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HOST-PATHOGEN-ENVIRONMENT INTERACTION IN FREE RANGING POIKILOTHERMS: THERE IS A SEASON TO LIVE AND A SEASON TO DIE

SUMMARY

Traditionally, the investigation of the dynamic of diseases has relied on two main elements of this interplay, and more specifically the host and the pathogen. For as complex as this interaction can be, the two-player system it is at the most a significant oversimplification of this process. More recently, there has been an acknowledgement of the role that the environment is also playing in this process. This is further evident and critical when it comes to free-ranging animals and even more when poikilotherms are considered. Here, are summarized some of the most relevant concepts of host-pathogen-environment interaction through the lenses of specific examples in the three poikilotherms vertebrate groups, fish, amphibians and reptiles.

Key words. Host-pathogen-environment interaction, infectious diseases, poikilotherms, disease ecology.

RIASSUNTO

Interazione ospite-patogeno-ambiente nei pecilotermi selvatici: c'è una stagione per vivere e una stagione per morire. Tradizionalmente, lo studio della dinamica della malattia si è fondato su due elementi principali di questa condizione, e cioè l'ospite e il patogeno. Per quanto questa interazione possa già essere complessa, nella realtà dei fatti, questa rappresenta per lo più una grande ipersemplificazione di questo processo. Più recentemente, è stato riconosciuto il ruolo che l'ambiente con i suoi vari aspetti riveste in questo contesto. Questo è tanto più evidente nel caso degli animali selvatici e ancor più per i pecilotermi. Qui, vengono riassunti alcuni tra gli aspetti principali che riguardano l'interazione ospite-patogeno-ambiente attraverso la lente di specifici esempi che riguardano tutti e tre i gruppi dei vertebrati pecilotermi, i Pesci, gli Anfibi e i Rettili.

Parole chiave. Interazione ospite-patogeno-ambiente, malattie infettive, pecilotermi, ecologia della malattia.

Host-pathogen interaction is a complex and relatively poorly understood process, which is at the basis of disease. More specifically, following the infection of the host, if the whole host's countermeasures will overcome the virulent factors of the pathogen, then no disease will occur. Differently, if the pathogen's virulence factors will be able to evade the host' countermeasures, then disease will occur. In between there is an infinite degree of intermediate scenarios, which will eventually define the life or death of the host. For as complex this interaction can be, and especially, when it comes to free-ranging animals, a third degree of complexity is added by environment and everything that it comes with it, including seasonality and temperatures. Accordingly, host-pathogen interaction, becomes a host-pathogen-environment interaction. Furthermore, when the host is a poikilotherm, an individual that by definition, has a body temperature, which is significantly dependent on the environmental temperature, it becomes obvious that host-pathogen-environment interaction acquires additional complexity.

Vertebrate poikilotherms include fish, amphibian and reptiles and their entire physiology depends on the environmental temperature, including their immune response. However, it would be extremely reductive to limit our considerations to temperatures, given that seasonality is an additional environmental factor, which can critically influence the overall poikilotherm physiology, including the efficiency and efficacy of their immune response. The interplay of all these factors and its outcome will define the chance of survival or death these animals and significantly contributes to define the significance of specific pathogens within the disease ecology of poikilotherms.

Here are presented specific paradigmatic examples of host-pathogen interaction in vertebrate poikilotherms (fish, amphibian and reptiles), which will help to highlight the critical and relevant impact of environmental factors on poikilotherm survival, when it comes to host-pathogen interaction and how, it really exists a season to live and a season to die for these animals, probably more than for any other vertebrate living being.

FISH–AEROMONAS SALMONICIDA SALMONICIDA

Aeromonas salmonicida, subsp. *salmonicida*, is a Gram negative rod and it is the etiologic agent of a disease named "furunculosis", which can cause severe mortality in salmonids. The main virulence factor of this pathogen is a type three secretion factor (T3SS), which is a transmembrane secretory apparatus (FREY & ORIGGI, 2016). Thanks to this molecular device, the bacteria can inoculate into the cells of the infected host a number of toxins critical for the occurrence of the bacteria-associated disease. The entire molecular

machinery is encoded by a large thermosensitive plasmid. The critical relevance of the presence and functionality of this molecular device has been shown in multiple experiments, where the loss of the entire plasmid or part of its functionality, translated into a complete loss of virulence of the infecting organism (Frey & Origgi, 2016). Accordingly, the same bacterial strain with the functional T3SS is lethal, whereas once devoid of the T3SS is non-pathogenic anymore and even if inoculated at high titer in the host, it will not cause disease or death. The mechanics that is behind this, it is only partially understood and it appears that a relevant activity of the T3SS, which is significantly detrimental for the host, is the impact of the T3SS itself on the host immune system. Accordingly, it has been shown that the presence of a functional, but even a defective T3SS, is associated with a substantial immune-suppression of the host, which can last at least for few days and which translates in the possibility for the bacteria to spread systemically in the host causing eventually its death (Origgi *et al*., 2017). One of the major peculiarity of the T3SS is that of being encoded by a thermosensitive plasmid. This mean that the entire integrity and consequently functionality of the plasmid is dependent on the external temperature. Interestingly, if the bacteria are grown at temperature under 20°C, the plasmid remains integer and the bacteria maintain their virulence. Differently, if the bacteria are incubated at 25°C, within six hours only, the entire plasmid is lost by all the bacteria grown at that temperature. The bacterial strain then technically "cured", and accordingly, not virulent anymore (Frey & Origgi, 2016). This is a paradigmatic example of how temperature variation can clearly impact the virulence of an infectious organism and how this can reflect on the survival of the infected host. This feature becomes of extreme interest also at the light of the ongoing global warming. It is unclear how bacterial virulence variation might be impacted by a rapid or slower change in the environmental temperature. Environmental temperature variation might not necessarily bring to virulence loss, but might provide evolutionary pressure to the thermosensitive plasmid, which might end up increasing its temperature tolerance and this might pose additional threat for the sensitive hosts.

Amphibians–Ranid Herpesvirus 3 And Bufonid Herpesvirus 1

It is relatively recent that infectious agents have been acknowledged as significant stressor contributing to the ongoing global amphibian decline. It is universally recognized the significant role that in this direction of few pathogens such as *Ranavirus* and chytrid fungi. At the same time, it is relatively unlikely that the several thousands of amphibian species might be

threatened or eventually lethally infected by only three infectious agents. It is relatively recent the discovery of two novel Batrachoviruses infecting frogs and toads, respectively (ORIGGI *et al.*, 2017, 2018). Batrachoviruses is a genus within the family Alloherpesviridae comprising all the known amphibian herpesviruses. Ranid herpesvirus 3 (RaHV3) and Bufonid herpesvirus 1 (BfHV1) shares a number of features including the association with skin proliferative lesions, the seasonal occurrence of the associated disease and the presence of putative immunomodulatory factors. The infected animals show multifocal, and variably extensive gray (RaHV3) or brown (BfHV1) patchy, skin thickening. Histologically, the lesions are characterized by prominent epidermal hyperplasia with the presence characteristic eosinophilic intranuclear inclusions. Even more interestingly, a mild to incospicuous cellular immune response is observed in the affected skin both in frogs and in toads. Investigations carried out with molecular methods (In situ hybridization) have shown how the viral replication is virtually confined within the upper portion of the thickened epidermis, the same portion of skin, which appears to undergo to a progressive degeneration and eventually sloughing off, leaving the basal portion of the epidermis, comprising the germinal layer, virtually unaffected (ORIGGI *et al.*, 2021). Curiously, the inflammatory cell infiltrate is also compartmentalized, with most of it, limited to the basal layer, paradoxically sparing the upper layers, where virtually, all the viral activity is apparently occurring. Anecdotal investigations reported that le skin lesions are commonly observed at the emerging from hibernation and then are not seen anymore as the good season progresses. The histological observations, would be consistent with a transient lesion, which would occur right after hibernation and would disappear later on. Herpesviruses are known to establish lifelong infections in their susceptible hosts and accordingly, it would not be unrealistic the hypothesis of both RaHV3 and BfHV1 infecting their respective hosts and causing a seasonal disease, functional to viral replication and spread. It is unclear, though, if the lesions and the infection might cause a long-term subclinical effect, which could eventually impact anyway the affected population in some way and that eventually could cause a population decline. Interestingly, both viral features, host physiology and seasonality are likely to be tightly embedded and at the base of the pathogenesis o the associated disease. As mentioned before, the clinical disease is detetcable macroscopically at the end of the hibernation, when the immune response of frogs and toads is significantly compromised because of the physiological leucocytes depletion that is associated with the hibernation. This physiological condition provides a temporal window to the virus, which can infect a host which is naturally and physiologically, temporarily immunocompromised. Additionally, the occurrence of the disease overlaps with the mating season

and accordingly, with the increase of the amount of circulating sexual hormones, universally recognized as immunomodulating compounds. Furthermore, the end of the hibernation is also associated with the highest annual peak of cortisol, which is by definition and immune-suppressive molecule. The additional element of the puzzle that could further enrich this array of predisposing conditions is the viruses themselves. It is well known that the best characterized of the Batrachoviruses, Ranid herpesvirus 1 (RaHV1) is well known to be highly temperature sensitive, and that is known to form viral inclusion in infected tissues only when kept at low temperature, whereas the virus is not detectable soon after raising the incubation temperature only of few Celsius (Mckinnell & Tarin, 1984). Accordingly, it is likely that RaHV3 and BfHV1 would be favored in their growth by the low temperatures of late winter. Finally, both these viruses have been shown to putatively encode within their genome a number of immunomodulatory factors (Origgi *et al.,* 2017, 2018). It is then possible that environmental factors (temperature and seasonality) along with intrinsic viral ones (immunomodulatory genes) and of the host (hormones and physiology) becomes integral part of the pathogenesis of RaHV3 and BfHV1 associated disease, showing clearly how host-pathogen interaction is only a partial picture of the whole infectious dynamic and how the environment is a critical player in this complex process. In conclusion, the low temperature would favor viral replication and a poor host cellular immune response because of the post-hibernation immune depletion. This would be enhanced by the intrinsic viral immunomodulating factors, partially explain the compartmentalized host immune response. The physiologic conditions of the animals would then provide an additional predisposing condition favoring the viral activity. Finally, the damage of the upper layer only of the skin would allow a cyclic disease to occur, likely not resulting in a short term fatal occurrence, but unclear if that might pose long term survival questions for the affected amphibians.

Reptiles - Testudinid Herpesviruses

Several infectious agents have been reported and investigated in captive and free ranging reptiles, however, herpesvirus infections appear to be over-represented in chelonians. It is not clear if this is secondary to a bias concerning the higher number of chelonians kept as pets and consequently, more likely to be presented to a veterinarian then snakes or lizards, or if the interaction between chelonians and herpesviruses have an actual specific biological background. Among the best characterized chelonian herpesviruses are those infecting tortoises, the members of the family Testudinidae. At least

four distinct genotypes of Testudinid alphaherpesvirus have been documented to date. They are all phylogenetically related and have been associated with obvious clinical diseases (ORIGGI *et al.*, 2015). Three of the four genotypes have been unambiguously associated with mortality of the infected host, and genotype 1 and 3 are the most widespread globally and account for the highest number of death. The classic clinical presentation of herpesvirus infected tortoises is a severe glossitis and stomatitis, associated with nasal and/or oral discharge. The most severe macroscopic lesions are normally associated to the upper digestive/respiratory tract and occasionally extend deep in the respiratory tract. Histologically, the hallmark of the disease is the presence of the classic intranuclear eosinophilic inclusions, which can be detected in every epithelial tissue and in the brain (ORIGGI *et al.*, 2015). Mortality is variable according to the species affected. More specifically, spur thigh tortoises (*Testudo graeca*) can be infected and develop disease, however, the mortality is overall low. Differently, Hermann's tortoises (*T. hermanni*) are highly sensitive to the disease and mortality can approach 100% of the naïve infected tortoises. There are not definitive explanations concerning the different sensitivity of distinct tortoise species to the virus, however, the most widely accepted hypothesis is that *T. graeca*, might represent the original host that Testudinid alphaherpesvirus 3 (the one considered putatively most virulent) coevolved with. According to recent analysis, within the genotype 3 there would be at least two distinct genogroups associated with a different degree of virulence (ORIGGI *et al.*, 2015). As previously described, herpesviruses are characterized by lifelong infections, with clinical disease alternated to periods of dormancy of the virus (latency) when no clinical diseases is detected. However, the virus can reactivate anytime the immune system of the host becomes impaired for any possible reason. Additionally, the virus can be intermittently shed by the infected host even with no apparent of mild clinical signs (MARENZONI *et al.*, 2018). Interestingly, it has been observed that the peak of herpesvirus associated mortality in tortoises occurs right after the end of hibernation and just prior the beginning of it. Similarly, to what described for amphibians, in poikilotherms, pre and post hibernation are critical times, when the immune system undergoes profound physiological changes, which might significantly modulate the efficacy of the immune response to pathogens. Accordingly, it is possible that a "window of opportunity" would occur for the virus during these times, increasing its virulence and lethality. Cortisol increase and sex hormones elevation, would provide an additional favorable background to the virus. Furthermore, similarly for the Batrachoviruses, chelonian herpesviruses have most likely adapted to the temperature of their hosts, with optimal replication temperature at 28° C. The relatively low temperatures occurring at the beginning of the spring and at the

beginning of the fall, would be more favorable to the replication of the virus then during the host day of summer, when herpesvirus-related mortality is essentially not recorded. Finally, similarly to what described for Batrachoviruses, Testudinid herpesviruses are also characterized by the presence of putative immunomodulatory genes, which are presumptively considered important players within the host-pathogen interaction.

Conclusions

The examples provide here are few of the many available, which can clearly exemplify how the outcome of an infection is not necessarily just the net result of the interaction between the host and the pathogen. The environment, together with some critical elements of it, such as temperature and seasonality are pivotal factor, which can push in one direction or the other the final outcome of the infection. Furthermore, the environmental factors are not necessarily playing only a direct effect (such as for the temperature on viral replication) but also indirect, such as the seasonality and the effect that it has on the host physiology. It is of paramount importance to critically evaluate each piece of this complex puzzle to really understand the significance of the pathogen and the environmental role when it comes to disease, especially when it comes to free ranging animals and even more when it comes to vertebrate poikilotherms. It really exists a "season to live and a season to die". The challenge of the future is surely a reinterpretation of all we have learned within the infectious disease arena and complement it, integrate it and revised it at the light of what we are learning and we will learn about host-pathogen-environment interaction. This is the main route toward the understanding of pathogens' disease ecology and accordingly of their actual significance within a constant changing and evolving environment.

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