Interstate Shellfish Sanitation Conference (ISSC)
National *Vibrio parahaemolyticus* Workshop Report
September 6-7, 2017 – Baltimore, MD

I. Purpose

According to the data provided by the Centers for Disease Control and Prevention (CDC), the number of *Vibrio parahaemolyticus* illnesses associated with shellfish consumption has increased in recent years. In an effort to address these illnesses, the ISSC has incorporated several control strategies. Significant increases in illnesses occurring in 2013 and subsequent years were discussed by the Executive Board recently and it was determined that a National *Vibrio parahaemolyticus* workshop would be helpful to better understand the risk posed by *Vibrio parahaemolyticus.*

II. Introduction

V.p. is a naturally occurring bacterium that is not associated with pollution. Since 2013, there have been approximately 400-500 confirmed cases associated with shellfish consumption and another 100 or more associated with wound infections. Approximately 1% of *V.p.* infections are fatal (typically immuno-compromised individuals with liver disease, cancer, or those taking drugs to suppress the immune system.) Considering under-reporting and under-diagnosing, CDC estimates that the actual number of infections is around 45,000 annually in the U.S.

III. Relevance to Molluscan Shellfish

The purpose of the National Shellfish Sanitation Program (NSSP) is to promote and improve the sanitation of shellfish moving in interstate commerce through federal/state cooperation and uniformity of State shellfish programs. One of the primary goals of the NSSP is the reduction of foodborne illnesses associated with shellfish. *V.p.* is a vibrio that causes a significant number of illnesses through the consumption of raw molluscan shellfish.

IV. Format and Meeting Objectives

The ISSC invited a panel of experts, academia, and scientists to present *V.p.* information and discuss existing *V.p.* controls and other potential strategies to address the increase in *V.p.* illnesses. The ISSC membership was requested to submit questions or concerns regarding *V.p.* as well as any relevant studies that may be helpful in understanding issues associated with *V.p*.

V. Expert Panelists

The ISSC invited several panelists with expertise in *V.p.* and illness investigation to participate in the meeting. The panelists are listed below. Many of the panelists

provided short presentations addressing specific areas of expertise. Summaries of the presentations are included in Section VI. Also included in Section VI are links to visual aids used by the panelists during their presentations.

A. Debra Barnes
New York State Department of Marine Resources

B. Chris Schillaci
Massachusetts Division of Marine Fisheries

C. Kristin DeRosia Banick
Connecticut Department of Agriculture

D. Clara Hard
Washington Department of Health

E. John Bowers
U.S. Food and Drug Administration

F. Cheryl Whistler
University of New Hampshire

G. Steve Jones
University of New Hampshire

H. John Jacobs
National Oceanic and Atmospheric Administration

I. Jessica Jones
U.S. Food and Drug Administration

J. Erin Burdette
Centers for Disease Control and Prevention

K. Andy Depaola
ADP Consulting/Retired U.S. Food and Drug Administration

L. Bob Rheault
East Coast Shellfish Growers Association

M. **Bill Dewey**
Taylor Shellfish Farms

N. **A.J. Erskine**
Bevans Oyster

O. **Laurie Stewart**
Washington Department of Health

P. **Seth Levine**
Virginia Department of Health

Q. **Jenna Iberg Johnson**
Louisiana Office of Public Health

R. **Rachel Noble**
University of North Carolina

S. **Keith Skiles**
Virginia Department of Health

T. **Enrico Bueanoventura**
Health Canada

VI. Presentations

A. National *V.p.* Illness Data — Erin Burdette

National *V.p.* trend data was provided for 2011-2015. The period was used to illustrate the significant *V.p.* illness increase which began in 2013 and continued through 2015. The presentation provided regional reporting totals and included data regarding percentages of cases that involved shellfish with single harvest area traceback information.

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B. State Epidemiological *V.p.* Reporting Procedures — Jenna Iberg Johnson, Seth Levine, Laurie Stewart

These presentations provided a detailed description of the illness reporting procedure within their respective states. The presenters addressed the following questions:

1. How is the illness reported to you?
2. Who and how the illness is investigated?
3. What other parties are advised when you receive notification of illness?
4. What roles do other parties play when illnesses are reported?
5. What are the notification time lines for *V.p.* in your State?
6. What determines the timeliness of COVIS form submission to CDC?

The presenters were asked to estimate time necessary to complete the COVIS form following notice of illness. The estimates were from 24 hours to thirty (30) days with most being completed in three (3) to seven (7) days.

Click here to access visual aids presented by Jenna Iberg-Johnson.

Click here to access visual aids presented by Seth Levine.

Click here to access visual aids presented by Laurie Stewart.

C. Illness Investigation — Eric Hickey

The presentation outlines the procedures used by the Massachusetts Department of Public Health in conducting shellfish illness investigations. The presentation addressed communication procedures between the state epidemiologists and the health department staff responsible for investigating illnesses. The presenter discussed efforts by the Department of Public Health to educate county health staff responsible for illness reporting.

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D. Shellfish Production Trends — John Jacobs

The presenter provided shellfish production data from the NOAA Commercial Fisheries Statistics. The presenter explained that the data is difficult to interpret with regard to national trends. States provide shellfish production data in several different formats with some states not responding at all. The NOAA data from Washington, Texas, Virginia, Massachusetts and Louisiana was more complete and showed significant relatively consistent production from

Louisiana, Texas and Washington with increasing production from Virginia and Massachusetts. Additional data from New Jersey and Massachusetts was provided by the ISSC. The New Jersey and Massachusetts data provided monthly totals. The data shows increased production during warmer months in Massachusetts and peak production typically in early summer in New Jersey. The NOAA data is total oyster production and makes no distinction for the percentage of that which was destined for the raw consumption market. Washington State has made an effort to collect data for live oyster vs shuck meat production during the vibrio months. Massachusetts indicates most of their production goes for raw consumption.

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E. Invasive and Endemic *V.p.* Strains Cheryl Whistler

Most *V.p.* infections in the Northeast are caused by two lineages: Pacific-originating ST36 (O4:K12) and Atlantic endemic ST631 (O11:KUT). The Atlantic US Coast has at least 3 distinct ST36 populations, each founded by a single bacterium, suggesting three separate introduction events. ST631 is the first reported major endemic pathogen to have evolved from the North Atlantic *V.p.* population, and it acquired its "pathogenicity island" from a Pacific strain (potentially ST36). The various strains that cause illness in the Northeast, are predominately tdh+/trh+ or tdh-/trh+. Molecular tests have been developed for specific lineages of *V.p.* and can be used in conjunction with standard testing methods.

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F. Environmental Conditions Associated with Elevated *V.p.* Concentrations Steve Jones

The presentation discussed an array of environmental conditions that appear to influence *V.p.* concentrations in oysters. Some of which probably reflect direct influence and serve as potential indicators and to better understand the ecology of *V.p.* Others may be correlative and represent integrated environmental measurements that could potentially serve as indicators of *V.p.* population dynamics and risk assessment. The presenter indicated that projected warming trends will no doubt change the relative importance of these factors for influencing *V.p.* concentrations in oysters. Other biological factors (plankton, predators, phage) which are probably highly significant influences on *V.p.* populations once the water temperature is warm during summer were

discussed. The presenter suggested that we are beginning to understand the ecology of pathogenic *V.p.* strains, including the roles of environmental selection and gene flow.

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G. Invasive and Endemic *V.p.* Strains in Massachusetts — Chris Schillaci

The presentation addressed the pathogenic strain has been identified as being responsible for illness increases since 2013. Locations within the state where these strains are most prevalent were illustrated. An outline of the state *V.p.* control plan was provided. Included was an assessment of the effective of post harvest controls.

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H. State *V.p.* Management Strategies — Kristin DeRosia-Banick

CT has two control plans in place for oysters: one for 2013 outbreak area and one for the rest of CT growing areas, both of which require shading. In the outbreak area, oysters must be rapidly cooled to an internal temperature of 50°F within one hour of harvest from June 1 - September 30, when water temperatures are above 68°F. In all other areas, 5 hours from harvest to refrigeration and 5 hours to cool to internal temperature of 50°F from June 1 - September 30. No more than two illnesses annually have been reported and confirmed from growing areas under control plans since 2013. All illnesses associated with water temperatures >68F.

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I. State *V.p.* Management Strategies — Debra Barnes

Following the outbreak in 2012, progressively stricter controls have been put in place in NY each year. In 2014, 1h to icing or 5h to refrigeration (depending on harvest area) with product cooled to 68F resulted in 15 cases linked to NY. In 2015, 1h to cooling (icing, refrigeration, or slurry) with product cooled to 60F resulted in 7 cases linked to NY. In 206, immediate cooling with product cooled to 60F resulted in 3 illnesses linked to NY product. Illness number and control plans apply to oysters and clams. Clam production ha recently increased in New York.

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J. State *V.p.* Management Strategies — Chris Schillaci

Control plan requires oysters to be iced within particular time of harvest or prior to leaving the point of landing, whichever occurs first. Icing is defined as 3x2x2 loose ice around each bag or submerged in an ice slurry <45F. Time to ice is 2hr ~May 15 - Oct 15 and 1hr in Western Cape Cod Bay and Katama Bay July 1- Sept 15. If any oyster processing has a greater than 2h exposure time, oysters must be re-submersed for a minimum of 14d prior to harvest. In one area where "frequent" illnesses have occured, transplantation to other locations is considered less conducive to high *V.p.* abundance (colder water, deeper, less productive) permitted. Under these controls, illnesses were ~27 in 2015 and 10 in 2016, with an increase in landings.

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K. Invasive and Endemic *V.p.* Strains/Environmental Conditions Associated with Elevated *V.p.* Concentrations/State *V.p.* Management Strategies — Clara Hard

A majority of PNW clinical *V.p.* isolates were historically ST36 (O4:K12), with other endemic clades ST43, ST417, and ST65 contributing to illnesses. The clinical isolate population is shifting with a majority of cases from O1:K56 in 2012. Most clinical strains are trh+, with the majority tdh+ as well. Many of the environmental strains are ST3, which are primarily tdh+/trh-. Temperature by itself is not a strong predictor of *V.p.* densities in water. In a 2008-2009 study, the highest water temperature was recorded months prior to highest *V.p.* levels in water (note: levels not measured in shellfish). Salinity, other nutrients (except silicate) and phytoplankton populations, did not show any effect on *V.p.* densities in water, but silicate was negatively correlated to *V.p.* concentrations. Additionally, studies indicate growing area substrate affects *V.p.* levels in shellfish.
Control plan is based on risk categories determined by single-source *V.p.* cases from previous five years. All categories' plans are based on air temperature, water or tissue temperature, and time to internal temperature of 50F. Risk category 3 allows a maximum of 5h to 50F from May 1 – Sep 30, 3h when air temp is >80F, 1h when water temp is 64-66F, and harvest is not allowed for 24h from July 1-Aug 31 when water temps are >66F. Illnesses for 2015-2016 were ~45-55 under new control plan, down from ~80 in 2013-2014.

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L. State *V.p.* Management Strategies — Keith Skiles

The state of Virginia is currently implementing a *V.v.* Control Plan with includes three (3) harvest options as follows:

- No harvest after:
  - 11:00 AM during the month of May
  - 10:00 AM during the months of June, July and August
  - 12:00 PM during the month of September
- On-board icing or refrigeration within:
  - 5 hours during the month of May
  - 3 hours during the month of June
  - 2 hours during the months of July and August
- Restricted Use Harvesting

Virginia has experienced significant increases in production in recent years without significant increases in illness.

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M. Environmental Conditions Associated with Elevated *V.p.* Concentrations
Rachel Noble

Strong association between water temperatures and *V.p.* in water and oysters. Also a correlation with *V.p.* and pigments associated with diatom populations. Trend towards higher *V.p.* levels in farmed oysters versus wild.

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N. Recent *V.p.* ISSC and VARB Research Summary Jessica Jones

Vibrio levels higher in oysters than clams from the same harvest area. Vibrio levels return to background after 7-14d resubmerging following routine desiccation, depending on study. Vibrio levels in intertidal oysters return to background after one tidal cycle, unless handled and stored. After 5h of ambient storage, vibrio levels significantly increase in oysters from all coasts. When compared to refrigeration alone, icing allows for more rapid cooling and would result in less post-harvest growth. MA data suggests high variability in *V.p.* (total, tdh, trh) levels across harvest areas, and also variations in correlations of environmental parameters with *V.p.* levels. EO water does not seem to be an effective means of reducing *V.p.* levels, but cool water "depuration" in artificial salt water shows >3log reduction over two days. "Relaying" to higher salinity and/or cooler waters show promise for *V.p.* levels reduction.

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O. Ecological Forecasting for Vibrios — John Jacobs

The presenter shared ecological forecasting tools that are being developed by NOAA with assistance from other collaborators. Short –term predictive guidance is available for expected Vp concentrations in oysters at time of harvest as well as continued growth post-harvest. Other tools are directly linked to state Vp control plans offering site specific guidance of when control measures may go into effect or Vp growth expected under different post-harvest practices. These tools are available for many areas around the country and are accessible through the NOAA web page (https://products.coastalscience.noaa.gov/vibrioforecast). John also shared data on the growth rate of ST36 strains at different temperatures in comparison to non-ST36 strains, other studies, and the FDA model. While only a limited number of strains were examined, ST36 growth was similar to FDA model predictions with the exception of potentially higher growth at low temperature.

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P. *V.p.* Risk Assessment and Calculator Development — John Bowers

Key assumptions used during development of the risk assessment are that (1) water temp alone determines the total *V.p.* levels at harvest, (2) pathogenic strains are tdh+, al tdh+ strains are equally virulent and grow the same as total *V.p.*, (4) growth rate is determined by temperature alone and there is no variability, (5) illness under-reporting and under-diagnosis is as described in Mead et al 1999, and (6) servings determined by NFMS landings data with the assumption that a serving size is 12 oysters and 50% are consumed raw. The RA is good at predictions of exposure (levels at retail), and seasonal distribution of illness. However, the RA is poor at predictions of regional distribution of illness. The prediction of total illnesses cannot be validated. The calculator reproduces select inputs/outputs of the *V.p.* RA in an Excel worksheet. Updates to the calculator in the current form would be of limited value and will likely change to a web-based tool.

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Q. *V.p.* Risk Assessment Updates — Andy DePaola

The first step was to test the skill assessment of the RA. In Mississippi study, observed *V.p.* levels slightly higher than RA predictions. In various studies, *V.p.* growth was similar to that predicted by RA, except for Asian oysters and Fernandez study with C. gigas. Skill assessment determined that outbreak strains may increase risk (AK model). Regionalization of risk models is needed.

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R. Canadian *V.p.* Management System — Enrico Buenaventura

The recent 2015 Canadian *V.p.* outbreak triggered a major shift in the *V.p.* policy and led to a review of the *V.p.* control program in Canada. The presenter provided an overview of recommended parameters that can be used as part of the harvester/processor risk management plan in Canada during the high-risk *V.p.* seasons. Historically, May 1st has signaled the start of the high-risk *V.p.* season in Canada while its end may vary depending on the Canadian harvest area location. The end of the high-risk *V.p* .season should not be declared over prior to October 1st; i.e., when, at point of harvest, the water and oyster temperatures have consistently demonstrated to be <15oC and that the *V.p.* levels of live oysters are consistently < 3 MPN/gram. Furthermore, following the 2015 vibriosis outbreak, a guideline of 100 MPN/gram for *V.p.* in oyster shellstock intended for raw consumption, offered for sale in Canada, was adopted. Since its implementation, *V.p.* illnesses have dropped significantly. Bill Dewey indicated that Taylor Shellfish of Washington has a company in British Columbia and that they have been able to meet the 100 MPN/gram guideline via holding shellfish at depths with lower water temperatures.

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X. Questions Answered by Panelists

Illness Data and Reporting

1. What is the distribution of reported *V.p.* illnesses in the US?

   Erin Burdette - Most *V.p.* cases (all transmission) are reported from CA, WA, NY, FL, MA, OR, VA, NJ.

2. Are increasing numbers of reported illnesses a result of higher risk, greater exposure, or changes in reporting?

   Seth Levine – there have been changes in reporting, including CIDT in 2017 that may lead to more cases reported. Laurie Stewart – have not seen a consistent rise in cases in WA; however, CIDT will result in more probable cases reported. Jenna Johnson – no change in LA for oyster consumption cases.

   Vibriosis became nationally notifiable in 2007. Since then it is thought that public awareness and clinician awareness has increased. It is probable that increased Vibrio levels in the environment (greater exposure) are a contributing factor, along with a shift in product of harvest for raw

consumption. The invasive strains in the NE also indicate there is a higher risk associated with these strains.

3. Are increases in illnesses associated with particular regions of the US?

   The increases in 2012/2013 in the NE, predominately due to ST36 were region-specific. Otherwise, not really.

4. Is there correlation between increases in reported illnesses and regions with improved/more/better reporting?

   Seth – there is likely more awareness among residents and clinicians in coastal states.
   Laurie – coastal state labs may be better prepared to confirm Vibrio infections.
   Jenna – the use of electronic lab reporting could increase reported cases.

   As stated in #2, it is most likely that the use of CIDT will lead to an increase of probable cases being reported.

5. What is the distribution of reported *V.p.* illnesses between immunocompromised persons and healthy individuals?

   Erin – 41% of cases report pre-existing conditions.

6. How much shellfish is harvested for the raw market (regionally, seasonally, by harvest practice, by species, etc. …)?

   Eric Hickey - 90% of MA harvest is for raw market.
   Bill Dewey – there has been a significant shift toward single-oyster production for raw consumption in WA.
   AJ Erskine – more oysters cultured for the shucked market in VA.
   Bob Reault – for NJ and north, very little going to shucked market.
   Steve Fleetwood – most raw production is during the summer (April – Oct).

   Can FDA start to push states on reporting how much product goes to raw market? The MO does not require production reporting by product type. The language states "if available".

7. For each State (or region) how much of the raw market comes from which States (or regions)?

   Can't answer this without better landings/production data.

8. Is the increase in illnesses due to an increase in production and/or raw consumption, so that the risk per serving is not increasing?

Can't answer this without better landings/production data.

9. How will you ever determine what the raw market is? Shucked product is consumed raw all the time. Shellstock is often cooked. It is difficult to get a handle on how food is actually consumed.

   See responses to #6-8 above.

10. What is the extent of *V.p.* illnesses underreporting?

    Erin B. – paper from Elaine Scallan (2011) is the primary reference; for foodborne *V.p.*, there are an estimated 35,000 cases and 100 hospitalizations from *V.p.* annually. The multiplier is 142.4:1 (estimated:reported).

    Many factors contribute to the underreporting factor for vibrio. It is a difficult organism to culture, so it can often go undiagnosed. Also, severity of illness is a contributing factor; people experiencing mild symptoms often do not seek medical attention. For more information, go to https://www.cdc.gov/foodborneburdon/2011-methods.html.

11. Are there any appreciable regional differences in underreporting?

    Erin B – no data available.

12. How has underreporting been trending over time?

    No data available.

13. How is underreporting determined?

    See response to #10. Details are provided and/or referenced in the Scallan 2011 paper.

14 The multipliers for under-diagnosis and under-reporting are very different for the CDC and the FDA. One estimates 2.2 X the national infection rate of the other. Why is the FDA's 2005 *V.p.* Risk Analysis suggesting 20,000 illnesses per year while the CDC is more like 45,000?

    Jessica J. – When the risk assessment was developed, CDC (Mead et al 1999) estimated the multiplier to be 20. FDA's 2005 *V.p.* Risk Analysis (RA) estimated 2800 illnesses per year based on this multiplier and the average number of culture-confirmed and oyster-associated illnesses occurring from 1998-2002. CDC has since updated that multiplier estimate (Scallan et al 2011) and the number of culture-confirmed and oyster-associated illnesses has increased compared to 19998-2002, which accounts for the difference. If

FDA were to update the RA, the current CDC multiplier would be used as would a more recent estimate of the annual number of culture-confirmed illnesses.

15. How are *V.p.* cases reported, including any regional or State differences?

All states report *V.p.* to CDC using the COVIS form. While there may be differences in process, timeliness and scope of investigations, the overall output appears to be equivalent.

16. Have changes occurred in reporting that could have influenced reported cases in recent years?
See responses to #2. The COVIS form has been modified slightly since 2007. The COVIS form now allows for 10 tags to be included on the form and photocopies of tags are requested. It is unlikely that minor changes have influenced reports cases in recent years.

17. How will reporting be affected by the new Culture Independent Testing methods that are more widespread now?

CIDT reporting is new. We won't really know for a few years. States and CDC will be considering CITD cases as probable (unconfirmed) unless cultured (which CDC strongly encourages). Most states will be conducting initial traceback and requesting tags on these cases. Some states are encouraging (or requiring) a sample or an isolate be sent to state lab for confirmation of CIDT positives.

18. What percentage of *V.p.* patients reported using Prilosec, Nexium or antacids?

CDC data states 10% of *V.p.* cases; LA, 15%; WA, 10-29%; VA, 64% (ANNUAL DATA FROM 2016); NY, 50% (during the 2012 outbreak). A complicating factor is that different strains may have different tolerance to stomach acid.

19. Would development of a whole genome sequencing library be useful in effective tracebacks? Could this be done?

This is being done with other pathogens through FDA's Genome Trakr Network. To make a system like this work for traceback, it requires a lot of sequence data (1000s of sequences). So, the level of refinement that exists in the current database is not available to determine where a strain came from, but we could work toward it. A caveat being that *V.p.* share genes so frequently that it is uncertain if we could ever achieve growing area level refinement.

Pathogen Ecology and Virulence

20. Why is there an apparent shift in illnesses in relation to geographical area and water/air temperature? Why are there more cases in the shoulder months in the Gulf states in recent years? Why do illnesses in the Mid- and North Atlantic decline during the hottest months (July – August)?

    V.p. cases from the "more pathogenic" strains occur near same latitude (NW, NE), and we are starting to see more cases in shoulder months in other latitudes (Gulf). This leads to a hypothesis that these pathogenic strains are outcompeted by less pathogenic populations and other species, or are otherwise reduced in importance within the oyster and harvest area ecosystem, at warmer temps. So, it is possible that there is a change in *V.p.* populations or competition/symbiosis of other organisms (microbiome or diatoms/alagae) with geography and/or temperatures that drive the shift in illnesses.

    Seed requires a check for MSX and Dermo; is it possible that these Vibrios are being transferred by seed? See response to #35.

    New Hampshire oysters did not have detectable levels of tdh+ until they were temperature abused. This means that the more pathogenic strains may thrive under higher temperatures relative to other strains.

21. What are some plausible explanations for why, according to CDC data, the historical seasonal distributions of reported oyster borne Vibrio parahaemolyticus cases between the major regions of the country different from each other?

    See responses to #1, 3, 4, 20.

22. Why does the illness rate in NJ appear to drop off in July and August as temps are peaking? Do we have a testable hypothesis?

    If we are assuming (response to #20) that there is a certain temperature window in which the pathogenic strains peak, NJ is a little different. Most of the summer harvest comes from Delaware Bay, which is shallow and the water temperature, so could be exceeding the upper temperature window that these strains prefer. There is no really good way to test this hypothesis, however.

23. What are the pathogenic strains (subpopulation of total *V.p.*) that are capable of causing illness?

    Refer to C. Whistler, C. Schillaci, and C. Hard presentations.

24. What is the virulence (dose-response or potency) of the pathogenic strains?

Occurrence of illness outbreaks in recent years due to introduction of non-indigenous strains indicates there is a distribution of virulence among the different strains causing illness. Cases are frequently reported with only one oyster being consumed. Average or effective dose-response within a region depends on the number and abundance of pathogenic strains in that region, and their individual virulence. Potentially, relative virulence of one strain versus another is related to their relative frequency of occurrence among environmental versus clinical isolates. If sufficient data were available this, in conjunction with other epidemiological and consumption data, might be used to estimate individual dose-response for pathogenic strains. At present sufficient data does not appear to be available. It was noted that the strains associated with the Alaska (2004) and Texas (1999) illnesses involved virulent strains that proved to be much more virulent than strains associated with recent *V.p.* illnesses.

25. Is virulence influenced in any substantive manner by environmental conditions or other factors?

    Refer to C. Whistler, C. Schillaci, and C. Hard presentations; response to #2.

26. What are the appropriate markers and/or tests for these pathogenic strains?

    Refer to C. Whistler, C. Schillaci, and C. Hard presentations.

27. How can emerging pathogenic strains be identified?

    The use of FDA's Genome Trakr system could aid in this as long as the appropriate strains (clinical and oyster/environmental) were submitted with the appropriate metadata. Now that specific pathogenicity islands have been identified, tools to track them, rather than relying on WGS could be developed.

28. Why are illnesses so sporadic? If you have virulent strains in a load that has been temperature abused, why don't most people get sick from that whole lot? Also consider different varieties of oysters, age class, culture methods, originating from seed stock vs wild/natural propagation.

    Although we are not certain why, significant oyster-to-oyster variability occurs. This variability is well documented.

29. What is the geographic range of these pathogenic strains?

    Refer to C. Whistler, C. Schillaci, and C. Hard presentations; response to #20.

30. How has the geographic range been trending over time?

See answer to Question 20. Most of the identified strains have been identified in clinical samples associated with illnesses involving oysters from Washington, Massachusetts, Connecticut, New York and Virginia. It is unclear how many other states have attempted strain identification.

31. How is abundance influenced by environmental conditions?

    It appears that certain temperatures play a role. Other environmental factors show little correlation.

32. To what extent do blooms (i.e., rapid and transient increase) in *V.p.* abundance occur and, if so, what environmental conditions influence that?

    Weather conditions seem to affect abundance of TLH, TRH, TDH, but it could be a matter of the feeding of the shellfish. In summary, we do not know.

33. Does the presence/growth of other Vibrio spp. influence the populations/growth rates of *V.p.*?

    We do not know.

34. Do certain pathogenic strains out compete other vibrio when environmental factors change?

    There are certain times when pathogenic strains emerge. It is unknown if this is the result of competition or more favorable environmental conditions.

35. Are regional pathogenic strains spread from importation of seed from different geographic areas? Are regional pathogenic strains potentially spread from growing area to growing area through wet storage?

    Numerous potential vectors have been suggested as being responsible for the recent occurrences of the more pathogenic strains, but little evidence is available to confirm the actual vector.

36. What explains the high level of variation between *V.p.* levels in oysters taken from the same bag/batch/lot? Is it simply feeding variability in the organism or is there some sort of host pathogen interaction?

    We do not know

37. Is there a correlation (regionally or generally) between total and pathogenic strains of *V.p.* that predicts risk of illness?

    Seems to be area specific, but a precise correlation does not exist.

Pathogen Exposure

38. Do the outbreak / pathogenic strains grow at the same rates at different temperatures as the strains that were used to create the 2005 *V.p.* Risk Assessment?

    There is very little information available to answer this question. John Jacobs indicated that ST36 grew at the rate at temperatures above 59°F (15°C) and that at lower temperature ST36 grew faster.

39. How do variations in temperature affect these pathogenic growth rates?

    See answers to questions #38.

40. Is there a relationship between total *V.p.* and pathogenic strains in shellfish species prior to harvest?

    John Jacobs indicated that in data from a study with Washington shellfish, when total *V.p.* levels were above 10, 000/g, only 7% were tdh positive.

41. How is this relationship affected by environmental conditions?

    Correlations do not seem to exist in a matter that could be readily applied to management. Chris Schillaci indicated correlations with temperature and tlh and trh occurrence in some areas. Additionally, Ph was negatively correlated with tlh in some areas. There were no strong correlations between environmental conditions and tdh. See Jessica Jones presentation.

42. Is the growth rate of pathogenic strains in shellfish species post-harvest similar to the growth rate of total *V.p.*?

    Very little information available to answer this question. See answer to question 38.

43. What factors or environmental conditions (e.g., salinity) affect growth rates of pathogenic strains?

    See answers to questions 31, 32, 33, 34, 38, 39, and 41.

44. Are there any appreciable regional and seasonal differences in pathogenic strains?

    The prevalence of pathogenic strains seems to be related to a temperature range that appears to begin at around 68°F. It appears that summertime water temperature in more southeastern locations around the US reach a warmer temperature that does not result in significance illnesses caused by

the more pathogenic strains. Although the exact upper temperature is not known it is believed to begin around 80°F. It may be that at higher temperatures the pathogenic strains are outcompeted by other less pathogenic strains. This could explain why illnesses associated with the more pathogenic strains occur in spring and fall in more southern states. Most of the *V.p.* illnesses are occurring in Washington, British Columbia and the New England area. The summertime water temperatures in these locations tend to be within the temperature window at which most *V.p.* cases occur.

See questions 21 and 22.

45. How variable are growth rates of pathogenic strains?

Very little information is available to address this question. See answer to question 38.

46. Are pathogenic growth rates different in different shellfish species (C. gigas v. C. virginica v. M. mercenaria)?

There is little information available regarding growth rate in different species of shellfish. Pumping rates and filtering capabilities are different for different species which would suggest that different levels of *V.p.* would be found in different species. This does not suggest higher growth rates necessarily, but that more *V.p.* is being consumed by the animal.

47. Do total and pathogenic populations behave similarly in response to refrigeration and/or icing?

They behave similarly to the same temperature. The temperature drop is faster in ice and results in numbers being lowered at a faster rate.

48. What are the survival and decline rates?

There is no definitive answer but studies indicate survival depends on temperature and estimates of survival of total *V.p.* at selected temperatures are available.

49. How quickly are *V.p.* depurated from shellfish when harvest practices involve some type of resubmergence?

It depends on the practice, the location and the temperature of the water. The amount and type of disturbance affects how quickly the animal returns to normal pumping. Taylor Shellfish reported having good results reducing total vibrios holding oysters below the thermocline on the British Columbia farms in 12-15°C water for 5-7 days. They have seen similar results in a

recirculating refrigerated wet storage system in Washington held at 10°C for 5 days.

50. How is this influenced by environmental conditions?

See question 49.

51. Do total and pathogenic populations purge at the same rate(s) from shellfish that are resubmerged?

See question 49.

52. What factors affect rates of purging from resubmerged shellfish (handling prior to resubmerging or the environment in which they are resubmerged)?

See question 49.

53. Most depuration studies looked at oysters in artificial SW or in filtered UV treated SW. Is the depuration rate improved by having food in the water? Is depuration faster at 50°F or at higher temps?

It seems that shellfish pumping is increased when food is present. Shellfish normally pump at a higher rate in higher temperatures, however, lower temperatures affect survival of *V.p.* thus reducing *V.p.* levels in shellfish.

Current Monitoring Programs and Methods

54. What is the extent of state monitoring for total and pathogenic *V.p.*?

Several states are sampling for *V.p.* with many including tlh, tdh and trh. These states include Virginia, New York, Connecticut, Massachusetts, New Hampshire, Washington, New Jersey and California.

55. What pathogenicity markers are they using and how are they doing it?

See question 54.
In Massachusetts, analysis is being conducted on trh and tdh positive samples to identify pathogenic strains.

56. Are environmental strains being monitored?

See question 55.

57. How expensive is environmental strain monitoring?

Cost at University of New Hampshire is $400 per sample plus university indirect cost.

58. How effective is environmental strain monitoring in predicting risk?

It is very difficult to find pathogenic strains in the environment because levels fluctuate constantly, much the same as illnesses. Illness may come from one shellfish. Levels of pathogenic strains are not consistent from animal to animal. The presence within an animal indicates risk. Determining the risk for the growing area is more complex.

Current Controls and Effectiveness

59. What is happening to *V.p.* populations under current handling practices (including harvest, post-harvest, cooling, etc.)?

Connecticut, Massachusetts and New York indicated a reduction in illnesses that were linked to shellfish from the area where the time to temperature were reduced to one (1) hour. Massachusetts indicated a drop in illnesses when controls were changed from two (2) hours to one (1) hour. While Connecticut indicated that the reduction was due to the more stringent time to temperature controls, New York and Massachusetts suggested its reduction may be the result of a significant reduction in the levels of pathogenic strains present in the harvest area.

Additional work is necessary to definitively differentiate the effectiveness of time to temperature controls. The level of pathogenic strains in the harvest area must be known to effectively evaluate effectiveness of controls.

60. Was the 2013 outbreak strain mitigated by controls or has it gone away?

See question 59.

61. Have illnesses trended down over the last two years since the Conference has implemented Model Ordinance Chapter II @.02 A.?

The requirements of Chapter II @ 02.A. became effective September 30, 2014. *V.p.* cases increased through 2015. The 2016 data is not available at present.

62. Is the tiered regulatory response requirements effecting the number of *V.p.* illnesses?

See question 61.

63. How are states implementing Model Ordinance Chapter II @.02 A.?

Several states have met threshold and have had closures.

64. Are there controls that have had success and how is success being measured?

See question 59.
Taylor Shellfish has an operation in British Columbia which is holding shellfish at 50°F during high risk months and the Canadian standard of 100 MPN/gram is being met.

Other

65. What is the temperature profile of shellfish when exposed during various harvest practices (submerged, intertidal, resubmerged, etc. …) and subject to specified NSSP time- temperature requirements?

Multiple studies demonstrated increases in internal temperatures of oysters exposed to ambient air. No studies appear to exist looking at submerged oysters. The studies with internal temperature data have shown *V.p.* growth similar to predicted growth rates outlined in the *V.p.* risk assessment, based on the observed temperature profiles.

66. How variable are these temperature exposure profiles?

The application of different temperature controls offer variability. Rapid cooling with ice slurry is consistent while using mechanical refrigeration is dependent upon the capability of the refrigeration unit being used.

67. Do the *V.p.* calculators only consider growth rates of total *V.p.*?

Yes.

68. What are the keys to cutting the cost per sample so we can do more studies more affordably? Are there specific roadblocks to developing cheaper assays?

Finding cheaper technologies.

69. What do we know about the fate of ingested *V.p.*, where it ends up, is it digested or tolerated, does it hang out and multiply on tissues, do hemocytes attack *V.p.* cells?

Very little is known regarding the fate of *V.p.* once it is ingested by the shellfish.