ON THE INTERFERENCE OF CLINICAL OUTCOME ON RABIES TRANSMISSION AND PERPETUATION

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ABSTRACT: Rabies is a viral zoonotic infectious disease that affects mammals and is caused by genotypes/species of the Lyssavirus genus (Rhabdoviridae, Mononegavirales), with the genotype 1 (classic rabies virus – RABV) being the most prevalent. Despite continuous efforts, rabies is still an incurable disease that causes thousands of deaths amongst humans worldwide. Due to a wide range of hosts and the different evolutionary paths of RABV in each host, several host-specific variants have arisen in an ongoing process. The result of RABV replication in nervous tissues may lead to two opposite clinical outcomes, i.e., paralytic/dumb form and encephalitic/furious one. The paralytic form creates dead-end hosts mainly amongst herbivores, while the furious form of the disease allows for augmented transmission when manifested in gregarious carnivores, as their natural aggressive behavior is accentuated by the disease itself. The aim of this article is to propose a theoretical model intended to explore how the rabies virus intrinsically modulates the immune system of different host classes, the pathological changes that the virus causes in these animals and how these elements favor its own perpetuation in nature, thus providing a basis for better prediction of the patterns this disease may present.

KEY WORDS: rabies, transmission, disease ecology, epidemiology, immune response.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Few transmissible diseases have been so perennially feared and historically documented as rabies. After being long accepted as invariably incurable, virtually ineradicable and restricted to undeveloped countries, rabies has recently returned to the attention of infectious disease researches after the first report of a human surviving anti-rabies treatment (1). Control programs are currently being applied worldwide with variable degrees of vigilance on the part of health authorities while cases of rabies have been recently reported in developed countries such as Germany, the USA and Japan (2, 3, 4).

Furthermore, in 2007, World Rabies Day marked the beginning of a promising effort to diminish the disease burden worldwide (5).

There is a consensus on most external topics on rabies among the scientific community. When organ transplantations were proven to be a hidden transmission route, the manner by which the rabies virus is transmitted from an infection source to a new susceptible host has recently been updated (6, 7).

The molecular biology and genetics of the rabies virus have reached an outstanding degree and have provided the basis for an astonishing amount of information on molecular diversity that can be promptly applied to the molecular epidemiology of rabies.

Viral replication and intracellular development that lead to apoptosis in the neurons affected by rabies have also stimulated a research filed that has experienced rapid development in the last decade. Immunological aspects have also greatly advanced in the field of rabies in the past few years.

Nonetheless, each subject within rabies is often seen as a separate compartment and integrative models that intend to unify this topic are scarce.

This article aims to propose a theoretical model to explore: how the rabies virus intrinsically modulates the immune system of different host classes; pathological changes that the virus provokes in these animals; and how such alterations favor its own perpetuation in nature under a host-parasite coevolutionary point of view, based on previously published data in each of these fields and their extrapolations. It is not the objective of this article to provide an exhaustive review on rabies.
RABIES VIRUS

The classical description of the rabies virus (RABV) depicts a lipid-enveloped structure with an average diameter of 75 nm and length up to 250 nm with a spiked bullet-shaped body (8). Spikes on RABV virions are formed by glycoprotein G, which enables the virus-cell attachment and is thus the major target for neutralizing antibodies (9).

Also embedded in the envelope is the matrix M protein, which plays a crucial role in virion structure. Internally to the G-M polymeric structure there is a ribonucleoproteic core formed by nucleoprotein N, phosphoprotein P and RNA-dependent RNA-polymerase, denominated large (L) protein (8).

All five of these proteins are coded by a single-stranded RNA with about 12,000 nucleotides, arranged in the N-P-M-G-L order, with a pseudo non-coding gene between genes G and L (10).

RABV is classified in the species/genotype 1 of the genus Lyssavirus, family Rhabdoviridae of the order Mononegavirales, with six other species/genotypes already accepted and four other proposed ones (11).

A closer dissection of RABV species shows that a diverse range of subspecies taxa exist and that these, in a major concept to be developed in further paragraphs, tend to have host-specificity, resulting, for instance, in typical RABV variants from different bat species or diverse terrestrial carnivores (12).

AN EVOLVING HOST-PARASITE INTERACTION

The arousal of host-specific rabies virus variants derives from an ancient host-parasite relationship. It is reasonably argued that bats were the very first animals to host the rabies virus, which evolved from an ancestor rhabdovirus from plants (13). It can be proposed that the evolutionary track that not only led to the current host relationship in rabies, but also still occurs in sporadic time and manner, can be divided into four putative steps.

In step 1, a new host is primarily infected by a heterologous rabies virus lineage, i.e., a rabies virus previously not found in this new host species jumps the species barrier, a fact quite common in rabies epidemiology (14-16). Indeed, this is essential to the great success and wide distribution that the rabies virus presents among mammals, caused by an event known as spillover (17).
This first step is clearly affected by the degree and frequency of contacts between the two different host species and the population density of each one, since a higher population density allows a faster adaptation of a given RABV variant (18, 19).

In step 2, if the new host has a great ability not only to maintain rabies virus replication, but also to transmit it to individuals of its own species, a new cycle for that lineage can be initiated. This is commonly true in gregarious predatory mammals, such as carnivores and vampire bats, which constantly transmit rabies to individuals of their own species due to fights, for example. But it is not valid for their prey, represented mainly by herbivores, which are not efficient rabies transmitters and are dead-end hosts to the virus.

In step 3, if the new host population is ethologically and geographically isolated from other rabies-virus transmitters, no novel rabies virus lineage will be introduced into the host population, except for the spillover from step 1, when the newly introduced lineage becomes now the only one that can be found in the new host.

Though different rabies virus lineages may infect the same animal or different individuals from the same host species, the frequency of spillover of a given lineage can be proposed as being responsible for the fixation of this lineage in the new host, i.e., the higher the frequency, the higher the fixation probability.

In step 4, the new internal environment of the host – mainly the availability of rabies virus receptors, i.e., cholinergic receptors at neuromuscular junctions and the neural cell adhesion molecule (NCAM) found in non-neural cells – and its immune system will play a major role in the natural selection of the newly introduced rabies virus lineage, which, like any other RNA virus, behaves as a quasispecies, on which selection, in fact, will be exerted (20-23).

A major point here is that since each host species presents different immune systems and cholinergic receptor moieties that react differently to the same antigen, one can expect that natural selection of rabies virus in a given bat species would be different from that observed in Canis lupus – considering the same rabies virus initial lineage or even from other bat species – which indicates that RABV phenotypes change according to different replication environments (18, 24).

A finding related to the aspect of rabies virus phosphoprotein P, responsible for the binding of the nucleocapsid to the axonal cytoplasmic light chain of dynein (LC8), may also contribute to the selection process described in step 4. The binding of viral P to cellular LC8 performs an essential function in intra-axonal rabies virus
nucleocapsid transport, a key feature of the virus propagation to and from the central nervous system (25).

Strikingly, it has been found that the LC8-binding domain of P in bat-related strains differs from carnivore-related strains by virtue of a Ser-to-Ala substitution at position 145 of the protein, which suggests that a host-parasite coevolution has led to a modulation in the speed at which rabies virus spreads in each of these species and that, after a spillover event, the dissemination of rabies virus would be slower than the one found in the natural host of that strain (26).

The results of steps 1 to 4 are that, after long periods of evolution, rabies viruses are theoretically compartmentalized in specific mammal hosts and possess antigenic and genetic markers that separate them from other lineages.

**Different hosts, different clinical outcomes: effects on rabies transmission**

In the field of rabies virus replication, one can expect two diverse classes of clinical outcomes – each interfering by different mechanisms in the disease transmission – the paralytic/dumb and the encephalitic/furious rabies.

Ruminants and other herbivores are the main prey of large carnivores such as species from Canidae and Felidae families and, in Central and South America, they are also victims of vampire bats, such as *D. rotundus*.

These herbivores, after being attacked by a *D. rotundus* that carries its typical rabies virus lineage or by an infected carnivore, acquire by bite the virus and, if it successfully reaches the nervous system, the prey may present the paralytic/dumb clinical outcome of the disease or even the furious form (27-29). The first type possess a more direct effect on rabies modulation in a given ecosystem, as it leads to inability to move, which, in herbivores, would eliminate their ability to escape predators, making them easier prey.

The first extrapolation that can be derived from the mentioned equation is that the higher the prevalence of rabies in a herbivore population, the higher the availability of food to carnivores, leading to a decrease of herbivores due to predation, or death provoked by rabies itself, and a parallel increase in carnivore populations, now favored by higher food availability.

But a concurrent and hidden consequence of increased ingestion of rabies-infected tissues by carnivores is the augmentation of anti-rabies immunity in these animals due to natural oral immunization. It is well known that oral transmission of rabies virus,
though it rarely leads to rabies, results in the stimulation of long-lasting specific humoral response, which consequently increases the immunity level among carnivores (30, 31).

Since the carnivore-to-carnivore cycle of rabies from carnivore-specific rabies lineages is a cause of death among these animals and thus an important manner of population control, it is possible to speculate that a higher immunity level may lead to a reduction in rabies impact in these animals, as many of them are naturally protected against the disease (32-35).

Therefore, an inferred consequence of both higher food availability and lower sensitivity to rabies is the rise of carnivore populations. However, this situation might have a reverse effect on rabies prevalence among carnivores, since the number of susceptible animals can begin to augment, which simultaneously increases the probability of rabies virus transmission even if its frequency is low at this starting point.

Rabies virus infection in carnivores may exacerbate their naturally aggressive behavior in the encephalitic/furious form, though these animals are also susceptible to the dumb form of the disease (22, 36). Furious rabies can enhance the carnivore-to-carnivore transmission, giving rise to epizootic rabies among them. The consequence of this is diminished predatory activity of carnivores whereas their mortality rate is expected to rise, regardless the clinical outcome of the disease.

As a result of death among predators, there is an increase in herbivore populations which leads to more individuals susceptible to rabies, which guides, in turn, to equilibrium and to the resumption of the cycle, when epizootic rabies among herbivores is imminent.

Rabies virus modulates the host behavior, depending on its species, and thus regulates the host population, balancing epizootic episodes with demographic fluctuation and disease burden, so that molecular events must be found in both the host and the virus.

A very interesting molecular clock for RABV is expressed by the pseudo-gene, which has been found to evolve with a higher substitution rate in canids but a lower substitution rate in mongooses, which may indicate that canids have fast-evolving RABV lineages due to a higher transmission rate among them on account of naturally aggressive habits (37).
An evidence for the intrinsic relationship between host habits and rabies pathogenesis was corroborated when raccoons were infected with raccoon-specific and canine-specific rabies strains (38). The adverse outcomes of this experiment were, in the host-specific RABV, aggressive behavior, extra-CNS infection, higher intensity of Negri bodies and longer survival; and, in the second case, the results were paralytic rabies with a predominant brainstem disease and a shorter survival period. These findings lead to the conclusion that the adaptation of RABV strains in, for instance, carnivores – animals that rely on their natural aggressiveness to hunt and survive – results in continued intra-species transmission. Consequently, RABV strains in carnivores adopt the aggressive behavior of their natural hosts as an evolutionary advantage. Concomitantly, these strains were selected to positively modulate hosts’ aggressiveness and increase animals’ survival period, so that they can remain a source of infection to other susceptible organisms.

**Viral and host factors affecting rabies outcomes**

The external ecological outcome of the disease provokes a debate on the origin of both types of rabies, the paralytic/dumb and encephalitic/furious forms, in order to establish the implications of rabies strains that influence their own transmissions. Not only has it been recognized that human rabies patients with the furious or paralytic form of the disease may present indistinguishable rabies virus strains – when taking into account the gene coding for the following proteins: N that plays a role in nucleocapsid assembly; G that harbors RABV ability of binding cell receptors and has a virulence marker at amino acid position 333; and P, responsible for the interaction with cellular dynein light-chain LC8 domain that leads to axonal virus transport – but also, more striking, it has been proven that a same rabies virus strain can cause human patients to develop any of the clinical outcomes (39, 40).

The search for viral genomic elements involved in the pathogeny of RABV has brought to light that RABV glycoproteins not adapted to a given host may have a decreased neuroinvasiveness that results in a higher viral clearance due to a more intense immune response (41). This permits the speculation that a non-specific host would present a more intense inflammatory response during the course of RABV infection.

Thus, it is not surprising that G protein has been correlated with apoptosis when it was evidenced that lesser G expression provoked low apoptosis and, consequently,
lower G-mediated anti-rabies immune response when a host-adapted G is considered; while a higher G expression, which occurs in a non-host adapted RABV, should have the opposite effect, resulting in a strong immune response, reduced spread to the brain after peripheral infection and ultimately avoiding the encephalitic form of the disease (42).

The consequence of the latter paragraphs is the clear indication that the ultimate outcome of the disease and, consequently, the behavior and eco-epidemiological role of the host, depend chiefly on the host itself.

Corroborating this point, an outstanding finding – derived from an elegantly designed experiment in euthymic and T cell-depleted (nude) mice – reported that rabid paralysis, developed previous to death, was derived from a cytotoxic T lymphocyte in euthymic mice, while in nude mice rabies was not accompanied by paralysis if the infection source was a RABV strain from *Epitesicus fuscus* bat (43).

This finding complements previous reports that inadequate anti-rabies immunization induces faster death than no immunization due to the balance between protection and disease enhancement by the immune response (44, 45).

In view of this information, it can be posited that carnivores continuously exposed to RABV by the consumption of rabid prey develop anti-rabies immune status sufficient to naturally vaccinate them and, if they develop rabies, T cell activity would not be enough to provoke a peripheral neural inflammation that causes paralysis.

Thus, ultimately, a low level of anti-rabies T cells in carnivores could be responsible for their continued ability to keep moving and perpetuate rabies even if they have a rabies virus-saturated central nervous system when presenting the furious form of the disease.

Indeed, some wild wolves may develop only a short-lived or no response of neutralizing antibodies after contact with rabies virus (46). If this low, though protective, response against rabies virus can be extrapolated to carnivores in general, it could be an argument in favor of the low immune response derived from the absence of paralytic rabies in some rabies cases in such animals, also arguably based on the low immunogenicity of a host-adapted rabies virus G protein as a consequence of the aforementioned continued carnivore-to-carnivore transmission.

Looking back to the different manifestations of rabies in humans infected with a same RABV strain, it is reasonable to argue that the immune status of patients may be the
basis of such phenomenon, since immunosuppression can lead to the furious form while a normal immune response can cause the paralytic one.

Further evidence that favors an immune host-dependent modulation between paralytic/peripheral and furious/limbic rabies comes from studies on human rabies patients, which led to the conclusion that inflammatory demyelinating peripheral nerve dysfunction is the pathologic basis for paralysis (47).

The adaptation-modulated immunogenicity of G protein is also favors this last hypothesis. Herbivores do not maintain an herbivore-to-herbivore cycle; instead, they acquire rabies from a reservoir, thus eliciting a stronger anti-G response that can be considered as not adapted, in opposition to what occurs with carnivores.

Further supporting evidence comes from data regarding the immune response of herbivores to rabies virus. It is known, for instance, that bovines are able to keep a protective titer of anti-rabies antibodies, higher than 0.5IU/mL, up to 15 months after contact with rabies virus antigens by vaccination (48). Given the lack of similar data from studies on other animals, the extrapolation of results on bovines leads to the conclusion that herbivores possess a prolonged immune response against the rabies virus.

Obviously, a prolonged high titer of antibodies cannot be simply correlated with effector immune cell activity that would explain higher cell-mediated neuritis and paralysis in herbivores, thus stronger evidence should be brought to light.

Additionally, evidence may be found in interferon $\alpha/\beta$ effector molecule, known as Mx dynamin, which interferes with the replication of some viruses. The bovine Mx dynamin strongly represses rabies virus spread in a virus strain-independent way, while the human Mx dynamin does not inhibit all strains (49).

The information that can be obtained from this observation is that herbivores hold back rabies virus replication at some degree, from different reservoirs, but they are incapable of suppressing the inflammatory process. Since IFN plays a major role in the development of the inflammatory process, a higher activity of Mx dynamin isoform indicates elevated IFN activity and, therefore, a stronger inflammatory process during rabies infection, explaining why some herbivores present a neuritic-dependent paralysis instead of the encephalitic furious form of the disease.

Some species of hosts present stronger anti-rabies immune response that may clarify the paralytic outcome of the disease. Additionally, the dynamics of rabies
transmission that affects its spread and perpetuation is the result of a model linking RABV spillover, host immune response, disease outcome and host etology.

CONCLUSIONS
The prediction of rabies trends among wild animals and human populations may take great advantage of information on how the RABV influences its own transmission and the course of the disease, allowing a better forecast of patterns that rabies may present if host diversity is available in a given area.

As already suggested, the understanding of behavioral ecology of rabies host species is essential for applying effective control measures including vaccination (50). This information is more valuable if in a rabies spreading model, the frequencies of rabies virus antibodies and rabies virus detection and typing are registered, which permits an even more accurate prediction.

Many speculations mentioned in this article must be viewed with caution, as they are based on extrapolations from preliminary data and they will surely have benefit from ongoing experimental data on comparative immunology; pathogenesis and virulence factors of rabies virus; and mathematical rabies-spreading models.

Nonetheless, the continuously growing impact of rabies demands the best use of knowledge accumulated hitherto and the integration of diverse research areas for a conscientious reaction until rabies finds its final day.

REFERENCES


