Chronic and persistent viral hemorrhagic septicemia virus infections in Pacific herring

P. K. Hershberger[1,*], J. L. Gregg[1], C. A. Grady[1], L. Taylor[1], J. R. Winton[2]

[1]Marrowstone Marine Field Station, US Geological Survey – Western Fisheries Research Center, Nordland, Washington 98358, USA

[2]US Geological Survey – Western Fisheries Research Center, Seattle, Washington 98115, USA

ABSTRACT: Chronic viral hemorrhagic septicemia virus (VHSV) infections were established in a laboratory stock of Pacific herring *Clupea pallasii* held in a large-volume tank supplied with pathogen-free seawater at temperatures ranging from 6.8 to 11.6°C. The infections were characterized by viral persistence for extended periods and near-background levels of host mortality. Infectious virus was recovered from mortalities occurring up to 167 d post-exposure and was detected in normal-appearing herring for as long as 224 d following initial challenge. Geometric mean viral titers were generally as high as or higher in brain tissues than in pools of kidney and spleen tissues, with overall prevalence of infection being higher in the brain. Upon re-exposure to VHSV in a standard laboratory challenge, negligible mortality occurred among groups of herring that were either chronically infected or fully recovered, indicating that survival from chronic manifestations conferred protection against future disease. However, some survivors of chronic VHS infections were capable of replicating virus upon re-exposure. Demonstration of a chronic manifestation of VHSV infection among Pacific herring maintained at ambient seawater temperatures provides insights into the mechanisms by which the virus is maintained among populations of endemic hosts.

KEY WORDS: Viral hemorrhagic septicemia · VHS · Pacific herring

INTRODUCTION

Widespread in the NE Pacific Ocean, viral hemorrhagic septicemia virus (VHSV) is a rhabdovirus that is highly virulent for Pacific herring *Clupea pallasii*, Pacific sardines *Sardinops sagax*, Pacific sandlances *Ammodytes hexapterus*, and other forage fishes, frequently causing disease (VHS) and mass mortalities in free-ranging populations (reviewed by Hedrick et al. 2003). Once exposed, laboratory stocks of Pacific herring typically experience acute disease followed by rapid mortality (Kocan et al. 1997), and epizootics in the wild are likely initiated as a result of a combination of environmental stressors and concentration or amplification mechanisms associated with viral shedding from infected individuals within a school (Hershberger et al. 2010a). Individuals that survive these epizootics become refractory to disease, indicating the involve-ment of a protective adaptive immune response (Kocan et al. 2001, Hershberger et al. 2007, 2010b).

An apparent paradox exists when considering the persistence of VHSV in natural populations of Pacific herring where it is thought that high VHSV shedding rates from infected individuals contribute to virus perpetuation through a process of continuous transmission from infected to uninfected individuals (Kocan et al. 2001, Hershberger et al. 2010a). This means of perpetuation can only persist within a narrow range of the basic reproduction number (R_0), the average number of infected individuals resulting from a single infected host (Ostfeld 2009). R_0 values much greater than one would result in an epizootic cascade followed by a paucity of susceptible hosts, and R_0 values much below this level would eventually result in virus attrition due to ineffective transmission; in either case, the pathogen would eventually be extirpated from the host popula-

tion. Recognizing the unlikelihood that this narrow R_0 range persists for extended periods in dynamic natural systems like the NE Pacific, it is hypothesized that alternative or complementary VHSV perpetuation strategies exist in wild populations of Pacific herring. Thus, while only acute manifestations of VHS have been reported, lower levels of mortality are difficult to observe in the ocean, and the occurrence of subacute forms of the disease could easily go unnoticed. Recognition of subacute forms of VHS in Pacific herring, analogous to those known to occur in cultured rainbow trout *Oncorhynchus mykiss* in Europe (Smail 1999), could offer an alternative means by which the virus may be maintained in populations without the need for exclusive reliance on transmission of acute disease within a narrow R_0 range.

Here, we report the results of long-term laboratory studies of VHSV-exposed Pacific herring that resulted in atypical disease manifestations that were characterized by tempered and prolonged mortality. The purpose of this study was to describe the kinetics of these subacute VHS outbreaks and to determine whether survivors were protected against future infections or disease.

MATERIALS AND METHODS

Establishment of subacute and persistent VHSV infections. Sub-acute and persistent VHSV infections were initiated among laboratory-reared, age 1+ yr, specific pathogen-free (SPF) Pacific herring (n = 3668). The fish were housed in a 2.5 m diameter (3200 l) circular tank provided with flowing, processed (sand-filtered and ultraviolet-irradiated) seawater at ambient temperatures that ranged from 6.8 to 11.6°C during the year-long experiment. Viral exposure occurred by discontinuation of the supply water to the tank and addition of a relatively low dose of stock VHSV (Genogroup IVa, isolate no. 99-292 from Atlantic salmon in British Columbia, Canada); supply water to the tank was restored after 1 h. The titer of waterborne VHSV during the 1 h exposure period (1190 plaque-forming units [pfu] ml^{-1}) was confirmed by plaque assay of aliquots of the tank water onto polyethylene glycol pre-treated *epithelioma papulosum cyprini* (EPC) cells (Batts & Winton 1989). A second tank containing a control colony of SPF herring (n = 4076) was handled similarly, except that an aliquot of phosphate buffered saline (PBS) was added in lieu of VHSV; absence of waterborne VHSV in the control tank during the 1 h PBS exposure was confirmed by plaque assay. Mortalities were removed from both tanks daily; tissues (brain and pooled kidney/spleen samples) were aseptically removed from subsamples of the mortalities

and stored at –80°C until viral titers were enumerated by plaque assay of tissue homogenates. Subsamples of fish were periodically removed from the tanks and used for additional experiments. All survivors were euthanized 350 to 379 d post-exposure with an overdose of buffered tricaine methane-sulfonate (MS-222, Western Chemical). The brain and a pool of kidney and spleen tissue were removed from each of the survivors in the VHSV-exposed colony (n = 738) and a subsample of those in the control colony (n = 20/348). Tissues from all survivors were stored at 0 to 4°C (not frozen) for <24 h prior to determination of VHSV titer by plaque assay.

Long-term persistence of VHSV in survivors. To investigate the duration of viral persistence in Pacific herring, subsamples were removed from the VHSV-exposed and control colonies 224 d post-exposure and transferred to smaller (260 l) triplicate tanks (n = 30 herring tank^{-1}), where they were held for 14 d, a more stressful condition that has been effective at amplifying low levels of VHS in herring and other species (Kocan et al. 2001, Hershberger et al. 2006). Mortalities were collected daily, and tissues (brain, kidney, spleen) from all mortalities were pooled by replicate tank. After 14 d, all survivors were euthanized with an overdose of buffered MS-222; tissues (brain, kidney, spleen) from all survivors were pooled by replicate tank. Presence of VHSV in all tissue samples was determined by plaque assay.

Susceptibility of persistently infected and fully re-covered herring to acute VHS. To determine whether groups of Pacific herring undergoing persistent VHS infections were protected from acute manifestations of the disease, subsamples of live herring were removed from the VHSV-exposed and -unexposed colonies 106 d after the initial exposure and separated into 4 experimental groups: negative controls, positive controls, once-exposed controls, and re-exposed treatment. Each experimental group consisted of triplicate 260 l tanks containing 30 to 31 herring. The negative control group consisted of herring from the PBS-exposed colony that were re-exposed to PBS in the triplicate tanks. The positive control group consisted of herring from the PBS-exposed colony that were exposed to VHSV for the first time in the triplicate tanks. The once-exposed control group consisted of herring from the VHSV-exposed colony that were exposed to PBS in the triplicate tanks. The re-exposed treatment groups consisted of herring from the VHSV-exposed colony that were re-exposed to VHSV in the triplicate tanks. Challenge procedures were similar to those described for the first exposure with the minor exception that the mean waterborne exposure titer was 6.5×10^3 pfu ml^{-1}. Dead and moribund herring were sampled from the tanks daily, and all survivors after 21 d were eutha-

nized with an overdose of buffered MS-222. All sampled fish were archived at −80°C, and VHSV presence was determined by plaque assay of tissue pools (brain, kidney, spleen).

To determine whether Pacific herring that had recovered from persistent VHSV infections were protected from future infection or disease, subsamples of herring were removed from the VHSV-exposed and -unexposed colonies 343 d after the initial exposure and separated into 4 experimental groups, analogous to those described previously. Exposure and challenge conditions were similar to those described previously, with the minor exceptions that triplicate tanks with a volume of 760 l were stocked with 59 to 61 herring, and the mean VHSV exposure titer was 4.4×10^3 pfu ml^{-1}. Dead and moribund herring were sampled from the tanks daily, and all survivors were euthanized after 43 d. Viral enumeration procedures were similar to those described previously.

Statistics. Among replicated treatments, all statistical comparisons were performed using arcsine-transformed data; differences between groups were assessed using single-factor analysis of variance (ANOVA) followed by the Dunnett's test for multiple comparisons. Statistical comparisons were considered significant when $p < 0.05$.

RESULTS

Establishment of chronic and persistent VHSV infections

Chronic and persistent VHSV infections were initiated in a colony of Pacific herring and were characterized by a slight increase in VHS mortality 2 to 4 wk post-exposure followed by near-background levels of mortality that persisted for several months (Fig. 1). A slight increase in mortality was observed at 25 to 28 wk post-exposure that was unrelated to VHS, because an analogous mortality pattern occurred in the colony of negative controls and VHSV was not recovered from the tissues of any mortalities that occurred during this period. Prevalence of VHSV was 100% among mortalities occurring during the first 6 wk post-exposure and declined to 8% by Week 24, after which the virus was no longer detected (Table 1). VHSV was detected in both brain and kidney/spleen pools from most virus-positive mortalities (n = 80/109); however, VHSV
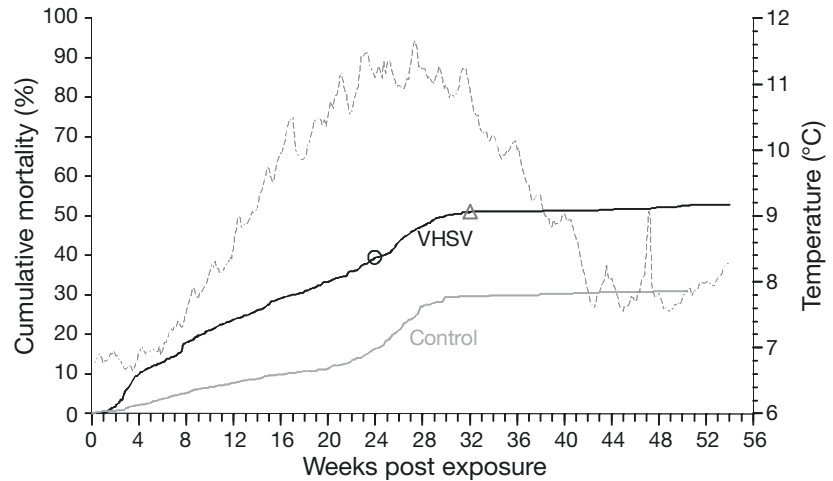

Fig. 1. *Clupea pallasii.* Chronic mortality following exposure of a colony of Pacific herring to viral hemorrhagic septicemia virus (VHSV) in a large tank. Circle indicates the occurrence of the last VHSV-positive mortality (167 d post-exposure). Triangle indicates the last day when positive survivors were known to exist in the exposed colony (224 d post-exposure). Dashed line indicates the daily ambient temperature (°C) in the tanks, reported as the daily mean of temperatures that were logged every 30 min

was recovered exclusively from brain tissues of 23 mortalities and exclusively from pooled kidney/spleen tissues from 6 mortalities. Geometric mean VHSV titers in brain tissues were typically greater than or equal to those in kidney/spleen tissues (Table 1). Virus was not detected in the tissues (brain or kidney/spleen pools) from any VHSV-exposed survivors that were euthanized 350 to 379 d post-exposure (n = 738), nor was virus detected in any subsampled mortalities (n = 177) or survivors (n = 20) from the negative control colony.

Long-term persistence of VHSV in survivors

VHSV persisted in the colony of exposed Pacific herring for at least 224 d post exposure. After transferring subsamples of previously exposed herring to triplicate tanks, VHSV was detected in tissue pools (brain, kidney, spleen) from mortalities (viral titer = 1.28×10^7 pfu g^{-1}) and survivors (viral titer = 1.28×10^4 pfu g^{-1}) in one of the triplicate tanks. Virus was not detected in any tissue pools from herring (mortalities or survivors) in the other 2 replicate treatment tanks or in any herring from the negative control triplicates.

Susceptibility of persistently infected and fully recovered herring to acute VHS

Groups of Pacific herring undergoing persistent VHSV infections (106 d post-exposure) were partially protected from acute manifestations of the disease.

Table 1. *Clupea pallasii*. Recovery of viral hemorrhagic septicemia virus (VHSV) from the tissues of dead Pacific herring. Calculation of geometric mean VHSV tissue titers were calculated from positive tissues only. nd: no data (subsamples of dead herring were not assayed for VHSV during these weeks); na: not applicable (none of the subsampled mortalities tested positive for VHSV); pfu: plaque-forming units

Weeks post-exposure	Mortalities		Kidney/spleen		Brain	
	Total	Subsampled	% positive	Geometric mean titer (pfu g^{-1})	% positive	Geometric mean titer (pfu g^{-1})
1	6	nd	nd	nd	nd	nd
2	60	23	100	3.3×10^7	100	2.2×10^7
3	163	8	100	$>4.0 \times 10^4$	100	$>4.0 \times 10^4$
4–5	199	nd	nd	nd	nd	nd
6	47	3	67	1.8×10^4	100[b]	5.3×10^4
7	55	23	52	2.2×10^4	87[c]	2.1×10^5
8	128	2	0	na	100[d]	9.4×10^4
9–15	332	nd	nd	nd	nd	nd
16	55	29	14[a]	1.4×10^6	17[d]	9.1×10^5
17	28	25	40[a]	1.8×10^5	40[b]	1.3×10^5
18	29	5	0	na	20[b]	8.0×10^2
19	35	18	28[a]	4.9×10^5	22	7.9×10^6
20	43	37	19	6.4×10^5	24[d]	4.2×10^5
21	39	21	24[a]	1.6×10^4	24[b]	2.2×10^5
22	47	28	18	4.8×10^4	18	3.3×10^4
23	57	25	8[a]	4.0×10^4	24[e]	6.1×10^4
24	57	24	4[a]	1.2×10^3	8[d]	7.1×10^3
25+	433	199	0	na	0	na

[a]VHSV was detected in the kidney/spleen pool but not the brain from 1 fish. VHSV was detected in the brain but not the kidney/spleen pool from [b]1 fish, [c]7 fish, [d]2 fish, or [e]4 fish

Upon re-exposure to VHSV, cumulative mortality in the re-exposed treatment group (36.3%), was significantly less (p < 0.01, ANOVA, Dunnett's test) than that of positive controls (85.8%), and similar to that of once-exposed controls (32.2%) and negative controls (20.4%; Fig. 2). Virus was detected in pools of tissues collected daily from mortalities in all VHSV-exposed groups, including the twice-exposed treatment, once-exposed control, and positive control groups (Table 2). Among pooled survivors at the end of the experiment, VHSV was detected only in the positive control and re-exposed treatment groups (Table 2).

Groups of Pacific herring that recovered from chronic and persistent VHSV infections (343 d post-exposure) were largely refractory to VHS (Fig. 3). Cumulative mortality among the re-exposed treatment group (3.7%) was similar to that of negative control (1.4%) and once-exposed control (2.3%) groups, but significantly less (p < 0.05; ANOVA, Dunnett's Test) than that of positive controls (18.6%).

Among mortalities, VHSV was detected only in the re-exposed and positive control treatments (Table 2).
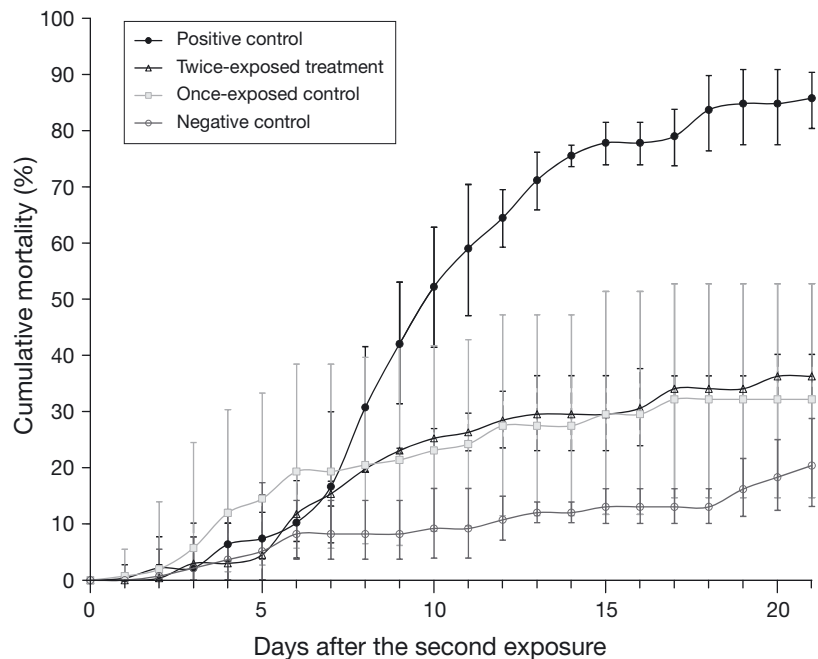

Fig. 2. *Clupea pallasii*. Susceptibility of chronically infected Pacific herring to acute viral hemorrhagic septicemia. All data points represent back-transformed percentages corresponding to the means of arcsine-transformed proportions from triplicate tanks; error bars indicate 2 SD from the mean

Table 2. *Clupea pallasii.* Detection of viral hemorrhagic septicemia virus (VHSV) in tissue pools from daily mortalities and survivors after re-exposure of persistently infected (second VHSV exposure occurred 106 d after the initial exposure) and recovered (second VHSV exposure occurred 343 d after the initial exposure) Pacific herring. All daily mortalities and all survivors at the end of the experiments were pooled by replicate tank. Tissues pools from each fish consisted of kidney, spleen, and brain. Numerals indicate the number of VHSV-positive tissue pools/total number of tissue pools

	Persistently infected	Recovered
Re-exposed treatment		
Mortalities	17/25	5/8
Survivors	2/3	0/3
Once-exposed control		
Mortalities	6/16	0/4
Survivors	0/3	0/3
Positive control		
Mortalities	29/36	22/22
Survivors	2/3	0/3
Negative control		
Mortalities	0/16	0/3
Survivors	0/3	0/3

VHSV was not detected in tissue pools obtained from survivors from any treatment group at the end of the experiment (Table 2).

DISCUSSION

In addition to acute manifestations of VHS in Pacific herring which are well-documented (Kocan et al. 1997, 2001, Hershberger et al. 2006, 2007, 2010a,b), we found that the disease can also manifest in this species as a chronic form that is characterized by viral persistence within infected hosts for extended periods with near-background levels of VHS-specific host mortality
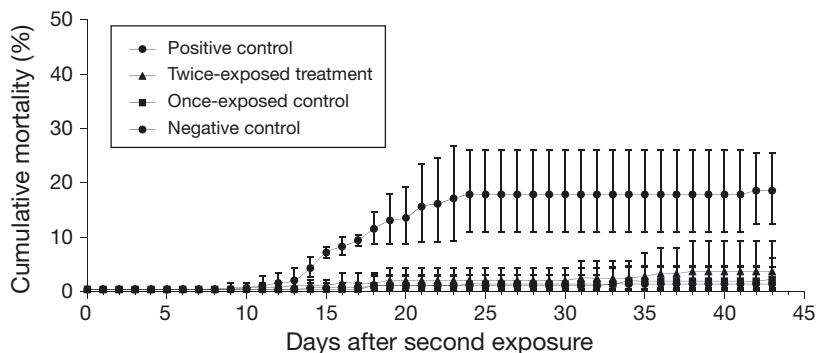

Fig. 3. *Clupea pallasii.* Susceptibility of Pacific herring to viral hemorrhagic septicemia after recovering from chronic infections. All data points represent back-transformed percentages corresponding to the means of arcsine-transformed proportions from triplicate tanks; error bars indicate 2 SD from the mean

(Fig. 1, Table 1). Although the duration of infection within individual fish was not enumerated, long-term persistence of the infection was demonstrated within a population of previously exposed Pacific herring. Analogous persistent infections occur with European strains of VHSV (Neukirch 1986), infectious hematopoietic necrosis virus (LaPatra et al. 1995), and other rhabdoviruses, including rabies virus (Mattos et al. 2001), and vesicular stomatitis virus (Sabin & Olitsky 1937).

In this study, the mechanisms of VHSV persistence in groups of previously exposed Pacific herring likely involved a combination of chronic infection in neurological tissues and continuous transmission or cycling of the virus between individuals within the colony. For example, chronic neurotropic infections provide a likely means of VHSV persistence within individuals for extended periods; however, perpetuation of the virus within a collective population requires an additional mechanism involving effective fish-to-fish transmission. This population-level maintenance of the virus is likely driven by continuous cycling of VHSV from infected to uninfected or even to recovered individuals within a laboratory colony or within a wild population; the involvement of chronically infected individuals in this cycle, particularly as a source of sufficient levels of shed virus to allow fish-to-fish transmission, requires further investigation. However, the ability of previously exposed individuals within a population to become re-infected following their second exposure to the virus, in lieu of any appreciable mortality, was demonstrated (Table 2).

The factors that determine the trajectory of VHS (either acute or chronic) in Pacific herring are unknown, but likely involve environmental conditions during the periods of exposure and early infection. Qualitative observations indicate that experimental tank size is often important in determining the VHS trajectory in laboratory experiments, even when water exchange rate, loading density, and viral exposure titer are normalized between tanks of different sizes (P. Hershberger & J. Gregg pers. obs.). Water temperature is likely an important determinant of VHS trajectory because persistence of infection in rainbow trout is inversely related to temperature (Vestergård Jørgensen 1974, 1982a,b), and VHSV isolations from wild fish populations tend to occur during periods of low water temperature (Meyers & Winton 1995, Amos et al. 1998, Hedrick et al. 2003, Altuntas & Ogut 2010). At temperatures below about 4°C, VHS proceeds in a slower and more chronic form in which the virus can be de-

tected in the brain of rainbow trout for as long as 379 d post-exposure (Neukirch & Glass 1984, Neukirch 1986). Losses due to VHS are generally highest at temperatures between 9 and 12°C, at which the disease becomes increasingly acute (OIE 2009); however, temperature optima for VHS epizootics are somewhat dependent on host species (Enzmann et al. 1993). The disease rarely occurs at temperatures above 18°C even after experimental challenge (Castric & de Kinkelin 1984, Arkush et al. 2006), possibly because the fish immune response to VHSV increases with temperature (Konrad 1986), the synthesis of protective interferon (de Kinkelin et al. 1982) occurs more quickly at elevated temperatures (Dorson & de Kinkelin 1974), waterborne stability of VHSV decreases with temperature (Parry & Dixon 1997, Hawley & Garver 2008), and *in vitro* replication of VHSV decreases rapidly at temperatures greater than 20 to 25°C (de Kinkelin & Scherrer 1970, Isshiki et al. 2001, Arkush et al. 2006). Additionally, abrupt changes in temperature to chronically infected Japanese flounder have been reported to result in exacerbation of VHS (Iida et al. 2003); however, an analogous response among chronically infected Pacific herring did not occur (data not shown).

While the brain has been shown to be a potential site for long-term persistence of fish rhabdoviruses such as VHSV (Kruse & Neukirch 1989), isolation of virus from the brain of chronically infected Pacific herring has practical implications for surveillance among populations of wild fishes. For example, if viral assessment of brain tissues was not included in these experiments, 21% (23/109) of the VHSV-positive herring would have been misdiagnosed as negative by sampling only kidney/spleen pools (Table 1); conversely, only 5.5% (6/109) of the VHSV-positive herring would have been misdiagnosed as negative by sampling only brain tissues. Additionally, VHSV titers in the brain of infected Pacific herring were generally as high as or higher than those in kidney/spleen pools, a pattern of viral tropism that is similar to reports of chronic and neurotropic forms of VHS in European rainbow trout VHS (Smail 1999). These results support the recommendations of the OIE (2009) that standard procedures for determining VHSV prevalence in wild fish populations should include collection of brain tissue as well as samples from the heart, kidney, and spleen for viral assays.

After surviving a sub acute outbreak of VHS, Pacific herring became solidly immune to the acute form of the disease (but not necessarily to re-infection), and the level of protection conferred was similar to that of herring surviving acute VHS manifestations (Kocan et al. 2001, Hershberger et al. 2007, 2010b). Although the immunological mechanisms of this adaptive response remain unknown, the resulting immunity following recovery from either the acute or chronic form of the disease has important implications in forecasting the potential for future VHS epizootics in wild populations. For example, immunological screening tools can be used to quantitatively assess the levels of herd immunity among herring populations resulting from prior exposure to VHSV. While the original manifestation of VHS (acute or subacute) does not appear to be critical in determining the strength of the resulting herd immunity, the fraction of the population mounting an immune response and the duration of immunity conferred by either form of the disease require further investigation.

LITERATURE CITED

Altuntas C, Ogut H (2010) Monthly occurrence and prevalence of viral haemorrhagic septicaemia virus (VHSV) in whiting *Meralangius merlangus.* Dis Aquat Org 88: 107–113

Amos K, Thomas J, Hopper K (1998) A case history of adaptive management strategies for viral hemorrhagic septicemia virus (VHSV) in Washington State. J Aquat Anim Health 10:152–159

Arkush KD, Mendonca HL, McBride AM, Yun S, McDowell TS, Hedrick RP (2006) Effects of temperature on infectivity and of commercial freezing on survival of the North American strain of viral hemorrhagic septicemia virus (VHSV). Dis Aquat Org 69:145–151

Batts WN, Winton JR (1989) Enhanced detection of infectious hematopoietic necrosis virus and other fish viruses by pretreatment of cell monolayers with polyethylene glycol. J Aquat Anim Health 1:284–290

Castric J, de Kinkelin P (1984) Experimental study of the susceptibility of two marine fish species, sea bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*), to viral haemorrhagic septicaemia. Aquaculture 41:203–212

de Kinkelin P, Scherrer R (1970) Le virus d'Egtved. I. Stabilité, développement et structure du virus de la souche danoise F1. Ann Rech Vet 1:17–30

de Kinkelin P, Dorson M, Hattenberger-Baudouy AM (1982) Interferon synthesis in trout and carp after viral infection. Dev Com Immunol (Suppl 2):167–174

Dorson M, de Kinkelin P (1974) Mortalité et production d'interféron circulant chez la truite arc-en-ciel avec le virus d'Egtved: influence de la temperature. Ann Rech Vet 5: 365–372

Enzmann PJ, Konrad M, Parey K (1993) VHS in wild living fish and experimental transmission of the virus. Fish Res 17:153–161

Hawley LM, Garver KA (2008) Stability of viral hemorrhagic septicemia virus (VHSV) in freshwater and seawater at various temperatures. Dis Aquat Org 82:171–178

Hedrick RP, Batts WN, Yun S, Traxler GS, Kaufman J, Winton JR (2003) Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus. Dis Aquat Org 55:211–220

Hershberger P, Hart A, Gregg J, Elder N, Winton J (2006) Dynamics of viral hemorrhagic septicemia, viral erythrocytic necrosis, and ichthyophoniasis in juvenile Pacific herring. Dis Aquat Org 70:201–208

Hershberger PK, Gregg J, Pacheco C, Winton J, Richard J, Traxler G (2007) Larval Pacific herring, *Clupea pallasii* (Valenciennes), are highly susceptible to viral hemorrhagic septicemia and survivors are partially protected after their metamorphosis to juveniles. J Fish Dis 30:445–458

Hershberger P, Gregg J, Grady C, Collins R, Winton J (2010a) Kinetics of viral shedding provide insights into the epidemiology of viral hemorrhagic septicemia in Pacific herring. Mar Ecol Prog Ser 400:187–193

Hershberger PK, Gregg JL, Grady CA, Collins RM, Winton JR (2010b) Susceptibility of three stocks of Pacific herring to viral hemorrhagic septicemia. J Aquat Anim Health 22:1–7

Iida H, Mori KI, Nishizawa T, Arimoto M, Muroga K (2003) Fate of viral hemorrhagic septicemia virus in Japanese flounder *Paralichthys olivaceus* challenged by immersion. Fish Pathol 38:87–93

Isshiki T, Nishizawa T, Kobayashi T, Nagano T, Miyazaki T (2001) An outbreak of VHSV (viral hemorrhagic septicemia virus) infection in farmed Japanese flounder *Paralichthys olivaceus* in Japan. Dis Aquat Org 47:87–99

Kocan R, Bradley M, Elder N, Meyers T, Batts W, Winton J (1997) North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory-reared Pacific herring. J Aquat Anim Health 9:279–290

Kocan RM, Hershberger PK, Elder NE, Winton JR (2001) Epidemiology of viral hemorrhagic septicemia (VHS) among juvenile Pacific herring and Pacific sandlances in Puget Sound, Washington. J Aquat Anim Health 13:77–85

Konrad M (1986) Infektionsversuche im Zusammenhang mit epidemiologischen Untersuchungen zur viralen haemorrhagischen Septikaemie (VHS) der Regenbogenforelle (*Salmo gairdneri* Richardson). PhD dissertation, Eberhard-Karls-Universität, Tübingen

Kruse P, Neukirch M (1989) The significance of the rainbow trout brain and excretory kidney for the propagation of viral haemorrhagic septicaemia (VHS) virus. In: Ahne W, Kurstak E (eds) Viruses of lower vertebrates. Springer-Verlag, Berlin, p 367–378

LaPatra SE, Lauda KA, Jones GR, Shewmaker BS, Morton AW (1995) Characterization of IHNV isolates associated with neurotropism. Vet Res 26:433–437

Mattos CA, Mattos CC, Rupprecht CE (2001) Rhabdoviruses. In: Knipe DM, Howley PM (eds) Fields virology, Vol 1, 4th edn. Lippincott Williams & Wilkins, Philadelphia, PA, p 1245–1277

Meyers TR, Winton JR (1995) Viral hemorrhagic septicemia virus in North America. Annu Rev Fish Dis 5:3–24

Neukirch M (1986) Demonstration of persistent viral haemorrhagic septicaemia (VHS) virus in rainbow trout after experimental waterborne infection. J Vet Med Ser B 33:471–476

Neukirch M, Glass B (1984) Some aspects of virus shedding by rainbow trout (*Salmo gairdneri* Rich.) after waterborne infection with viral haemorrhagic septicaemia (VHS) virus. Zentbl Bakteriol Mikrobiol Hyg A 257:433–438

OIE (World Organization for Animal Health)(2009) Manual of diagnostic tests for aquatic animals, 6th edn. OIE, Paris

Ostfeld RS (2009) Climate change and the distribution and intensity of infectious diseases. Ecology 90:903–905

Parry L, Dixon PF (1997) Stability of nine viral hemorrhagic septicaemia virus (VHSV) isolates in seawater. Bull Eur Assoc Fish Pathol 17:31–36

Sabin AB, Olitsky PK (1937) Influence of host factors on neuroinvasiveness of vesicular stomatitis virus. J Exp Med 66:15–34

Smail DA (1999) Viral hemorrhagic septicemia. In: Woo PTK, Bruno DW (eds) Fish diseases and disorders, Vol 3. Viral, bacterial and fungal infections. CABI Publishing, New York, NY, p 123–147

Vestergård Jørgensen PE (1974) A study of viral diseases in Danish rainbow trout, their diagnosis and control. PhD dissertation, Royal Danish Veterinary and Agricultural University, Copenhagen

Vestergård Jørgensen PE (1982a) Egtved virus: temperature-dependent immune response of trout to infection with low-virulence virus. J Fish Dis 5:47–55

Vestergård Jørgensen PE (1982b) Egtved virus: occurrence of inapparent infections with virulent virus in free-living rainbow trout, *Salmo gairdneri* Richardson, at low temperature. J Fish Dis 5:251–255