Journal of Great Lakes Research 41 (2015) 520–529

Contents lists available at ScienceDirect

Journal of Great Lakes Research

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

Applying multi-scale occupancy models to infer host and site occupancy of an emerging viral fish pathogen in the Great Lakes

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ARTICLE INFO

Article history:
Received 29 May 2014
Accepted 22 December 2014
Available online 24 January 2015

Communicated by Ed Rutherford

Index words:
Multi-scale occupancy model
Reversible Jump Markov chain Monte Carlo
qRT-PCR
Viral hemorrhagic septicemia virus
Viral surveillance
Virus isolation

ABSTRACT

Emerging pathogens in wildlife are being described at an increasing rate, but the methods used to describe their dynamics in wildlife populations have been slow to develop. Understanding pathogen prevalence and risk factors for infection are critical first components of developing wildlife disease management. However, the estimation of these attributes can be biased by imperfect detection of the pathogen. In this study, we adopt a multi-scale site occupancy model to estimate the probability of pathogen detection when the diagnostic test is imperfect, host detection is imperfect, and detection can be measured at multiple scales. In addition, this model allowed the comparison of different diagnostic tests for pathogen presence. We then applied this model to the detection of viral hemorrhagic septicemia virus (VHSV) in wild fish populations in the Great Lakes. We show that VHSV is still widely distributed in the Great Lakes, and that a multi-scale model can identify additional risk factors to those identified by previous logistic regression approaches. We also estimate detection probabilities using molecular and traditional virological methods. Although our approach has several limitations, it has important implications in the management and modeling of VHSV and other emerging pathogens in aquatic wildlife.

Introduction

Estimation of pathogen prevalence and risk factors for infection are critical components in management of diseases in wild populations. There is evidence that pathogens can drive species diversity (Holt and Dobson, 2006) and have an effect on community dynamics at multiple ecological scales (Tompkins et al., 2011). However, the estimation of both prevalence and risk factors is complicated by imperfect detection of the pathogen. At an organismal level, a diagnostic test can miss a truly infected individual (a false negative result), resulting in an underestimation of true prevalence. Alternatively, a diagnostic test may detect a pathogen in a host that is truly not infected (a false positive result), resulting in an overestimation of pathogen prevalence. The same phenomenon can occur at the population and/or community level as well, where non-detection at the population level may not mean that the population is truly free of the pathogen. This can be especially problematic if infected individuals are not sampled with the same frequency as healthy individuals.

Site occupancy models are a robust suite of ecological models that separate the observation process of species detection from the state process of site occupancy (MacKenzie et al., 2002, 2006). These models have been applied to the issue of imperfect detection of pathogens (Thompson, 2007; Lachish et al., 2012) to show that prevalence can be underestimated given different detection methods. However, traditional occupancy models estimate occupancy at one scale, whereas, viral surveillance is often interested in quantifying both the risk factors of individuals and the prevalence within a population and comparing that prevalence among locations. This multi-scale inference can be performed in two ways: (1) additional multilevel structure can be incorporated using random effects or (2) an additional state variable can be incorporated in the site occupancy procedure. The latter formulation was developed by Nichols et al. (2008) and Mordecai et al. (2011) and has been used to simultaneously account for imperfect detection and availability for detection (i.e., site use) when estimating the occurrence of a species across the landscape. Applying this model to pathogen presence would address many of the current limitations to estimating pathogen prevalence and risk factors in wild populations.

Viral hemorrhagic septicemia virus (VHSV) is the etiologic agent of the World Organisation for Animal Health (OIE) reportable disease of fish, viral hemorrhagic septicemia (VHS). Over the past thirty years,

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http://dx.doi.org/10.1016/j.jglr.2015.01.002
0380-1330/

the known host range of VHSV has expanded dramatically, reaching 82 species (World Organisation for Animal Health, 2009). During this time the known geographic range of this virus has also expanded to include the Atlantic coasts of Europe and North America, the Pacific coasts of Japan and North America, and, in 2003, the Laurentian Great Lakes (Elsayed et al., 2006). Surveillance efforts for VHSV have focused on active surveillance demonstrating freedom from disease, identifying risk factors, and passive surveillance (USDA-APHIS-VS Centers for Epidemiology and Animal Health National Surveillance Unit, 2009). Active surveillance efforts have resulted in detection of virus in new species (Frattini et al., 2011) and in additional locations (Cornwell et al., 2011). These efforts have allowed for preliminary modeling of infection dynamics, including predictive factors for infection (Cornwell et al., 2012). However, surveillance to date has not accounted for imperfect detection.

In this study, we adapt a multi-scale site occupancy model (Nichols et al., 2008; Mordecai et al., 2011) to estimate host occupancy and collection occupancy while accounting for imperfect pathogen detection. For the purposes of this study, we define host occupancy as the presence of virus in the fish and collection occupancy as the presence of virus at a collection site. Moreover, this method allows the direct comparison of multiple detection methods of virus presence within fish hosts. We applied this modeling strategy to a large-scale virus surveillance effort for VHSV across all five Great Lakes and connecting waterways in order to (1) identify potential risk factors of individuals, (2) estimate apparent prevalence in collections made in the study area, and (3) identify correlates of viral presence both within fish and within a collection.

Material and methods

Study design and sample collection

Fish were collected between 12 May 2010 and 1 July 2010. Six sites were chosen in each of the five Laurentian Great Lakes as well as one site in each connecting waterway (Niagara River, Detroit River, Lake St. Clair, and St. Mary's River) and 18 sites in the St. Lawrence River based on known or predicted availability of yellow perch (*Perca flavescens*) and round goby (*Neogobius melanostomus*) (Bain et al., 2010), availability of data on previous VHSV prevalence (Bain et al., 2010; Cornwell et al., 2011), distance from other sites, and availability of boat access (total number of sites = 51; Fig. 1). Round goby and yellow perch have been targeted in other surveillance efforts (Bain et al., 2010; Cornwell et al., 2011, 2012) because round goby tend to have a higher prevalence of VHSV while yellow perch tend to have a low to moderate prevalence of VHSV. A unique site was defined as a site greater than 1 km away from any other site. At each site, attempts were made to collect 60 yellow perch, 60 round goby, and 60 individuals of other species. This sample size was selected because if VHSV was not detected at a site, it allowed for 95% confidence that the site prevalence in that species or group of species was 5% or less (Simon and Schill, 1984; Fosgate, 2009). Fish were collected and dissected as described in Cornwell et al. (2012). Briefly, fish were collected by electrofishing. Electrofishing was conducted until an adequate number of each species was collected or until 4 h had elapsed, whichever came first. At sites where 60 yellow perch or round gobies were unable to be collected additional species were collected to attempt to reach a total number of fish collected per site of 180. The boat and all collection equipment were disinfected with a 1% solution of household bleach and allowed to dry between each sampling site. Collected fish were euthanized and transported frozen to Cornell University where they were dissected using instruments disinfected with 10% household bleach between each fish. A new sterile scalpel blade was used for each fish.

A small portion of each dissected tissue (anterior kidney, posterior kidney, spleen, heart, and liver; total weight approximately 0.05 g) was placed into a sterile, nucleic acid free 2 mL homogenizing tube (BioSpec Products, Bartlesville, Oklahoma) containing 200 μL RNAlater® (Ambion, Applied Biosystems, Carlsbad, California) and immediately frozen at −80 °C for VHSV testing by qRT-PCR. The remaining tissues were split evenly into three sterile, nucleic acid free microcentrifuge tubes. If the remaining tissues mentioned above were too small to add to three tubes, a portion of gonad or muscle was also added. Two microcentrifuge tubes were archived at −80 °C for future use and one microcentrifuge tube was sent to the USDA-APHIS National Veterinary Services Laboratories (NVSL). If a fish was dissected on Monday–Wednesday, samples were sent to NVSL packed on wet ice (i.e. they were not subjected to an additional freeze–thaw cycle) for immediate testing for virus isolation in cell culture. Due to shipping constraints, samples from fish dissected on Thursday and Friday were immediately frozen at −80 °C and sent to NVSL on dry ice the following Monday (these samples were subjected to an additional freeze-thaw cycle). Additionally, the large volume of samples sent required that some samples originally sent on wet ice be frozen at −80 °C upon arrival at NVSL. These samples were included with the dry ice samples for purposes of analysis because they were also subjected to an additional freeze–thaw cycle. There was no significant difference in the mean tissue weight (a low tissue weight was an indicator of the presence of gonad or muscle in the sample tube) between samples subjected to one or two freeze–thaw cycles ($t = 0.93$, $p = 0.35$). Although initially all samples sent to NVSL were tested regardless of the qRT-PCR results, eventually due to the large volume of samples being received, only samples that tested positive by qRT-PCR were tested by virus isolation in cell culture.

Testing for viral hemorrhagic septicemia virus

Two methods were used to test for VHSV: a qRT-PCR assay developed by Hope et al. (2010) and virus isolation in cell culture. For testing by qRT-PCR, viral RNA was isolated using MagMax magnetic bead extraction (Life Technologies, Carlsbad, California). Each sample was thawed and kept on ice until extraction. Ninety-six samples were extracted at a time, in approximately the order of collection, such that samples remained stored in RNAlater® at −80 °C for no more than one month. One 1.3 mm chrome steel bead (BioSpec Products) and 200 μL sterile HMEM-10 cell culture media (Minimal Essential Medium with Hanks' salts prepared with 10% fetal bovine serum, penicillin [100 IU/mL], streptomycin [100 μg/mL] and HEPES buffer [1 M 0.015 mL/mL] [Gibco, Invitrogen, Carlsbad California]) were added to each tube and samples were homogenized for 1 min using a Mini-Beadbeater-16 (BioSpec Products). Immediately after homogenization, samples were returned to ice until loading onto the extraction plate. Extraction was performed using a MagMax™-96 viral isolation kit (Ambion by Life Technologies) using the protocols described in the kit (AMB1836) and extraction program AM1836_DW_50_V2. Eluted RNA was immediately placed in sterile, nucleic acid free microcentrifuge tubes following extraction and frozen at −80 °C.

RNA quantity and quality were assessed just prior or just after samples were loaded onto the qRT-PCR plate using a NanoVue spectrophotometer (GE Healthcare, Piscataway, New Jersey). The qRT-PCR assay used was that reported by Hope et al. (2010) with slight modifications described in Cornwell et al. (2012). All samples were initially run in duplicate. If VHSV was detected in only one replicate, a third replicate assay was performed. For the occupancy model, each assay was considered a replicated survey of virus occupancy status.

Virus isolation in cell culture was performed at the National Veterinary Services Laboratories following OIE guidelines for virus isolation using *Epithelioma papulosum cyprini* cells (World Organization for Animal Health, 2009).

Data analysis

To compare the agreement between virus isolation and qRT-PCR with one or two freeze–thaw cycles, we performed traditional,