27	**AR**, MS, LA
1.41	1	1.41	> 1.00	27	MO, TN
3.16	1	3.16	> 1.00	27	KY, **MN**, WV
3.16	2.5	7.90	> 1.00	27	IL, IN, MI, NY, OH, PA, VT, WI

Resilience of Disease Freedom. States with continual introduction risks should be targeted for ongoing surveillance. The temporal rate of data depreciation, and the need for recurrent surveillance, can be estimated for a State by its IR. NSU estimated IR*, representing risks of VHSV IVb introduction via natural pathways, for all 50 States (Table 10, Figure 8). Results show that States connected by waterway, without barrier to fish movements, require the greatest amount of recurrent sampling. States with barriers to fish movement, but downstream of a VHS affected water body, require a moderate amount of recurrent sampling. All other States can justify minimal recurrent sampling.

IR* presumes adequate control of anthropogenic risk factors and thus is only applicable once States have achieved these benchmarks. Consequently, States should concurrently develop and secure infrastructures to minimize introduction risk, or re-assess IR using all risk factors and increase sample sizes accordingly. Recommended sample sizes (Table 10) also presume that the current information on disease distribution is accurate; if disease is detected in new States, HUCs or regions, IR* scores may change.

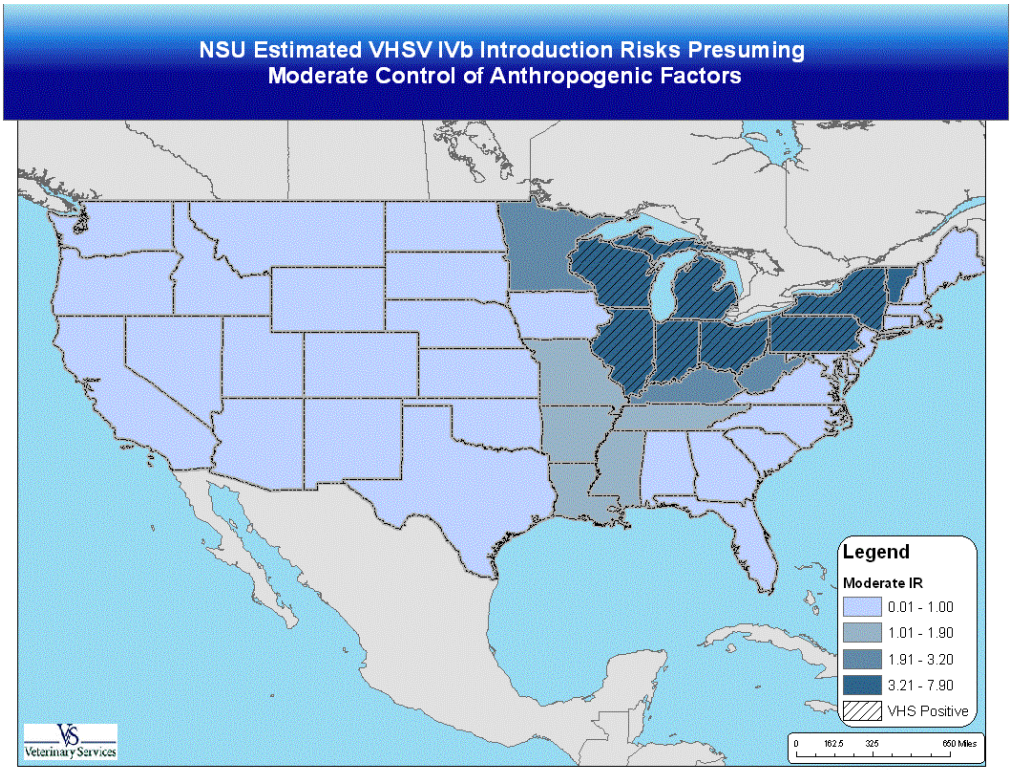

Figure 8: NSU estimated introduction risk given adequate control of anthropogenic transmission pathways. Hatchmarks denote States abutting or containing VHSV IVb known positive water bodies. IR ≤ 1 suggest temporal resilience of survey data and minimal statistical need for repeat structured surveillance.

Table 10: Recommended sample sizes for ongoing surveillance in non-regulated States, in lieu of a formal State assessment of risk factors. IR* is estimated from location factors, presuming adequate effort to reduce anthropogenic risks. The new site column is the number required, after completion of a baseline assessment, to statistically support disease freedom at a 10% detection threshold. The number is determined by discounting last year's target (presuming completion of the baseline assessment) by introduction risks (e.g., from Table 8: 27 – 27/IR, or 8 – 8/IR). States that have not addressed anthropogenic risks require greater sampling effort than reported. Regulated States (in parentheses) with mechanisms to mitigate intra-State transmission risks may be able to couple biosecurity conditions and ongoing surveillance to substantiate disease-free status for a non-affected portion of the State.

IR*	new sites	States
1.00	0	AK, AL, AZ, CA, CO, CT, DE, FL, GA, HI, IA, ID, KS, MA, MD, ME, MT, NC, ND, NE, NH, NJ, NM, NV, OK, OR, RI, SC, SD, TX, UT, VA, WA, WY
1.41	8	AR, LA, MS, MO, TN
3.16	19	KY, WV, (MN)
7.90	24	VT, (IL, IN, MI, NY, OH, PA, WI)

Regulated States. Surveillance results can inform decisions concerning the potential designation of intra-State control or buffer zones around more stable core regions (Tables 4-5). IR should be considered when evaluating potential zone boundaries. For example, States might consider excluding from protected zones both 4-digit HUCs abutting any of the Great Lakes, as well as known-affected four-digit HUCs, to reduce transmission risks and potentially also sampling requirements. For example, Minnesota might choose to exclude 0401 and 0402, abutting high-risk Lake Superior, from any designations of disease freedom or delineations of protected regions, even though confirmed detections of VHSV have not yet occurred in Lake Superior. To facilitate this type of decision process, Appendix 4 provides non-anthropogenic IR*, and boundaries and sampling history, for 4-digit HUCs in regulated States. Concurrent demonstration of biosecurity initiatives sufficient to mitigate risks of intra-State transmission is an essential condition for any disease-free or Split-state status. The expert panel risk evaluation (Appendix 1, Table 1) provides a template for consideration of appropriate State biosecurity initiatives.

Recommendations

Regulated Area Boundaries

Knowledge of VHSV IVb distribution helps to ensure effective boundaries for disease control. Ideally, the VHSV IVb regulated area is the minimum sufficient to contain all affected populations. To date, all VHSV IVb detections have occurred in regulated States from fish collected or originating from wild populations. And to date all detections have occurred within the jurisdictional boundaries of the regulated States.

All but one of the VHSV IVb detections occurred in the Great Lakes watershed (Region 04). The Great Lakes watershed predominantly drains into the Great Lakes. Consequently, risk of spread to other U.S. regions via downstream movement of Great Lakes water is limited. However, the Chicago Sanitary Canal, a shipping link between Lake Michigan and the Chicago River, does allow water from the Great Lakes to discharge into the Mississippi River watershed. Similarly, routes for fish passage connect several inland waters, otherwise upstream, directly to the Great Lakes. These include watersheds directly abutting Great Lake shorelines, and a network of locks and canals that connect the St. Lawrence River to Lake Champlain and the Hudson River.

Additionally, a single location in the neighboring Ohio River Region (Region 05) was found positive for VHSV IVb through muskellunge sampled April 2008 from the Clear Fork Reservoir in Ohio. However, Clear Fork Reservoir is only a few miles south of the Great Lakes watershed and, after draining into the Ohio River, ultimately joins with drainage from the Great Lakes watershed (e.g., via the Chicago Sanitary Canal) in the Mississippi River. Consequently, this finding only changed the risk status of West Virginia, which borders sections of the Ohio River that fish could naturally access from Clear Forks Reservoir. Kentucky and Pennsylvania also border the Ohio River, but barriers to fish migration likely limit the passage of fish from Ohio to Pennsylvania or Kentucky.

Consequently, the regulated area has been sufficiently large to capture all positive findings to date. However, because of watershed connectivity, it is recommended that both regulated and non-regulated locations near downstream or fish-accessible routes from known-affected waters continue to be monitored for new introductions. Susceptible populations in conducive habitats along the Ohio River, Lake Champlain and the Hudson River, and the Mississippi drainage, for example, though outside the VHSV IVb regulated area, should continue to be monitored for new evidence of disease.

While the distribution of positive findings suggests that the current regulated area is sufficiently broad, initial surveillance within the Great Lakes States suggests that the size of the regulated area may not yet be at an effective minimum. Certain Great Lake States are building baseline surveillance evidence sufficient to substantiate VHSV IVb freedom for some portion of their constituent watersheds. Surveillance results coupled with an evaluation of protective measures utilized to reduce and detect new introductions, whether by natural, regulatory, public, or industry actions, could provide evidence to support decisions concerning potential revision of Federal or intra-State zonation.

The adequacy of the existing boundary presumes disease freedom outside the zone. Since only 12 States outside the regulated region conducted, and fewer completed, baseline surveillance, this presumption is not fully tested. Completion of baseline assessment in non-regulated States, as well as States considering intra-State zonation, would further confirm, or refute, the presumed sufficiency of the existing boundary.

Future Surveillance

Baseline Knowledge Gaps. Baseline risk and/or surveillance evaluations should ideally be completed in the remaining non-surveyed States. Historical data, e.g., that associated with movement testing, research or other Federal or State-accessible datasets, could potentially be used to meet some or all of the survey requirements. For example, USFWS data (http://www.fws.gov/wildfishsurvey/) that has not already been co-listed in State Cooperative Agreement reports might augment confidence in disease freedom in some regions. Additionally, passive surveillance and risk evidence streams are also valued in VHSV IVb disease assessment. Consequently, even States unable to commit time or resources to active surveillance can begin to build confidence in disease absence through documenting and improving fish health regulatory and passive surveillance infrastructures.

The reported analysis assumes that the detection threshold (10% prevalence) targeted for Cooperative Agreements is acceptable to trade partners and constituents. The expected interconnectivity of sites for a disease affecting wild populations in open waterways reduces the likelihood of isolated disease occurrences. This potential for movement of fish, water and/or wildlife through natural channels to and between sites provides justification for allowing less coverage of sample sites (i.e., a higher detection threshold) without substantive loss in the ability to detect disease should it occur in a new region. However, a more typical prevalence threshold for site-level detection efforts, e.g., example defaults in OIE manuals, is 1-2%. Furthermore, a 10% prevalence threshold reflects disease that is likely well established in a watershed prior to its detection, a

compromise that is acceptable for a baseline review of disease distribution, but not one that is necessarily well-staged for immediate or early detections of new incursions. Consequently, increasing confidence in already surveyed regions, especially those considered higher risk of introduction, may be a useful exercise. Regardless, once a baseline confidence has been established, alternative or complementary surveillance methodologies should be used to improve speed and likelihood of detection of disease spread to previously free regions.

Baseline Knowledge Resilience. NSU estimated IR, IR*, reflects the expected stability of prior data for States that have achieved certain disease control benchmarks. Results suggest that most States, pending their completion of a baseline assessment of disease status and documentation of anthropogenic controls, could justify discontinuing active VHSV IVb surveillance entirely. States recommended for ongoing surveillance, due to a predicted potential for VHSV IVb introduction via natural movements of fish, water and/or fomites, include Arkansas, Louisiana, Kentucky, Minnesota, Mississippi, Missouri, Tennessee, and Vermont. However, as time evolves without incident, confidence in historical absence builds and further reduces sampling demands. Consequently, while continued active surveillance is important for some States, all States will benefit from implementation of mechanisms to reduce anthropogenic risks and improve passive surveillance for new disease outbreaks.

Split-State Status. Regulated States with epidemiologic separation of affected (or abutting) and non-affected watersheds may use surveillance to inform zonation decisions for intra-state disease management or potential split-State status. Proposed zone boundaries should reflect watershed use, watershed disease introduction risk, regulatory capacity, and survey evidence to substantiate disease freedom in the protected region. Surveillance to bolster regional confidence in disease freedom, evaluation of introduction risks, documentation of protective measures supporting biosecure boundaries, and implementation of an ongoing monitoring plan for early detection of any change in disease status would further efforts to implement and/or substantiate intra-State zonation.

Surveillance Design. Over time, active test-based surveillance could be phased out in exchange for risk-based monitoring and passive or active observational surveillance in most States. As historical evidence of VHSV IVb absence grows stronger, States can focus more of their resources on maintaining systems for early detection and response to existing disease spread and/or new disease incursions. Early detection efforts should emphasize training and availability of personnel and resources to observe, or to query those observing (e.g., involving anglers or commercial harvests), susceptible fish populations on a routine basis. Active observational surveillance centers on structured (trained, reliable frequency, recorded) observations at selected sites, with diagnostic tests reserved only for confirmation of suspicious events. A key distinction between active and passive observational systems is that locations and effort (e.g., negative as well as positive data) are tracked, and system sensitivity can be assessed, through an active system. Record keeping and standardized response criteria allow the negative data to act as a diagnostic test and statistically counteract loss of confidence in disease absence where risks of introduction are substantive. Consequently, States with un-mitigated

introduction risks and a calculated need for ongoing surveillance could conceivably maintain assurance of disease status through active observational surveillance. However, even without a formal accounting of observational effort, a functioning passive surveillance system contributes to confidence derived through historical absence.

Targeted selection is advocated over random selection of sampling units for VHSV IVb field surveillance, whether observational or test-based. Targeted selection of sites (e.g., by mortality event location, final location in drainage system, and history of live fish imports from infected regions) and fish (e.g., by susceptible species, clinical appearance and life stage), when possible, can increase the efficiency and minimize costs of surveillance by focusing efforts on presumed high prevalence or high susceptibility strata.

Conclusion

VHSV IVb appears currently localized to Great Lake States. However, baseline surveillance outside the Great Lakes region is only partially complete, and should be emphasized to support this preliminary conclusion. Available surveillance within the Great Lakes States, in contrast, has been fairly extensive and suggests that the boundary of the regulated area could potentially be narrowed in some sections. Ongoing surveillance combined with State assurance of epidemiologic separation from affected populations may justify State or split-State exclusions from the regulated region. Finally, while only a selection of States are predicted at risk from natural movements of fish and/or water from known VHSV IVb affected regions, varying State capacities to mitigate risks of anthropogenic transmission could lead to spread of the disease outside the Great Lakes States. Continued surveillance and/or infrastructures for disease control and monitoring will help to ensure that disease spread is both negligible and detectable outside the known affected regions.

Consequently, both to demonstrate as well as to maintain confidence in disease absence, States are encouraged to support any of the following surveillance-related activities: (1) regulatory and public education actions to reduce the potential for new anthropogenic introductions; (2) fish health infrastructure development to support passive surveillance and early detection; and (3) structured active surveillance in regions where baseline assessment is incomplete or where introduction risks, e.g., due to location, are difficult or impossible to mitigate.

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Appendix 1: Expert Panel Risk Factors for Watershed Evaluation

Answer the following questions separately for each 4-digit HUC in the State. If answers have changed in the last five years, please indicate both prior and current status.

1) Hydrologic connectivity with VHSV-affected watersheds

Is the watershed connected hydrologically to VHSV IVb-affected watersheds?

a. The watershed is connected hydrologically to VHSV IVb-affected watersheds, and fish can move freely between locations.
b. The watershed is downstream of VHSV IVb-affected watersheds, but barriers prevent natural fish passage between locations.
c. The watershed is upstream of VHSV IVb-affected watersheds, but barriers prevent natural fish passage between locations.
d. The watershed does not connect with VHSV IVb-affected watersheds by water route.

2) Linear distance to VSHV-affected watersheds

How distant is the watershed from VHSV IVb-affected watersheds?

a. The watershed is separated from VHSV IVb-affected watersheds by < 100 km.
b. The watershed is separated from VHSV IVb-affected watersheds by 100-500 km.
c. The watershed is separated from VHSV IVb-affected watersheds by > 500 km.

3) Known susceptible species

Does the watershed support VHSV-susceptible, VHSV-carrier, or diadromous fish species?

a. Yes, with known congregation areas (e.g., for spawning, stocking or farming).
b. Yes, but no known congregation areas.
c. No.

4) Conducive water temperatures

Can the watershed be characterized as a cool to cold water environment?

a. Yes
b. No

5) Fomites and waste exposure

Is there possibility of exposure to vessels, equipment, personnel, wastes or effluents from VHSV IVb-affected watersheds (e.g., recreational boaters, anglers, divers, installers of farming equipment, weed harvesters,or via processing of non-local fish) without prior cleaning and disinfection?

a. Yes, potential for exposure through shared traffic or wastes is substantial.
b. Yes, but educational campaigns or regulations limit chances of exposure.
c. No, shared traffic or waste is minimal to none.

6) Live fish transfer for bait

Is there recent history, or likelihood, of transfer of live bait from VHSV IVb-affected watersheds?

a. Yes, including fish from lots or facilities not tested (per federal surveillance, OIE, FHPR or AFS guidelines) for VHSV.
b. Yes, but only fish from lots or facilities testing negative (per federal surveillance, OIE, FHPR or AFS guidelines) for VHSV.
c. No. Enforced regulations and policy, or lack of transfers, prevent introductions.

7) Live fish transfer for culture or stock enhancement

Is there a recent history or likelihood of transfer of live fish to public or private waters or facilities (excepting those effectively compartmentalized from surrounding watersheds) from VHSV IVb affected watersheds?

a. Yes, including fish from lots or facilities not tested (per federal surveillance, OIE, FHPR or AFS guidelines) for VHSV.
b. Yes, but only from lots or facilities testing negative (per federal surveillance, OIE, FHPR or AFS guidelines) for VHSV.
c. No. Enforced regulations and policy, or lack of transfers, prevent introductions.

8) Frozen fish transfer

Is there recent history of use of frozen fish or fish products (unprocessed or raw) from VHSV IVb-affected regions for fish feed or bait?

a. Yes, including fish or products from lots or facilities not tested (per federal surveillance, OIE, FHPR or AFS guidelines) for VHSV.
b. Yes, but only fish or products from lots or facilities testing negative (per federal surveillance, OIE, FHPR or AFS guidelines) for VHSV.
c. No. Enforced regulations and policy, or lack of frozen fish use, prevent introduction.

9) Regulatory or policy framework governing fish health

Is oversight sufficient to ensure the timely investigation and reporting of VHSV outbreaks? (Sufficient presence is defined as 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.)

a. Yes
b. No

Appendix 2: Assessment of VHSV IVb Status in Wild and Farmed Fish Sites

A comparative analysis includes both wild and farmed fish sites in assessment of State or watershed VHSV IVb status, and also includes survey sites of unrecorded type (wild vs. farmed).

Table A: Wild and farmed fish surveillance-derived confidence in disease freedom for non-regulated States. The epidemiologic unit of analysis is the 8 digit HUC (HUC8). This analysis presumes that farmed fish facilities are suitable representatives of watershed status, which is only likely to be the case if facilities are farmed on non-biosecure water sources. Average HUC8 sensitivity is the average confidence achieved at a single site, and relates to the number of fish sampled.

State	Number of HUC8s sampled	Average HUC8 Sensitivity	Number of HUC8's per State	Confidence of 5% Prevalence Threshold	Confidence of 10% Prevalence Threshold
AR	26	0.95	59	0.81	0.97
NE	23	1.00	71	0.80	0.94
CO	25	0.96	94	0.78	0.94
ME	17	0.94	22	0.72	0.93
VT	12	1.00	18	0.67	0.90
SD	13	1.00	56	0.55	0.81
IA	9	0.98	57	0.40	0.65
MT	6	0.91	114	0.26	0.43
OK	4	1.00	67	0.17	0.36
TX	4	1.00	210	0.14	0.32
MS	3	0.99	59	0.15	0.28
MD	4	0.84	24	0.14	0.26
WA	1	0.08	73	0.00	0.01

Table B: Wild and farmed fish surveillance-derived confidence in disease freedom for regulated States. This analysis excludes sites sampled from positive and presumptive (abutting) 4-digit HUC watersheds.

State	Number of HUC8s sampled	Average HUC8 Sensitivity	Number of HUC8's per State	Confidence of 5% Prevalence Threshold	Confidence of 10% Prevalence Threshold
MN	43	1.00	85	0.94	1.00
IL	11	0.97	56	0.48	0.74
NY	9	1.00	57	0.41	0.66
MI	0	0	65	0	0
OH	6	1.00	45	0.25	0.53
WI	6	0.81	55	0.25	0.44

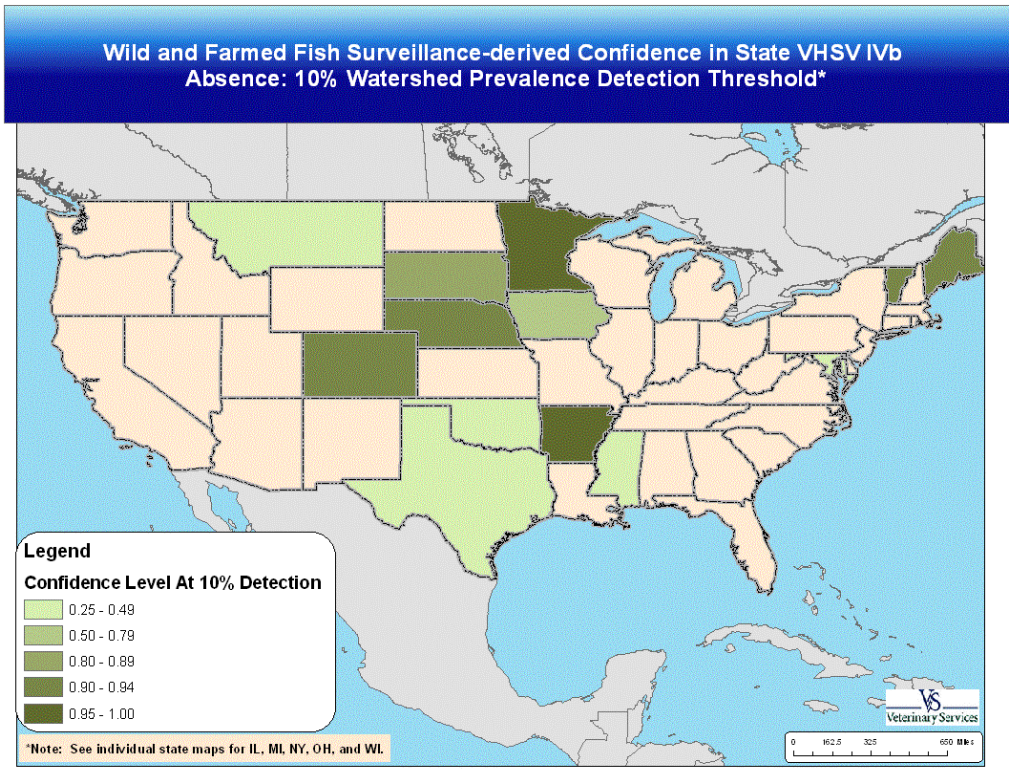

Figure A: Wild and farmed fish surveillance derived confidence in VHSV absence by State. A 10% prevalence detection threshold was assumed using 8-digit HUC watersheds as the epidemiologic unit of analysis. Minnesota, a regulated State, was included in this map as it neither contains nor abuts VHSV IVb known-affected 4-digit HUCs. The other regulated States were analyzed separately.

Appendix 3: Methods

Epidemiologic Units

Watersheds, defined at the eight-digit HUC level, were the epidemiologic unit for surveillance analysis. Fish in watersheds were sampled at places of known susceptible species occurrence (wild fish) or production (facilities) during periods of conducive water temperatures (typically spring and fall). A sample of random or moribund fish of susceptible species was collected in each location and submitted for VHSV IVb virus isolation, with confirmatory RT-PCR testing and genotyping of any culture positives (Bilateral VHSV Surveillance Working Group, 2007).

Expert Panel-Derived Risk Factors

The VHSV Bilateral Surveillance Plan developed for the United States and Canada (Bilateral VHSV Surveillance Working Group, 2007) targeted regions and populations at greatest VHSV IVb disease risk and/or uncertainty. To guide and standardize initial surveillance, members of the bilateral VHSV Working Group from USDA-APHIS-VS and the Canadian Food Inspection Agency's Aquatic Animal Health Division requested the assistance of select international experts on VHSV and fish health (VHSV Expert Panel). Through a modified integrative group process, the 30-member VHSV Expert Panel identified and estimated strengths of association called likelihood ratios, (LRs) for a preliminary set of risk factors perceived important to VHSV IVb emergence in freshwater systems of the United States and Canada (VHSV Expert Panel and Working Group, in press).

State completion of the resulting Expert Panel VHSV IVb risk evaluation (Appendix 1) was voluntary. Interested States or regions calculated a risk score (RS) for their constituent 4-digit HUC watersheds based on the presence or absence of the expert panel-identified risk factors. The product of applicable risk factor LRs for the State's highest risk 4-digit HUC was used as the RS for the State. An RS equal to 1 suggests no predictive significance; an RS greater or less than 1 suggests a detrimental (better conditions for disease) or protective influence, respectively.

Active Surveillance Sensitivity

Surveillance sensitivity was calculated from surveillance results for each State or region. Sites were defined as eight-digit HUC watersheds. On average, there are 61 eight-digit HUCs per State (range: 9-210); some which overlapped with neighboring States. Within a selected site (eight-digit HUC watershed), locations for fish sampling were chosen by susceptible species availability and convenience. Confidence in disease freedom for a given *site* was described by a binomial distribution (assuming an infinite population) with number of fish sampled (given all negative results), diagnostic test sensitivity and specificity of 85 and 100 percent respectively, and among fish, a pre-set prevalence detection threshold of 5 percent. Confidence in disease freedom for a *State* (or region) was described using a hypergeometric approximation (Cannon, 2001) with number of sites sampled (given all negative results), diagnostic sensitivity derived from the average confidence achieved in sampled sites, a specificity of 100 percent, and among sites, a pre-

set prevalence detection threshold of 10 percent. We also calculated confidence for States and regions at a detection threshold of 5 percent.

Evaluation of VHSV IVb distribution among fish culture facilities paralleled the process in wild populations. The key difference was that site participation in surveillance was entirely voluntary, without identified compensation for potential positive findings. This conceivably produces a selection bias favoring facilities that believe themselves to be unlikely to find disease. Consequently, it was recommended that participating States develop a registry of fish culture facilities with VHSV-susceptible species that are (1) currently or historically involved in live fish sales, exchange or stock enhancement with any other zones, and (2) willing (or State-mandated) to participate in VHSV surveillance. Resulting inferences then pertain to this select registry group, rather than all production facilities, in a defined State or region.

Passive Surveillance Sensitivity

Passive surveillance, based on trained veterinary and laboratory personnel and capacity, facilitates the detection and reporting of diseases of national or international concern. Over time, the absence of reported incidents through passive surveillance accrues confidence in disease freedom. OIE guidelines suggest that a 10-25 year history without incident can substantiate disease freedom claims based on historical absence, presuming basic biosecurity conditions are in place to recognize, investigate and communicate suspicious disease events. Thus, for States or regions with a functioning passive surveillance system, 25 years is a conservative estimate of the effort (measured in time) that is required to achieve a probability of disease accepted to be negligible. Given that the U.S. has been on alert for VHSV IVb since its first detection in 2005, States with a functioning fish health infrastructure have accrued 4 of 25 years of passive surveillance data: 16 percent (4/25) of the difference between un-informed and best-case (no further evidence required) prior probabilities. We used this algorithm (1 – 0.16), representing the proportion of passive surveillance evidence accrued, to revise our prior expectation of disease from a uniform distribution with an upper limit of 1 (centered at 0.5) to a beta distribution with an upper limit of 0.84 (and a mode of zero to represent negative observations), for States claiming a functional fish health infrastructure (Appendix 1, factor 9, answer a). A probability describing 4/25 years of effort is approximated by a beta (1, 1.6) prior.

Combined Probability of VHSV IVb Disease

Confidence in disease absence was estimated for all States using a combination of evidence streams (active surveillance, passive surveillance, and risk evaluation). Confidence derived through active surveillance was a central component of analysis. Where risk information was unavailable, confidence was derived using Cannon's formula (Cannon, 2001) for imperfect sensitivity. Confidence at a 10 percent detection threshold and a mode of zero were then used to derive a beta distribution for prevalence. The prior for this beta distribution was either specified as un-informed, e.g., beta (1, 1), or informed, e.g., beta (1, 1.6), by the lack or the availability, respectively, of passive surveillance data. Thus the initial prevalence distribution incorporates information from both passive and active surveillance streams.

Where risk evaluations were also available and predicted the absence of disease (RS < 1), surveillance and risk information were combined using Bayes' theorem. A Bayesian prior odds of disease was given by the initial prevalence distribution describing active and passive surveillance results. The Bayesian posterior odds of disease was the product of the surveillance-estimated odds of disease and the region's risk score (RS), representing the predictive strength of watershed risk factors (Gustafson et al., in press). Results are reported as a combined confidence in disease freedom at a 10 percent detection threshold.

Consequently, surveillance and risk evaluations provided complementary lines of evidence for disease probability estimation. The supplemental confidence in disease freedom that an RS < 1 provides is approximated by a sampling equivalent equal to the product of (1-RS) and a target sample size. For example, for a disease freedom standard requiring 27 sites (e.g., for 95% confidence at a 10% detection threshold), an RS of 0.25 reduces the necessary surveillance effort from 27 to 7 sites (27*0.25). However, because Bayesian revision is not a linear process (strong priors, e.g., arising from no data, are resistant to revision), substantive confidence is technically only realized after some (more than zero) field survey effort has been concurrently applied. This effort, represented as an informed prior probability, could potentially derive from active or passive surveillance.

Surveillance Gaps

States that did not yet complete an initial assessment of disease status were identified as gaps in baseline surveillance. Because risk evaluations were not available for all States, sampling effort recommended for baseline assessment of State disease status was derived from an NSU estimate of RS (RS*). The RS* presumed negligible anthropogenic risks (LR = 1, Factors #5-8), including a functional fish health infrastructure (LR = 0.8, Factor #9), a conducive temperature/species environment (LR = 3.0, Factors #3-4), but incorporated location scores describing hydrology and distance (Factors #1-2) characteristics (Appendix 1, Table 1). The location scores represent the potential for continued VHSV IVb exposure through natural pathways, such as water connectivity, fish passage or linear proximity (e.g., fomite transfer via wildlife or recreational traffic). Baseline sampling effort was calculated from the resulting RS* (product of described LRs) times the standard sample size. For example, a RS* of 0.12 suggests that a State with sufficient anthropogenic risk mitigations could demonstrate baseline disease freedom confidence through sampling 0.12 * 27 = 4 sites.

To identify areas requiring recurrent or ongoing surveillance, the temporal resilience of baseline confidence was based on an NSU-estimate of introduction risk (IR*). IR is the product of LRs for pathways open to disease transmission (LR > 1), and provides a rate for temporal discounting of historical survey data (Gustafson et al., in press). IR*, similar to RS*, presumes sufficient control of anthropogenic risk factors. The IR-revised value of historical data is estimated by dividing the State's previous sample size by IR. For example, an IR of 2.0 suggests that 27 sites sampled in a previous year retain a value of 14 (27/2) sites in the current year.

Sample sizes derived from either estimate apply to States with minimal anthropogenic risks. The RS* provides a target survey size, and the IR* provides a rate of historical discounting for historical survey data, given a protective regulatory environment and acceptable measures for control of anthropogenic risks. Thus, the resulting sample size calculations under-estimate needs where States have not yet addressed anthropogenic risks and over-estimate needs were States have exceeded average mitigations of anthropogenic risks. Consequently, for maximum confidence, States should complement surveillance with efforts to mitigate anthropogenic introductions and support systems for passive surveillance.

Appendix 4: Regulated States Introduction Risk and Sampling History

Table a: Introduction risks (IR*) for 4-digit HUCs based on predicted hydrology and distance scores. Hydrology and distance scores represent LRs from the VHSV IVb risk evaluation (Appendix 1, Table 1, Factors 1 and 2). Higher scores represent greater risks of introduction via natural pathways. Scores are based on current knowledge of VHSV distribution, and presume adequate control of anthropogenic risks. If VHSV is detected in a new 4-digit HUC, this could change the hydrology and/or distance scores for associated regions (e.g., Lake Superior detections would increase Minnesota distance and IR scores for neighboring HUCs). Results are shown for regulated States that provided scoring for individual HUCs. VHSV IVb known affected 4-digit HUCs (0403, 0406, 0408, 0409, 0412, 0413, 0414, 0415) in these States are excluded from this analysis.

State	HUC	Hydrology	Distance	IR*
Michigan	401	3.16	1.00	3.2
	402	0.71	1.00	1.0
	404	3.16	2.50	7.9
	405	3.16	2.50	7.9
	407	3.16	2.50	7.9
	410	3.16	2.50	7.9
	705	0.71	1.00	1.0
	707	0.71	2.50	2.5
	712	3.16	2.50	7.9
Minnesota	401	3.16	1.00	3.2
	701	0.71	1.00	1.0
	702	0.71	1.00	1.0
	703	0.71	1.00	1.0
	704	0.71	1.00	1.0
	706	0.71	1.00	1.0
	708	0.71	1.00	1.0
	710	0.32	1.00	1.0
	902	0.32	0.39	1.0
	903	0.32	1.00	1.0
	1017	0.71	1.00	1.0
	1023	0.32	1.00	1.0
New York	201	3.16	2.50	7.9
	202	3.16	2.50	7.9
	203	3.16	1.00	3.2
	204	0.32	1.00	1.0
	205	0.32	2.50	2.5
	501	0.32	2.50	2.5
Wisconsin	401	3.16	1.00	3.2
	402	0.71	1.00	1.0
	404	3.16	2.50	7.9
	703	0.71	1.00	1.0
	704	0.71	1.00	1.0
	705	0.71	1.00	1.0
	706	0.71	1.00	1.0
	707	0.71	2.50	2.5
	709	3.16	2.50	7.9
	712	3.16	2.50	7.9

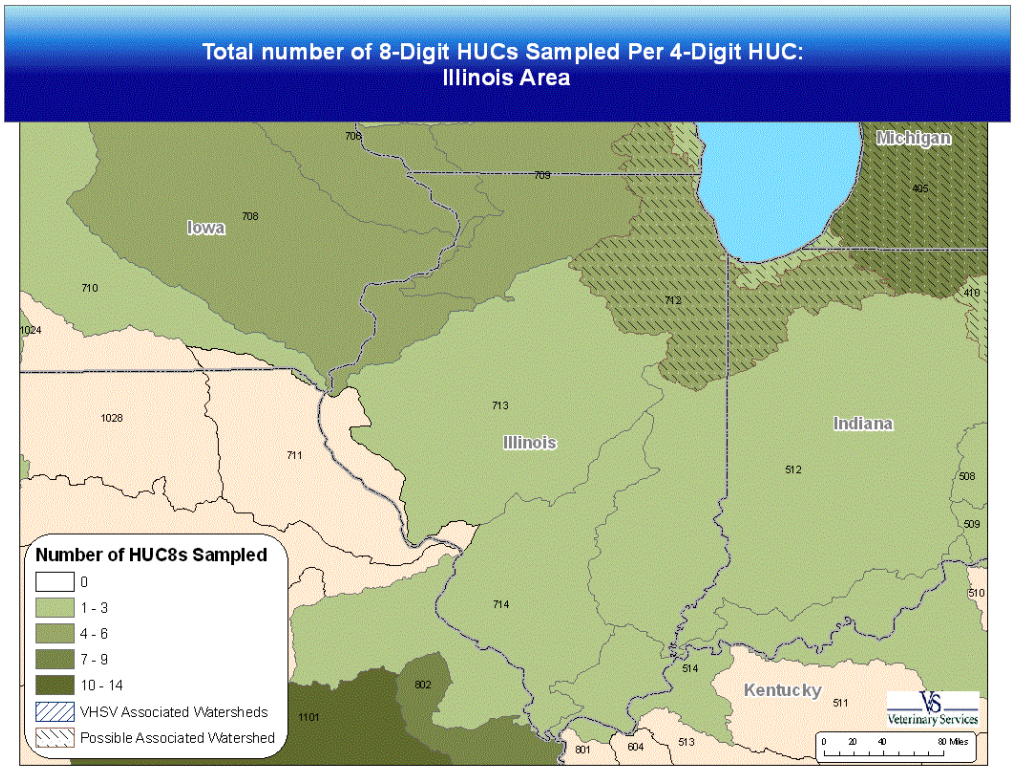

Figure a: Number of 8-digit HUCs sampled per 4-digit watershed in the Illinois area. Watersheds incorporating VHSV IVb known-positive locations and/or abutting known positive Great Lakes are hatch-marked. Greater sampling intensity generally corresponds with greater confidence in disease absence, unless the region is already denoted VHSV IVb affected. Confidence for the portion of the State that excludes the VHSV-affected (associated) and/or abutting (possible associated) HUCs is described in Tables 4 and 5. This map, combined with a similar map showing introduction risks by 4-digit HUC, would help site boundaries for intra-State disease management or zonation purposes.

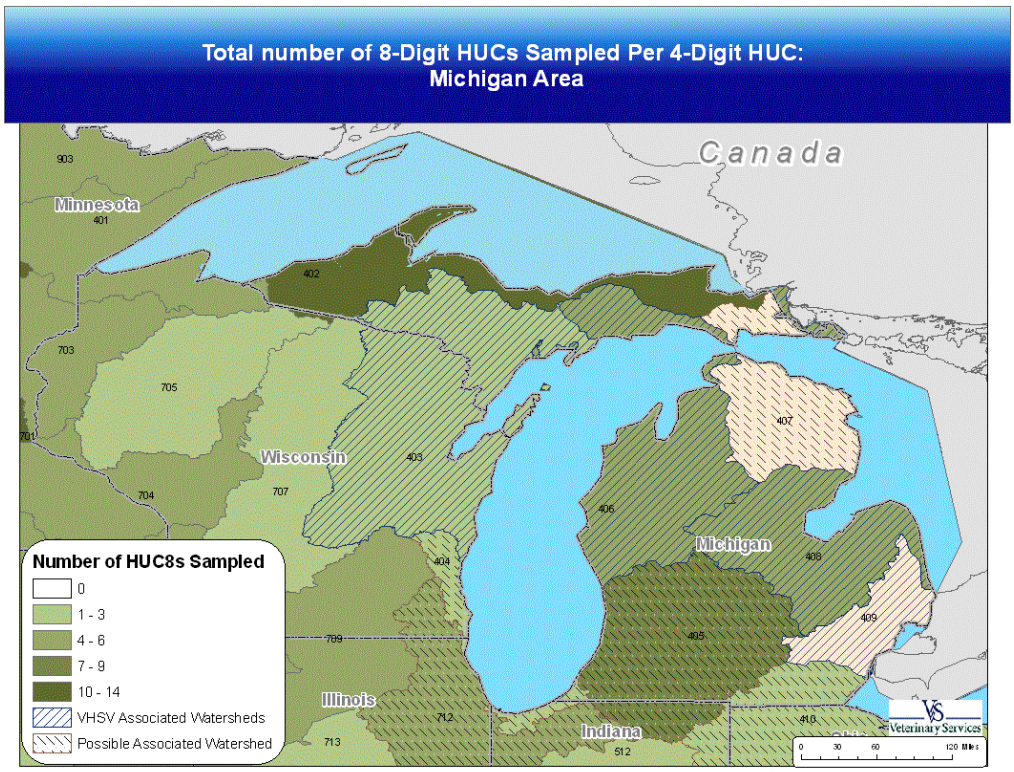

Figure b: Number of 8-digit HUCs sampled per 4-digit watershed in the Michigan area. Watersheds incorporating VHSV IVb known-positive locations and/or abutting known positive Great Lakes are hatch-marked. Greater sampling intensity generally corresponds with greater confidence in disease absence, unless the region is already denoted VHSV IVb affected. Confidence for the portion of the State that excludes the VHSV-affected (associated) and/or abutting (possible associated) HUCs is described in Tables 4 and 5. This map, combined with a similar map showing introduction risks by 4-digit HUC, would help site boundaries for intra-State disease management or zonation purposes.

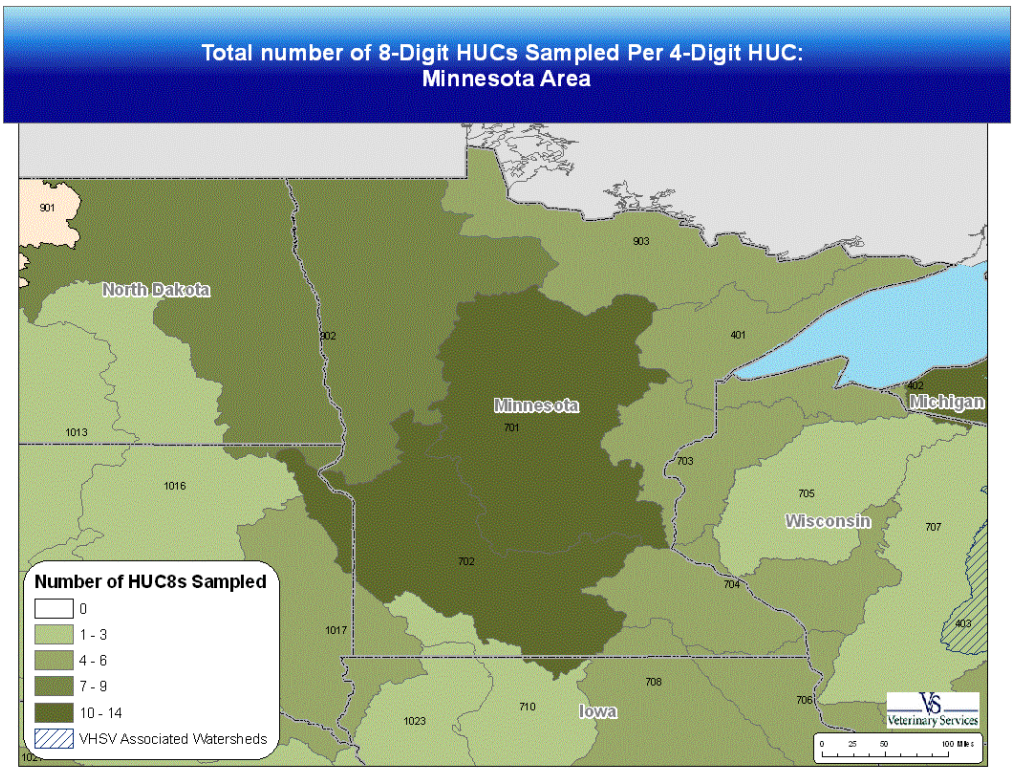

Figure c: Number of 8-digit HUCs sampled per 4-digit watershed in the Minnesota area. Watersheds incorporating VHSV IVb known-positive locations and/or abutting known positive Great Lakes are hatch-marked. Greater sampling intensity generally corresponds with greater confidence in disease absence, unless the region is already denoted VHSV IVb affected. Confidence for the portion of the State that excludes the VHSV-affected (associated) and/or abutting (possible associated) HUCs is described in Tables 4 and 5. This map, combined with a similar map showing introduction risks by 4-digit HUC, would help site boundaries for intra-State disease management or zonation purposes.

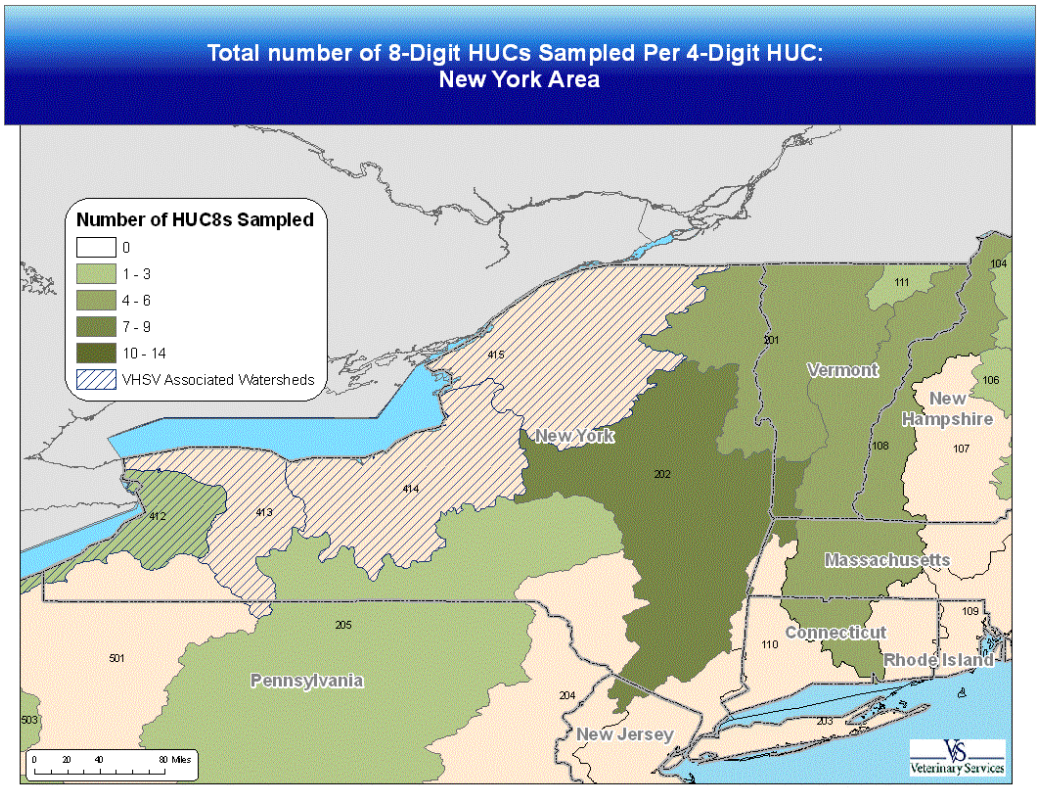

Figure d: Number of 8-digit HUCs sampled per 4-digit watershed in the New York area. Watersheds incorporating VHSV IVb known-positive locations and/or abutting known positive Great Lakes are hatch-marked. Greater sampling intensity generally corresponds with greater confidence in disease absence, unless the region is already denoted VHSV IVb affected. Confidence for the portion of the State that excludes the VHSV-affected (associated) and/or abutting (possible associated) HUCs is described in Tables 4 and 5. This map, combined with a similar map showing introduction risks by 4-digit HUC, would help site boundaries for intra-State disease management or zonation purposes.

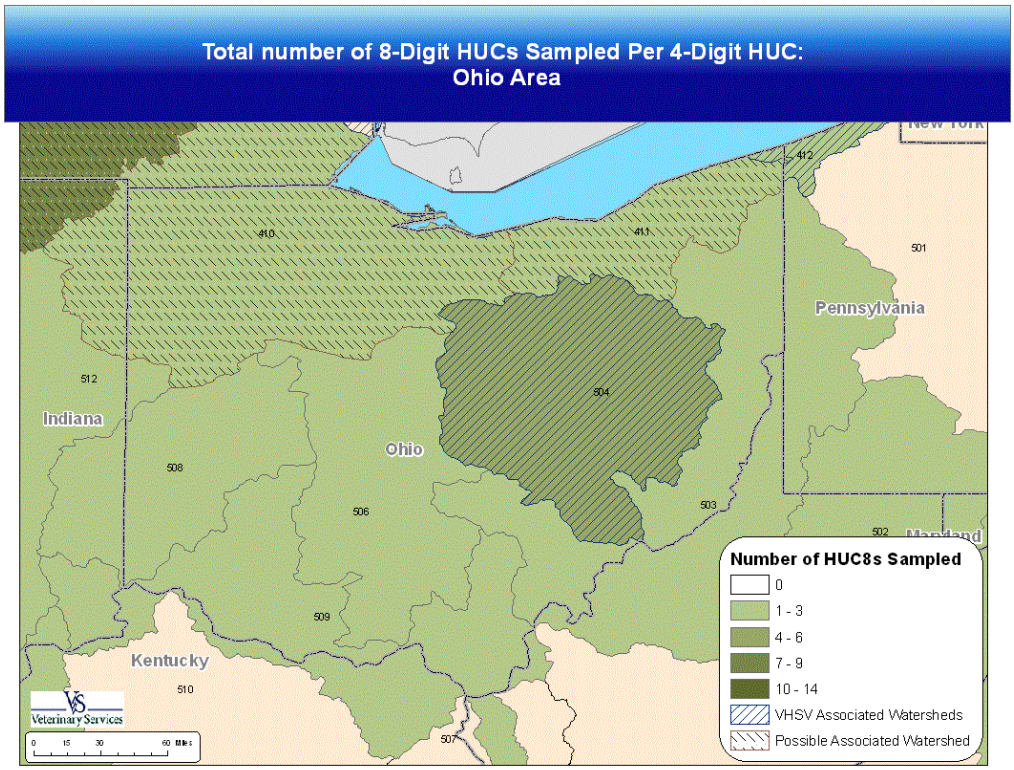

Figure e: Number of 8-digit HUCs sampled per 4-digit watershed in the Ohio area. Watersheds incorporating VHSV IVb known-positive locations and/or abutting known positive Great Lakes are hatch-marked. Greater sampling intensity generally corresponds with greater confidence in disease absence, unless the region is already denoted VHSV IVb affected. Confidence for the portion of the State that excludes the VHSV-affected (associated) and/or abutting (possible associated) HUCs is described in Tables 4 and 5. This map, combined with a similar map showing introduction risks by 4-digit HUC, would help site boundaries for intra-State disease management or zonation purposes.

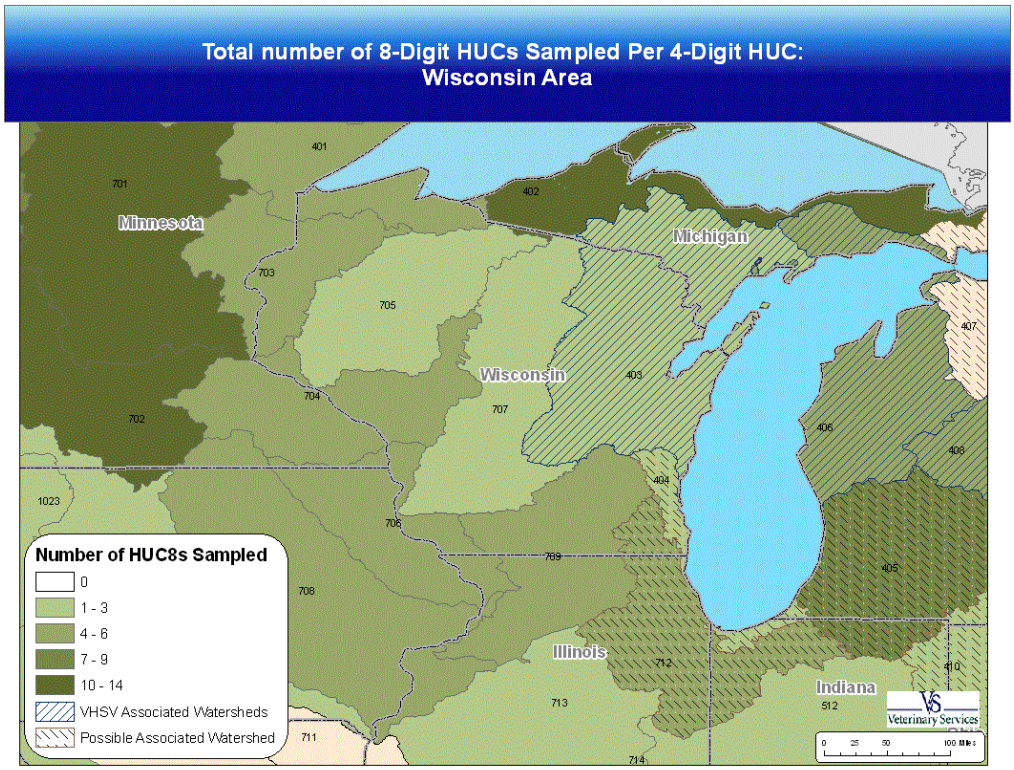

Figure f: Number of 8-digit HUCs sampled per 4-digit watershed in the Wisconsin area. Watersheds incorporating VHSV IVb known-positive locations and/or abutting known positive Great Lakes are hatch-marked. Greater sampling intensity generally corresponds with greater confidence in disease absence, unless the region is already denoted VHSV IVb affected. Confidence for the portion of the State that excludes the VHSV-affected (associated) and/or abutting (possible associated) HUCs is described in Tables 4 and 5. This map, combined with a similar map showing introduction risks by 4-digit HUC, would help site boundaries for intra-State disease management or zonation purposes.