CellPress

Review

Oysters and Vibrios as a Model for Disease Dynamics in Wild Animals

Frédérique Le Roux,[1,2,*] K. Mathias Wegner,[3] and Martin F. Polz[4]

Disease dynamics in the wild are influenced by a number of ecological and evolutionary factors not addressed by traditional laboratory-based characterization of pathogens. Here we propose the oyster, *Crassostrea gigas*, as a model for studying the interaction of the environment, bacterial pathogens, and the host in disease dynamics. We show that an important first step is to ask whether the functional unit of pathogenesis is a bacterial clone, a population, or a consortium in order to assess triggers of disease outbreaks and devise appropriate monitoring tools. Moreover, the development of specific-pathogen-free (SPF) oysters has enabled assessment of the infection process under natural conditions. Finally, recent results show the importance of microbial interactions and host genetics in determining oyster health and disease.

Trends

Knowledge of the genetic structure of the *Vibrio* population provides a framework for mapping disease properties and the analysis of the evolutionary and ecological dynamics of these organisms.

Specific-pathogen-free (SPF) oysters have recently been developed, enabling the study of disease ecology in ocean environments.

High-resolution environmental sampling, population and functional genomics, combined with high-throughput infection assays, can lead to identification of the functional unit of pathogenesis in oysters.

The unit of pathogenesis in an oyster can be a population, a consortium of populations, or a clone.

Oyster pathogenesis ultimately also involves the coevolutionary interplay between vibrios, oysters, and their microbiota.

Involvement of Vibrios in Oyster Disease

Vibrios are ubiquitous marine bacteria that are ecologically and metabolically diverse members of planktonic and animal-associated microbial communities [1,2]. They have been called 'opportuni-trophs' [3] due to their high metabolic versatility and genetic variability coupled with chemotaxis and **quorum sensing** (see Glossary), all of which allow high colonization potential [4,5]. Vibrios encompass the ancient and well-studied human pathogen, *Vibrio cholerae*, as well as some less thoroughly characterized opportunistic pathogens capable of infecting humans, including *Vibrio parahaemolyticus* and *Vibrio vulnificus* [2]. Even less well understood are the consequences of *Vibrio* infections in other animals, including fish, coral, shrimp, and mollusks; however, infections in these organisms can have important environmental and economic consequences [6]. In particular, vibrios have been suggested to be responsible for repeated mortality outbreaks in oyster beds (*Crassostrea gigas*) in France that have resulted in losses of up to 80–100% of production (Box 1). However the onset and progression of disease in the wild has been difficult to follow in the past. Hence, it is often not clear whether vibrios isolated from diseased oysters are the causative agent, secondary opportunistic colonizers, or commensals [7].

Vibrios appear to employ a diversity of molecular mechanisms to infect oysters, albeit our knowledge is based mainly on very few model strains. For example, infection with *Vibrio tasmaniensis* LGP32 [8] involves an intracellular phase in hemocytes, which are the oyster's immune-competent cells, and resistance to (i) antimicrobial peptides (AMPs), (ii) reactive oxygen species (ROS) and (iii) copper [9–12]. *Vibrio aestuarianus*, by contrast, interacts with hemocytes extracellularly and inhibits their phagocytotic abilities [13,14]. A common theme, however, is that metalloproteases have been linked to toxicity in *V. tasmaniensis*, *V. aestuarianus*, *Vibrio corallilyticus*, and *Vibrio tubiashi* [13,15,16]. Nonetheless, the disruption of the gene encoding metalloprotease in *V. tasmaniensis* does not result in an attenuation of virulence [17], and the

[1]Ifremer, Unité Physiologie Fonctionnelle des Organismes Marins, ZI de la Pointe du Diable, CS 10070, F-29280 Plouzané, France
[2]Sorbonne Universités, UPMC Paris 06, CNRS, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, CS 90074, F-29688, Roscoff cedex, France
[3]AWI – Alfred Wegener Institut – Helmholtz-Zentrum für Polar- und Meeresforschung, Coastal Ecology, Waddensea Station Sylt, Hafenstrasse 43, 25992 List, Germany

 http://dx.doi.org/10.1016/j.tim.2016.03.006

Box 1. Mortality Outbreaks in *Crassostrea gigas*

Due to its potential for rapid growth and a wide range of tolerance to environmental conditions, *Crassostrea gigas* has become the oyster of choice for aquaculture in many regions of the world, such as Canada, the USA, Mexico, France, Ireland, China, and Japan [70]. Oysters can be collected from the environment (wild oysters) or cultivated to juvenile size (see Figure 2 in main text) using wild or hatchery-produced seed. In hatcheries, bacterial infections of larvae are common, and are most frequently attributed to vibrios [71–73].

Thirty years after its introduction into France, mass mortalities have affected *C. gigas* threatening the sustainability of economically important aquaculture as well as biodiversity of wild populations. First, until 2008, oysters (juvenile and adults) were affected by what has been termed 'summer mortality syndrome' that occurred at a threshold temperature of 19 °C and was geographically restricted [74]. This syndrome was influenced by physiological stress associated with maturation, abiotic conditions, genetic traits of the host, and aquaculture practices [74]. In addition, summer mortalities have been associated with the presence of several infectious agents: an ostreid herpes virus [75], designated OsHV-1, and bacteria of the genus *Vibrio*, namely *Vibrio aestuarianus, Vibrio harveyi*, and strains belonging to the *Splendidus* super clade [18,20,76].

Second, since 2008, deaths of juvenile oysters have increased considerably, and current mortality levels range between 60% and 90% [77]. These events are more widespread because they occur at a lower threshold temperature (16°C) and extend to all coastal regions (Atlantic, Channel, and Mediterranean) [78]. Previous research efforts have mainly focused on the hypothesis that this disease results from the emergence of a new genotype of the herpes virus, OsHV-1 µvar [78]. However, using specific-pathogen-free (SPF) oysters, it has been observed that rapid colonization by diverse vibrios precedes the viral replication under field conditions [7]. It has also been demonstrated that the virus is neither essential nor sufficient to cause juvenile deaths, whereas bacteria are necessary for the disease process [7]. This emphasizes the need to investigate the role of bacteria, particularly vibrios, in this disease.

Third, since 2012, the number of reported cases of adult mortalities associated with the presence of *V. aestuarianus* has increased considerably [79]. Interestingly, during the 2008–2012 period, this bacterial species had been rarely isolated from moribund oysters, suggesting the possible (re)emergence of *V. aestuarianus* as an oyster pathogen.

Finally, to date, these recurring mass mortalities have not been observed in other European regions. Invasive populations of *C. gigas* in Northern Europe have only been affected by isolated disease events (e.g., Norway, in 2014, and Southern Wadden sea [48]) or have been spared entirely, so far (e.g., Northern Wadden Sea).

[4]Parsons Lab for Environmental Science and Engineering, MIT, Cambridge, MA 02139, USA

*Correspondence: frederique.le-roux@sb-roscoff.fr (F. Le Roux).

presence or absence of this gene is not diagnostic for pathogenicity [18]. Finally, it should be emphasized that, although all of these model strains were isolated from diseased animals, they have not been found in repeated surveys. In fact, naturally infected oysters are typically colonized by a diverse assemblage of vibrios [7,19–21] and it has been hypothesized that this diversity may contribute to pathogenesis [8]. Indeed, despite the widely shared concept in veterinary medicine that the functionally active unit of pathogenesis is a single strain, numerous examples of genetically diverse pathogens or polymicrobial disease have been described [22].

Considering the uncertainties outlined above, it is not *a priori* clear what represents the functional units of pathogenesis in oyster disease. Are such units clones that have emerged after recent acquisition of virulence factors, populations of closely related organisms with virulence as a core function, or a specific consortium where virulence (or severity of pathogenesis) is an emergent property of their specific interaction? Exploring these questions is important for diagnosing, predicting, and preventing disease outbreaks in aquaculture, and requires understanding of the complex ecological and evolutionary dynamics of infectious agents.

In this review, we propose that the oyster, *C. gigas*, and *Vibrio* pathogens can be used as a model to explore disease dynamics in wild animals. This is now possible because of several recent developments. First, vibrios have been subject to population genetic analysis, which has allowed the delineation of functionally and genetically cohesive ecological populations. These provide a framework for mapping of disease properties combined with analysis of their environmental dynamics and interpretation of selective mechanisms. Second, SPF juvenile oysters have recently been developed, enabling experimental infections under near-natural conditions but without the potential confounding effect of prior infections by unknown environmental