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Interspecies transmission and viral epidemics: integration of molecular and ecological approaches in the epidemiology of two RNA viruses (1989-2010s)

Abstract

At a crossroads between biology and medicine, epidemiology is the study of infectious and non infectious diseases in populations. In particular, epidemiology of infectious diseases relies on the articulation between the biology of the germ(s) and the biology of the host population(s). Virus-host interactions are studied by epidemiologists at different levels and from different perspectives. The concept of emerging infectious disease, elaborated in the 1990s, emphasizes the need to investigate both the molecular and ecological aspects of virus-host interactions. Molecular approaches in epidemiology focus on the genetic, subcellular and cellular aspects of the host-germ relationship at the individual and population levels, while ecological approaches insist on the spatial distribution of host and germ populations, their relationships with their environment, and their interactions with other species. This paper describes integration processes at work between ecological and molecular approaches in the epidemiology of two RNA viruses since the advent of the concept of emerging infectious disease. Based on these two case studies, it further explores the meaning of integration, and aims at identifying the specific goals, challenges, expectations and issues associated with integration in these contexts.

Key words: history of epidemiology, emerging infectious disease, integration, emergent RNA viruses, molecular approaches, ecological approaches

Introduction: understanding epidemics

"Epidemics have a unity of place as well as time – and even worldwide epidemics are experienced and responded to at the local levels as a series of discrete incidents" (Rosenberg 1992, p.278-279, footnote 1). In his 1992 *Explaining Epidemics*, Historian of science and social scientist Charles E. Rosenberg depicted the dramaturgic sequence of events that constitutes every epidemic, defined as the rapid spread or increase in incidence of a particular disease among a given population, at a given time and space. Each step of this sequence associates the biological chronology of the epidemic outbreak with its social chronology.

From a biological and medical perspective, Rosenberg identified two predominant styles of explanation of epidemics – explanation being articulated with control and sometimes prediction. While the "configuration" style sees epidemics as resulting from the disturbance of an equilibrium or specific configuration between a whole range of factors (environment, climate, individual condition or health, communal life), the "contamination" style explains epidemics as the result of a particular disordering event, "some morbid material" – or infectious agent – transmitted from person-to-person (*Ibid*., p.295). This analysis of explanation styles in epidemiology finds some echoes in the work of Erwin H. Ackerknecht who depicted the distinction, and sometimes opposition, between contagionist and anticontagionist (environmentalist, miasmatic) accounts of epidemics between 1821 and 1867 (Ackerknecht 1948). Despite a clear conceptual distinction between the holistic, interactive, environmental, contextual perspective of the configuration style and the monocausal, potentially reductionist perspective of the contamination style, both styles were often employed in combination, even if the emphasis was put on one or the other.

This paper describes the articulation between ecological and molecular approaches in the epidemiology of two RNA viruses since the end of the 1980s. It partly follows Rosenberg's distinction between contamination and configuration.

Ecological approaches refer to the study of virus-host interactions from the perspective of their geographical distribution, their environment as well as the interactions with other species, therefore adopting a holistic and interactive perspective.

Molecular approaches is here understood as a general term that aims at describing virus-host interactions at the molecular, genetic, subcellular and cellular levels. Targeting the *interaction* between virus and host, these molecular approaches are inadequately characterized as "monocausal", as they are not less interactive than are ecological approaches. However, molecular explanations sometimes focus on peculiar molecular, genetic or biochemical characteristics of the virus, thereby linking a viral epidemic outbreak with, for instance, a single mutation or a set of mutations affecting the virus genome and the products of its translation. In this regard, molecular approaches may emphasize the effect of one particular (e.g. genetic) cause among other factors explaining the outbreak, size, length or end of a viral epidemic.

Then, despite resemblances between the configuration/contamination framework and the ecological/molecular one, the two are not exactly the same. First of all, the configuration style sometimes assumed the epidemic was not caused by a germ, but only by miasma, that is by a corrupted air (see Ackerknecht 1948). On the contrary, ecological studies necessary include the role of the germ in the general picture.

Furthermore, as Rosenberg noticed, the configuration and contamination styles are "general positions" that, in a given context, can take on very different meanings and applications (*Ibid*., p.302). On the contrary, ecological and molecular approaches refer to specific sets of methods, concepts and tools. In the context of epidemiology, ecological approaches include, for instance, the tools, methods, techniques and concepts of community ecology, environmental ecology or behavioral ecology, while molecular approaches include, for instance, population genetics, phylogenetic epidemiology or landscape genetics.

Distinguishing between ecological and molecular approaches is useful for another reason. Both approaches are often associated with distinct priorities in the control or prevention of epidemics. As philosopher and historian of health sciences Pierre-Olivier Méthot and evolutionary biologist Samuel Alizon noticed, ecological approaches often contribute to the establishment of local, national and international programmes of disease detection and surveillance, while molecular approaches help fighting epidemics by prophylaxis – e.g. vaccines – or by therapeutics – e.g. antibiotics (Méthot & Alizon 2014, p.123-124).

In 1989, the concept of "emergence" entered the realm of epidemiology and public health. It was proposed during a conference entitled "Emerging Viruses, The Evolution of Viruses and Viral Diseases." This conference, held in Washington D.C. and chaired by virologist Stephen Morse, was crucial in emphasizing the need to better articulate molecular and ecological approaches of viral and more generally infectious emerging diseases. However, given the variety of tools, methods, concepts and priorities that are associated with each of these approaches, integration is not trivial and represents a major challenge, as it requires to articulate concepts, methods and tools inside a coherent explanatory framework, and to further discuss the relevant priorities in fighting or preventing epidemics.

This paper describes integration processes between molecular and ecological approaches in the epidemiology of two RNA viruses, in order to understand the mechanisms, as well as the specific challenges or issues involved in such an integration. Such integration processes take place in a specific historical context, the one following the elaboration of the concept of emergence, also named "emerging infections" or "emerging infectious disease". Section 1 traces the historical context surrounding the elaboration of this concept and underlines the impact it had on epidemiological thinking and practice, notably in reaffirming the need to articulate molecular and ecological approaches of infectious diseases and epidemics. Section 2 analyses the integration of ecological and molecular approaches in the understanding of viral epidemics, focusing on the understanding of interspecies transmission – and the correlative potential or actual rise of an epidemic outbreak – in the contrasted cases of two

RNA viruses, rabies viruses and influenza viruses. Section 3 investigates the purpose of integration, as well as the issues and challenges associated with it.

1. The impact of the emergence concept on epidemiological thinking and practice: the need for molecular and ecological approaches

Emergence, in the context of infectious diseases epidemiology, is a relatively new concept. Elaborated in the 1990s, in a context of general complacency and progressive neglect of infectious diseases inside the United States, and more generally inside the "global North" (Weir & Mykhalovskiy 2010), it emphasized the necessity for epidemiologists as well as public health officers and institutions to take into account and to articulate the molecular and ecological aspects of infectious diseases and agents. This section describes the historical context surrounding the invention of the concept of emergence and how this concept was forged and used to enhance the articulation between both ecological and molecular determinants of microbial diseases.

1.1. Towards the end of infectious diseases and epidemics? [1950s-1980s]

From the 1950s to the 1980s, indifference towards the challenges posed by infectious diseases and epidemics was growing in the United States and in the global North. This situation was due to many factors. Dating back to the work of Edward Jenner on smallpox in the 18th century, vaccines were considerably improved during the 20th century, thereby contributing to decrease the incidence and prevalence of some important infectious diseases like, for instance, tuberculosis (Plotkin 2005). Moreover, from their early developments in the first decades of the 20th century, antibiotics came to be seen as "magic bullets" (e.g. Williams 2009, Aminov 2010). Finally, prompted by the development of DNA synthesis inhibitors in antitumoral research during the 1950s, antivirals began to be synthesized or selected (Brun-Vezinet & Pépin 1992, p. 3), their design progressively shifting from a "serendipitous" to a "rational" methodology (de Clercq 2011, p.19). Vaccines, antibiotics, antivirals, as well as chemical pesticides used in vector control, represented major weapons to fight against infectious diseases, sometimes in epidemic forms.

These powerful weapons, combined with the belief – supported by the avirulence hypothesis promoted by Theobald Smith and others (Smith 1904, Smith 1934, Méthot 2012) – that infectious diseases were going to naturally decline, lead to the idea that the end of infectious diseases and epidemics was close (Snowden 2008). Furthermore, the announcement of smallpox eradication[1] in 1980 represented a great success and seemed to

[1] The disease no longer exists in nature, but strains of the virus are still preserved in laboratories. Then, "eradication" means eradication "from nature" and not "absolute and complete" eradication. Before smallpox eradication, numerous attempts of infectious disease eradication occurred, but remained often unsuccessful.

encourage a general belief in the possibility to eradicate many, if not all, human infectious diseases (Fenner et al. 1988, p.1104).

For all these reasons, chronic diseases progressively replaced infectious diseases as a primary cause of mortality, and then as a primary target for public health and epidemiology. In the context of such an "epidemiological transition" (Tulchinsky & Varavikova 2000, p.42-43) from infectious to chronic diseases in many Northern countries, the HIV/AIDS pandemic of the 1980s was sometimes treated as an "exception" (Weir & Mykhalovskiy 2010, p.32).

1.2. Questioning Public Health assumptions: the fixity of infectious agents and diseases [1960s-1980s]

However, the project of eradicating most of the infectious diseases, which already had endured severe critics in the past from researchers like Emile Duclaux (1902; see also Debru 1991, Morange 2006), was more and more challenged by growing bacterial and viral resistance to (respectively) antibiotics and antivirals (for the history and epidemiology of antimicrobial resistance, see for instance Cohen 1992, Barrett et al. 1998). It was equally challenged by the successful emergence or reemergence of several diseases, like tick-borne Lyme disease (1975), hemorrhagic fevers associated with Marburg and Ebola viruses, whose first detection respectively occurred in 1967 and 1976, Legionnaire's disease (1976), or Acquired Immunodeficiency Syndrome (1981). Already in the first part of the 20th century, famous researchers like Charles Nicolle (1939) had argued that "new" infectious diseases were always going to appear. "Plagues are as certain as death and taxes", argued Richard Krause in his 1981 book, *The Restless Tide: The persistent challenge of the Microbial World* [2], only one year after the great success of smallpox's eradication.

Even in cases where antimicrobials agents are still effective and where the disease is known, eradication may not be such an easily achievable goal, because fighting a disease is not equivalent with prophylaxis and therapeutics. Insisting on the role of individual and collective behavior in the emergence, maintenance and transmission of infectious diseases, hematologist and historian Jacalyn Duffin describes the limit of a strictly "medical model" of infectious disease and treatment, that would undermine the importance of social components of disease: "Syphilis continues to be sensitive to the 'magic bullet', penicillin, but the disease has not been eradicated, nor has it been controlled. The medical model treats infection inside the organism; however, prevention and eradication rely on the more difficult task of interfering with behavior" (Duffin 2009, p.172).

Malaria is a good example of this. Caused by a parasite belonging to the genus *Plasmodium*, malaria infects human hosts via a mosquito vector of the genus *Anopheles*. Even if some localized attempts to eradicate malaria's vectors were successful (see for instance the work of Fred Soper in Brazil and Egypt: http://profiles.nlm.nih.gov/ps/retrieve/Narrative/VV/p-nid/78), malaria today still claims more than 600 000 lives per year (Listios 1997, WHO 2013) and cause disease in more than 200 million people.

[2] Quoted in Morse 1993, p. XVIII

Antimicrobial resistance, "new" diseases and epidemics as well as doubts about the possibility to eradicate all or many infectious diseases, challenged a general assumption that prevailed in public health and epidemiological reasoning until the 1980s: germs are unchanging, static entities. Such an assumption could have been questioned by the work of molecular biologist Joshua Lederberg and geneticist Edward Lawrie Tatum on bacterial genetics in the 1940s and 1950s. Either by a process of conjugation (transfer of genetic material between bacteria by direct contact or through a bridge-like connection) or by a process of transduction (transfer of genetic material between bacteria using bacteriophages as intermediates), bacteria were shown to evolve and adapt. Surprisingly, the work of Lederberg and Tatum did not lead to question the immutability of germs, despite the Nobel Prize Lederberg received in 1958 (Weir & Mykhalovskiy 2010, p.32, see also Methot & Fantini, forthcoming). Rather, this assumption was challenged by the invention of a concept, the concept of "emerging infectious disease".

1.3. The elaboration of the concept of emerging infectious disease in the 1990s: articulating molecular and ecological factors

In May 1989, epidemiologist, public health officer and virologist Stephen Morse chaired a conference held in Washington D.C. on "Emerging Viruses, The Evolution of Viruses and Viral Diseases." The year before, Morse had convinced Lederberg of the necessity to organize such a conference (Weir & Mykhalovskiy 2010, p.32). This conference, before being published in 1993 under the title *Emerging Viruses*, led to the formation of an Institute of Medicine (IOM) Committee on "Emerging Microbial Threats to Health" in 1991, co-chaired by Lederberg and virologist Robert E. Shope. The Committee published its report in 1992, resulting in a book entitled *Emerging Infections. Microbial Threats to Health in the United States*, directed by Lederberg, Shope and public health officer Stanley C. Oaks. The 1989 conference, as well as the 1992 and 1993 books, were critical in conceptualizing emerging infections as a threat, thereby questioning existing national and international infectious diseases control arrangements. Sociologists Lorna Weir and Eric Mykhalovskiy precisely described the progressive internationalization of the 'emerging infectious disease' concept from 1989 to 1996, resulting in the elaboration of the World Health Organization (WHO) strategic plan "Emerging and other communicable diseases" (WHO 1996). By inventing a concept, the 1993 and 1992 books have had deep repercussions on public health expertise, its jurisdiction, temporality, spatiality, modes of surveillance/vigilance – through an "online early warning outbreak detection" technique – and its information/reports system (Weir & Mykhalovskiy 2010, Chapter 1). Social epidemiologist Nicholas King also insisted on the productivity of the emergence disease concept, which triggered the launch of the online journal *Emerging Infectious Diseases* in 1995 (King 2004, p.68). In the 1990s, disease emergence was both a scientific concept and a field of scientific investigation (see also Grmek 1993, Ewald 1994, Satcher 1995, Saluzzo et al. 2004, Gessain et al. 2006).

To Weir and Mykhalovskiy, the Emerging Infectious Disease (EID) concept is also an "active concept" (Weir & Mykhalovskiy 2010, p.29, see also Méthot 2011, Méthot & Fantini forthcoming) in the sense that it significantly altered the understanding of infectious diseases and agents. Notably, infectious agents were no more seen as generally static entities: "*Emerging Viruses* and *Emerging Infections* formulated a new programme for public health governance that drew on accepted science in microbiology and molecular genetics. In both [books] microbes are understood as genetically mutable rather than as fixed entities. From its inception the EID concept has been conceptually coordinated with contemporary genetic approaches to microbiology and molecular biology, aligning public health thinking and practice with genetic knowledge" (*Ibid*., p.33). Rooted in microbial genetics and molecular biology, the EID concept invited epidemiologists to study microbial evolution at the molecular and genetic levels. In this paper, such an approach of the epidemiology of emerging infectious diseases and agents is referred to as a "molecular" approach.

Yet, the concept of emergence did not insist on the sole molecular aspects of emerging infectious diseases. It was also conceived of ecologically. As King noticed, "the concept of emergence had intellectual roots in older understandings of environmental and disease ecology" (King 2004, p.65, see also Anderson 2004) and shared with them a holistic view of infectious diseases and epidemics, which emphasized the need to place germ-host relationships in ecological and social contexts.[3] Weir and Mykhalovskiy noticed that microbial adaptation and change at the molecular and genetic levels represented only one of six main factors in the 1992 IOM report, the others being ecological (*Ibid*., p.33-34). "Human demographics and behavior", "Technology and industry", "Economic development and land use", "International travel and commerce", "Breakdown of public health measures" are equally important factors to understand microbial epidemics and emergence (Lederberg et al. 1992, p.47). "Ecological" here is broadly understood, as it may refer to ecological interactions between species, ecological interactions between species and their environment, and ecological changes resulting from cultural, political, technical, industrial and economical behaviors. In 1991, Morse had coined a term to address the importance of ecological factors: "viral traffic" refers to the multiple ecological pathways a virus may take to emerge in a given population at a given time (Morse 1991).

Being multifactorial, "emergence" may refer to distinct situations. As a consequence, the meaning of the word "emergence" varies. For the sake of clarity, different typologies of infectious disease emergence have been proposed, some insisting on emerging *diseases* (Lederberg 1992, p. 34; Grmek 1993) while others focus on emerging *viruses* (Morse 1993,

[3] Given the affinity between disease ecology and the concept of emerging infectious diseases, one might wonder what makes this last concept original and new. To social epidemiologist Nicholas King, the difference between traditional disease ecology and the concept of emergent disease would is that the latter relies on Morse's conception of the role of society: society is "not only [seen] as the *cause* of new risks but also as the source of their *solutions*" (King 2004., p.66). Following Weir and Mykhalovskiy (2010), one could argue that the distinction also relies on the specific context surrounding the elaboration of the EID concept, and the impact it had on both concepts, institutions and practices.

p.12). For the purpose of this paper, whose focus is on viruses and their relation with their host rather than on disease detection, clinical characterization and nosology, I adopt the typology described in the 1993 book edited by Morse.

> [Viral disease emergence has] fundamentally three sources (which are not necessarily mutually exclusive): [1] evolution *de novo* of a new virus (more precisely, usually the evolution of a new viral variant); [2] introduction of an existing virus from another species; [3] dissemination of a virus from a smaller population in which the virus might have arisen or originally been introduced. (Morse 1993, p.12)

Viral emergence, which may result in major epidemic outbreaks, may then be the result of either (genetic) evolution, interspecies transmission (with or without significant genetic change) or dissemination among one or several human populations (with or without significant genetic change). Importantly, this typology underlines the fact that neither genetic and molecular factors nor ecological factors are always sufficient conditions for emergence to occur. In some cases, mutations as well as genetic drift are not conditions *sine qua none* for emergence to occur - in other words, "emerging" does not always mean "new", as a preexisting and unmodified germ might emerge in a given population by being simply transferred from another population or species. In other cases, ecological factors may only play a minor role in epidemic outbreaks and infectious disease emergence.

Which factors are involved, the roles they play as well as their relative weight compared to other factors, is a matter of context. However, the multifactorial and context-dependent nature of emergence appears to be the source of a puzzling difficulty: how, by what means and by who is the relative weight of the involved factors estimated?

1.4. Estimating the relative weight of molecular and ecological factors

In 1992, Lederberg, Shope and Oaks noticed that the role of viral (and more broadly microbial) traffic was often underestimated for the benefit of evolutionary studies at the genetic and molecular levels.

> In discussions about the emergence of "new" diseases, considerable debate has centered on the *relative importance* of de novo evolution of agents versus the transfer of existing agents to new host populations (so-called microbial traffic). It is sometimes presumed that the appearance of a novel, disease-causing organism results from a change in its genetic properties. This is sometimes the case, but there are many instances in which emergence is due to changes in the environment or in human ecology. In fact, environmental changes probably account for most emerging diseases. For example, despite the fact that many viruses have naturally high rates of mutation, the significance of new variants as a source of new viral diseases has been hard to demonstrate, and there

> appear to be relatively few documented examples in nature. (Lederberg et al. 1992, p.42-43. Emphasis is mine)

The issue at stake here is no more to define the concept of emergence but to describe how actual emerging events – in our case, viral epidemics[4] – are understood. Understanding actual viral epidemics is to estimate, in each case, the relative importance and weight of heterogeneous factors. As there is no *a priori* or general determination of the relative weight of molecular/genetic and ecological factors involved, contextual elements – peculiar biological characteristics of the germ and the host population, of the environment – have to be *integrated* inside a coherent explanation framework. The same epidemic event may be studied at multiple (geographic, temporal) scales and at multiple levels of analysis (molecular, genetic, cellular, populational, ecological, and so on). As King noticed, scales and levels are not given or preexisting to the scientific inquiry, as they are often articulated with explanatory goals, intervention strategies and funding's research. (King 2004, p.63).

The next section investigates integration processes between molecular and ecological approaches and factors at work in the epidemiology of two RNA viruses since the advent of the concept of emerging infectious disease. More precisely, it focuses on the way explanations of interspecies transmission, leading a virus to emerge inside a new species and potentially resulting in an epidemic, require a specific integration of molecular and ecological approaches.

2. Integration processes between ecological and molecular approaches in contemporary epidemiology of two RNA viruses: rabies and influenza

The choice to compare rabies and influenza A viruses epidemiologies first relies on the complex ecology and evolution of these viruses. Both ecological and molecular approaches are then needed for the understanding and successful control of rabies and flu epidemics.

Indeed, these RNA viruses circulate among multiple hosts, vectors and reservoirs. A reservoir host is often one of the main foci of ecological studies, as it is "a host species where the parasite predominantly multiplies" (Guégan & Choisy 2009, p. 35). A reservoir host is something different from a vector which corresponds to a host species that disseminates the infectious agent. The reservoir host, also qualified as the "natural" host, generally does not suffer from infection (although it is sometimes difficult to assess with certainty whether a reservoir host suffers or not from infection, as signs and symptoms may

[4] It is important to distinguish between the emergence of a virus into a new population – an event that can either result in an epidemic or not – and the emergence of a viral epidemic. These are two events that may occur at the same time or at different times. The distinction between them often relies on the criterion of "increase in incidence", although this criterion may not be enough to further distinguish between emerging diseases and epidemics (Méthot & Fantini, forthcoming, p. 17-19).

be hard to see or to decipher in animal populations). However, numerous situations may blur the distinction between vector and reservoir host – e.g. a reservoir host acting as a vector. Interactions inside and between different host species, environmental as well as social, behavioral and cultural factors, may play an important role in enabling the virus to jump the species barrier, diffuse and persist inside a new host population.

Moreover, a molecular understanding of the genetic evolution of RNA viruses is also critical, as rabies and flu viruses share a common property, which is fundamental in the biology of RNA viruses: due to the absence of an error-correcting polymerase activity, resulting in a high mutation rate per generation, RNA viruses have a very high evolutionary rate. As a consequence, RNA virus populations consist of "a repertoire of variants", many of which differ from the "master sequence", and may be described as "mutant clouds" or "viral quasispecies" (Domingo et al. 2012, p.159). These terms, "mutant clouds" and "viral quasispecies" underline the extreme genetic and phenotypic diversity of the viral progeny: the population of RNA viruses synthesized inside the infected cell largely differ from the virus that infected the cell and replicated in it.

Given such a diversity, RNA virus evolution follows specific and complex mechanisms. "Viral quasispecies evolution" essentially relies on mutation, recombination and, in the case of influenza viruses, reassortment events. Genetic recombination consists in the exchange of genetic information between two DNA or RNA molecules, leading to a new combination of alleles, thus increasing the genetic variation of populations. Reassortment, also named antigenic shift, relies on the segmented aspect of the genome of some RNA viruses, including influenza viruses. Each viral genome consists of a define number of segments. In the case of multiple infections inside the same host, gene segments from diverse origins – avian, human, equine for instance – can combine together to form original reassortments of the parental sequences. Antigenic shift can greatly facilitate the "jump" of species barriers, and is thus the subject of many investigations trying to anticipate potential influenza outbreaks (e.g. Ferguson et al. 2005).

These mechanisms provide RNA viruses with an important capacity of adaptation, often allowing the viruses to escape antiviral strategies: "major events in the biology of RNA viruses, such as their capacity to change their cell tropism or host range or to overcome internal or external selective constraints (immune responses, antiviral agents, etc.) have their origin in the repertoire of variants present and arising in mutant spectra" (Domingo et al. 2012, p.159). In other words, it is highly probable that the explanation – and control – of RNA virus epidemic outbreaks will require a clear understanding of the genetic diversity and quasispecies evolution of RNA virus populations.

In this regard, rabies viruses offer an interesting counterexample as their genetic evolution is rather limited, as detailed later in this paper. On the contrary, influenza A viruses are often described as paramount examples of a fast and complex genetic evolution. It is then interesting to compare the integration of molecular and ecological approaches in two RNA viruses that represent extreme cases.

2.1. Understanding successful species jumps of rabies viruses

Rabies is a very ancient disease that results in an inflammation of the brain (for a history of rabies, see Baer 1975, Chapter 1). Rabies virus (RABV) belongs to the genus Lyssavirus (genotype 1; family *Rhabdoviridae*), which group together enveloped, negative and single-stranded RNA viruses (Baer 1975, Bourhy et al. 2008). Despite the development and improvement of vaccines and the discovery of new (and sometimes asymptotic) reservoirs like bats before the 1950s, the control of rabies epidemics still represented a major challenge in 1975:

> Rabies is a unique virus in that it manages to exit in the saliva when its host is stimulated to bite – a mean accomplishment. Most people are not aware that the dog is still by far the worst offending species for man and that rabid vampire bats cause hundreds of thousands of cattle deaths in the Americas annually. With few exceptions the disease is no less a worldwide problem than it was centuries ago. (Baer 1975, p. XIII)

The two volumes of the *Natural History of Rabies* edited by George M. Baer in 1975 described molecular and immunological aspects of the virus (Volume I) and examined the different hosts of the virus as well as existing and possible control measures (Volume II). Importantly, ecological control measures (e.g. population reduction) as well as molecular ones (e.g. vaccination) are reviewed (Volume II, Parts II and III). This contribution also underlined the need to establish preventive – and not only reactive – control measures.

Yet, anticipative strategies require to clarify the mechanisms by which the virus is transmitted from one species to another. Furthermore, rabies viruses may interact with their host in two different ways. Either they form a stable relationship (as observed in some bat species and in carnivorous mammals like dogs, foxes, raccoons or skunks), characterized by successful transmission of the virus to other members of the same species or to another species, or they form an unstable relationship, as in the case of some cross-species transmission events – e.g. from dogs to humans[5] – resulting in sporadic cases of disease without further transmission (Holmes et al. 2002).

In the latter case, one might say that rabies viruses regularly *emerge* in human populations. This kind of emergence, also termed "spill-over events", is not leading to the maintenance of the virus in either an epidemic or an endemic state. It must then be clearly distinguished from the more durable emergence of rabies viruses in new host species, as in the case of a successful species jump from dogs to red foxes (*Vulpes vulpes*) that occurred in Northeast Europe during the 1930s (Bourhy et al. 1999). Why are some emergence events durable and others not? What are the factors leading to successful interspecies jumps?

[5] Human species acts as a dead end and the species jump is unsuccessful. Nevertheless, more than 50 000 human deaths are caused by rabies each year, especially in Asia and Africa.

2.1.1. Integrating molecular and ecological causes and factors in the understanding of successful and unsuccessful species jumps of rabies viruses

In 1999, a paper entitled "Ecology and evolution of rabies virus in Europe" investigated the presence of and factors leading to species jumps of rabies viruses in Europe. This paper was the result of a collaboration from researchers belonging to diverse research centers, including the Pasteur Institute of Paris in France (Rabies Unit, Lyssaviruses Laboratory[6], Infection & Epidemiology department), the National Veterinary Research Institute and the National Institute of Hygiene in Poland, the National Veterinary and Food Research Institute in Finland, and the Wellcome Trust Centre for the Epidemiology of Infectious Disease in the UK (Department of Zoology, University of Oxford).

Using phylogenetic analyses, Hervé Bourhy from the Pasteur Institute and colleagues showed that two (durable) changes of host species occurred during the spread of rabies virus across Europe: from dogs to foxes, and then to raccoon dogs – even if it remains unclear whether the source of the virus was infected foxes or infected dogs. Factors such as "density of susceptible hosts [here, raccoon dogs], as well as the close proximity of a donor species, are major ecological factors in the establishment of rabies virus in a new host species" (Bourhy et al. 1999, p. 2555). Behavioral factors of infected carnivores, such as aggressiveness and long-distance walking outside their territory, also played a great role in favoring contact and transmission of the virus.

Nevertheless, as genetic traits may also have favored the successful evolutionary adaptation of rabies viruses to raccoon dogs, the authors compared nucleotide sequences of the nucleoprotein (N) and glycoprotein (G) genes from distinct groups of rabies viruses circulating in Europe. The N gene was chosen because it encodes an internal (functional) protein involved in the regulation of transcription and replication. As a consequence, it could be an important factor in host adaptation. On the other side, the G gene may also be important in determining host range, but for another reason: it encodes an external protein important in pathogenicity and which reacts with cellular receptors of rabies virus (Bourhy et al. 1999, p. 2546-2548; see also Dietzschold et al. 1983).

However, the results did not allow any firm conclusion: "strikingly, both the G and N proteins are generally conserved with few amino acid replacements accumulating among the strains studied. In particular, very few amino acid changes were found to accompany the change in transmission from dogs to foxes or raccoon dogs, although it is also possible that key mutations reside in other genes" (*Ibid*., p. 2555). These results did not exclude that genetic changes played a role in allowing successful adaptation to new hosts. Yet, as they are very limited, it was also probable that ecological factors *alone* would explain the successful cross-species transmission of rabies virus.

Three years later, researchers from the Department of Zoology of the University of Oxford and from the Rabid Unit of the Pasteur Institute in Paris – some of them having already

[6] This laboratory is now named "Dynamic Unit of Lyssavirus and host adaptation."

contributed to the 1999 paper – explored in more details the "Genetic Constraints and the adaptive evolution of rabies virus in nature" (Holmes et al. 2002). In contrast with laboratory studies, where genetic variation in the N and G genes of rabies virus can be generated extremely rapidly, the evolution of rabies N and G genes in nature seemed strongly constrained (especially in the case of nonsynonymous substitutions[7]). The results confirmed the existence of a few amino acid replacements [yet occurring in the G gene only] in rabies viruses in nature (Holmes et al. 2002, p. 252).

The general conservation of the G gene was especially striking: unlike other glycoprotein genes of some RNA viruses – e.g. influenza A virus, HIV-1 or hepatitis C virus – associated with important rates of nonsynonymous substitutions, the G gene of rabies virus is highly conserved. Strong selection pressures (e.g. immune selection) constrain the evolution of G genes of viruses like influenza A virus, *yet* they are highly variable. Why is it not the case for rabies virus? How to explain that this RNA virus gene, able to quickly evolve in the laboratory, does not evolve rapidly in nature? Are the few amino acid replacements positively selected or the result of genetic drift?

To answer these questions, the authors ventured three different – although not incompatible – hypotheses. The first hypothesis relies on a comparison between rabies and vector-borne RNA viruses, that also present a relatively low rate of nonsynonymous substitution. As vector-borne RNA viruses need to replicate in both vertebrate and invertebrate hosts, nonsynonymous evolution may be strongly constrained by the necessity to maintain a range of very different hosts (Weaver et al. 1999; for an opposite view, see Novella et al. 1999). "Rabies viruses may represent an analogous example where genetic constraints are imposed by the need to replicate in very different cell types. Hence, although rabies virus has a strong neurotropism, replication *in vivo* does not only take place in neuronal cells" (Holmes et al. 2002, p.253). Thus, the intra-host ecology of rabies viruses, involving not only neuronal cells but also other cellular types, could explain the strong selective constraints upon rabies G gene, as this gene must permit the entry of the virus in diverse cellular types.

Going even further, the authors assume that the virus may then be "preadapted to replicate in a wide range of species. In other words, they hypothesize that the constraints imposed by the need to replicate in a range of cell types mean that rabies virus can jump with relative ease to other species that have similar cell types. Hence, strong purifying and weak positive selection would be the norm in nature" (Ibid., p.253). This hypothesis articulates genetic and ecological factors in different ways: intra-host ecology provides an explanatory basis for the general conservation of the G gene, where nonsynonymous substitutions are submitted to strong purifying selection as they may often be deleterious in

[7] A nonsynonymous substitution is a nucleotide substitution that alters the amino acid sequence of a protein, hence resulting in a biological change, whereas a synonymous substitution may be silent, having no functional or phenotypic consequences – but some synonymous substitutions are not silent.

restricting the broad tropism of the virus. In turn, the overall genetic stability of the G gene accounts for the ability of the virus to easily jump the species barrier.

Their second hypothesis explains the conservation of the G gene by assuming that the G glycoprotein may not be subject to strong immune selective pressures, unlike other envelope proteins of viruses. As documented by previous studies (e.g. Ceccaldi et al. 1989), rabies virus may evade immune pressure by rapidly reaching the central nervous system (CNS) whose cells are generally under weak immune surveillance. This second hypothesis also articulates genetic factors with intra-host ecological factors (rapid circulation of the virus escaping the immune pressures). The third hypothesis, also compatible with the two previous ones, emphasizes the role of stochastic processes, including genetic drift and population bottlenecks "which may occur among hosts during transmission, and within hosts as variants infect different cell types" (Holmes et al. 2002, p.254). Population bottlenecks consist in significant reduction of the size of a population, following (intra- or extra-host) environmental events. Population bottlenecks strongly reduce the genetic diversity of the population and may result in either its disappearance or its survival. Another kind of population bottleneck, referred to as a founder event, results from the isolation of a small proportion of a population and its subsequent separated evolution from the main population. Again, this hypothesis articulates genetic (genetic drift) and (intra- and extra-host) ecological factors (population bottlenecks) to account for the evolution and adaptation of rabies viruses.

These three hypotheses do not describe genetic features of rabies viruses as *sufficient* to explain the virus adaptation to new host species: intra-host ecological and environmental factors such as broad tropism, inside host viral circulation, population bottlenecks are equally important. Furthermore, extra-host ecological factors – density and proximity of donor and recipient (susceptible) species, behavior – still play a critical role in determining the success or failure of durable emergence. Then, ecological and molecular factors are both *ultimate causes* of rabies virus durable emergence, but ecological factors, especially extra-host ones, may be described as *proximate causes* that truly trigger successful species jumps.

The integration between molecular and ecological approaches here relies on an integration of *factors* and *causes*. The relative weight of each factor is determined by the results of investigations following either molecular (e.g. genetic evolution) or ecological (e.g. behavior) hypotheses. Ecological factors are often considered to be prominent because successful emergence – and potential epidemic – of rabies viruses directly depends on factors such as density of hosts, physical barriers or behavior. For instance, emergence is often successful in dogs or raccoons because the virus modifies their behavior, leading them to bite, and therefore to transmit the virus. On the contrary, humans are dead end for rabies virus, as they do not transmit the virus. Despite some behavioral or physiological changes (e.g. thirst), humans do not (generally) bite other humans.

However, another kind of integration takes place in the explanation of species jump of rabies viruses.

2.1.2. Integrating molecular data and techniques to ecological methods and explanations in the understanding of successful species jumps of rabies viruses

Molecular and ecological factors of interspecies transmission are still under investigation today and researchers are still trying to more precisely determine the "genetic basis of the traits that govern cross-species transmission" (Bourhy et al. 2008, p. 2680). Yet, in the absence of key mutations enabling successful interspecies jumps, ecological factors are the subject of particular attention.[8] In other words, the prominent role ecological factors play in the epidemiology of rabies viruses led researchers to concentrate most of their efforts on their investigation. Thus, a number of recent studies, focusing on reservoir dynamics, asymptomatic infections, behavioral and social factors as well as transmission routes, reinforce the ecological understanding of rabies epidemiology (e.g. Ronsholt et al. 1998; Hampson et al. 2009).

What role do molecular approaches play in such a context, where ecological factors have been acknowledged as being prominent? In a significant number of recent ecological studies, molecular approaches do not first aim at identifying one or several key mutations that would explain rabies virus adaptation to new hosts. Rather, molecular data and techniques are getting more and more *incorporated* into ecological approaches of rabies emergence to genetically track ecological virus-host, virus-virus and host-host interactions. The integration at work here is not only between molecular and ecological explanations, nor between molecular and ecological factors or causes. Rather, molecular data and techniques, as well as ecological data are integrated inside ecological explanations and methods.

For instance, correspondences between genetic data provided by molecular epidemiology and population genetics on the one hand, and ecological or environmental data provided by spatial epidemiology and surveillance networks on the other hand, are used to identify viral routes of transmission between different hosts (e.g. Biek et al. 2007).

Another major contribution of molecular data and techniques to disease ecology and epidemiology of rabies viruses is the progressive integration of landscape epidemiology and landscape genetics. In 2012, a paper entitled "Integrating the landscape epidemiology and genetics of RNA viruses: rabies in domestic dogs as a model" precisely aimed at describing how such an integration might work and what challenges would be associated with it. This study resulted from the collective work of researchers from different research centers in the UK (Institute of Biodiversity, Animal Health and Comparative Medicine, Medical Research Council, University of Glasgow; Centre for Virus Research, College of Medical and Life Sciences, University of Glasgow; Wildlife Zoonoses and Vector Borne Diseases Group, Animal Health and Veterinary Laboratories Agency).

[8] For instance, Bourhy and colleagues insisted on the crucial role of the dog in inter-species transmission: "while we found no significant evidence for adaptive evolution, our observations strongly suggest that the dog has served as the main vector for inter-species RABV transmission, generating viral lineages that then spread to other taxa" (Ibid., p.2679).

In this study, Kirstyn Brunker and colleagues promoted an integrative approach to understand the specific 'landscape' – including host movements, physical barriers, socio-cultural factors and population level effects – that permits the persistence of rabies transmission among domestic dogs, a persistence which enables rabies virus to regularly reemerge in human populations and challenges control and eradication efforts. Landscape epidemiology, defined as the study of the causes and consequences of spatial variation in disease incidence or risk across heterogeneous landscapes, aims at providing an ecological framework for the emergence or maintenance of rabies in domestic dogs. Such an approach insists on the geographical, physical, environmental characteristics of a defined landscape, to understand the ways these characteristics influence – and are influenced by – the ecological (including social, cultural) interactions between species, sometimes favoring the (re)emergence and maintenance of the virus inside a given population.

Landscape epidemiology is however an ecological framework that relies on the integration between "spatial" (including ecological) data and techniques on the one side, and molecular data and techniques on the other side.

> Revealing the landscape factors underlying these interactions [between virus, host and vector species] calls for an interdisciplinary approach that draws on a range of techniques across different spatial scales. Molecular markers provide a basis for this by genetically tracking spatial and temporal dynamics in pathogen and host populations. A landscape genetics approach to infectious disease therefore encompasses a range of analytical tools, including geographic information systems, remote sensing, population genetics, phylogenetics and statistical and mathematical modeling techniques. (Brunker et al. 2012, p.1899)

Landscape genetics, identifying "molecular markers", then becomes the basis for a molecular-grounded ecology of virus-host interactions. By "molecular-grounded ecology", I do not mean that the molecular level becomes preferentially chosen to account for ecological phenomena in viral epidemiology. Molecular data and tools are one way, among others, to trace viral transmission routes.

The integration of molecular data and tools with ecological data and methods inside an ecological framework answers specific challenges. Even if the role of host movements, physical barriers and population level effects in the transmission and persistence of rabies virus had already been investigated, it remained challenging to *quantify* these effects. Landscape genetics precisely offers a mean to quantify these ecological and environmental factors.

In 2008, researchers from the USA (Division of Biological Sciences, University of Montana; Biology Department, Fordham University) and Portugal (Centro de Investigacao em Biodiversidade e Recursos Geneticos, University of Portugal) already characterized landscape genetics as a "promising approach to understanding disease spread" (Archie et al. 2008, p.27). Commenting on the study of Rees and colleagues on the impact of a river in the dissemination and transmission of raccoon rabies (Rees et al. 2008), Elizabeth A. Archie,

Gordon Luikart and Vanessa O. Ezenwa analyse the benefits of using molecular markers in landscape epidemiology in their 2008 paper entitled "Infecting epidemiology with genetics: a new frontier in disease ecology" (Archie et al. 2008). Despite the fact that multiple landscape features limiting the spread of viruses and infected or susceptible hosts are often identified, "quantifying these effects can be challenging" (*Ibid*., p.27). The study led by Erin E. Rees and colleagues from Canada (Natural Resources DNA profiling & Forensic Centre and Wildlife Research and Development Section at Trent University; Department of Geography, Queen's University; Cissec Corporation) used landscape genetics and computer simulation to predict and assess the impact of Niagara river on the movements of raccoon populations – and therefore on the movements of rabies viruses. Comparing the simulated population genetic structure with the actual population genetic structure based on mitochondrial DNA from 166 raccoons, Reed and colleagues estimated that Niagara river represented a barrier preventing 50% of raccoons from crossing from one side to the other (Rees et al. 2008). Molecular data and tools provide epidemiology with a quantitative translation of the impact of ecological factors.

Finally, two types of integration have been described in the epidemiology of epidemic and emergent rabies viruses. The first kind of integration articulates molecular and ecological factors inside coherent explanatory frameworks to explain, control or anticipate virus (durable) emergence. The second kind of integration relies in the expression and quantification of ecological information by "molecular markers" to better understand the effect of spatial heterogeneity and the correlative ecological interactions between species on viral transmission. Molecular data and tools are then integrated with ecological data and methods inside a general ecological framework. Current research in rabies epidemiology highlights the existence of at least two important directions where integration between molecular and ecological approaches successfully occurs.

2.2. Emerging and re-emerging influenza A viruses

Causative agents of influenza, and notably Influenza A viruses that are responsible for flu pandemics, are, like rabies viruses, enveloped negative and single-stranded RNA viruses but they belong to the genus Orthomyxovirus (Webster et al. 1992). One of the main differences between rabies and influenza A virus relies upon the fact that the latter is a segmented virus: its genome is made of eight discrete gene segments, each coding for at least one protein. This characteristic provides flu viruses with the ability to reassort and is an essential aspect of both their genetics and ecology, as we shall see.

Wild waterfowl are the major reservoir of influenza A viruses, but these viruses possess a wide host range, from birds to various mammalian species including humans, pigs, horses, dogs and others (Webster et al. 1992). Past and current research on influenza A viruses tried to determine how, from the many influenza A viruses circulating in a great number of avian species, some acquire in certain circumstances the ability to infect mammals – and then humans – and sometimes even to durably adapt to some of these mammalian species. Here I

concentrate on scientific attempts to determine how avian flu viruses not only become able to infect humans but also how they *become epidemic or pandemic* in human populations. As researchers from the National Institute of Allergy and Infectious Diseases notice, "the factors underlying all such emergences are poorly understood" (Morens et al. 2012, p. 335; see also Parrish et al. 2008).

2.2.1. Molecular determinants of influenza virus adaptation: unraveling ecological routes

Following the discovery of influenza viruses by Robert E. Shope in 1931 and their subsequent isolation in 1933 (Smith et al. 1933), numerous studies on the molecular and genetic determinants of influenza virus adaption to humans were developed. On the contrary to rabies viruses, Influenza A viruses are often described as typical examples of RNA viruses whose genetic adaptability, essentially arising through mutation and reassortment, strongly impact interspecies-transmission (e.g. Webster & Rott 1987; Cox & Bender 1995; Suzuki 2005; Watanabe et al. 2012). Notably, some key molecular "steps" have been associated with the ability of the virus to jump the species barrier. Surface proteins HA (hemagglutinin) and NA (neuraminidase) – from which are established the different subtypes of influenza A viruses, e.g. H1N1, H5N1, H2N2, etc. – like other proteins like PB2, PB1-F2 and NS1 are considered central in conferring adaptation of viruses from avian origin to mammals, as these proteins interact with host factors or play important roles in viral replication, export, assembly, budding, and antagonism of the host antiviral response (Medina & Garcia-Sastre 2011).

The molecular and biochemical study of these proteins provides some insights in the way interspecies transmission may be restricted or favored – in case of mutation or reassortment. Notably, depending on the hemagglutin molecules on the viral coats of distinct influenza viruses, these viruses have affinities with different host species, as the hemagglutin molecules preferentially bind to the certain forms of the molecules on this host cell membrane. For instance, hemagglutinin molecules on the viral coats of avian influenza viruses preferentially bind to one form of molecule in the host cell membrane [sialic acid (SA)-α-2,3-Gal-terminated saccharides], whereas hemagglutinins on human influenza viruses prefer another [SA-α-2,6-Gal-terminated saccharides] (Kuiken et al. 2006, p.395). As SA-α-2,6-Gal-terminated saccharides are predominant in the human trachea, this may prevent avian influenza viruses to replicate in humans, while enabling human influenza viruses to replicate in humans. In other words, the molecular difference between viral HA from avian and human origins "provides an interspecies barrier preventing avian viruses from easily infecting humans" (Watanabe et al. 2012, p.14).

Yet, a modification of an HA from an avian virus may facilitate the entry of an avian virus in a human population. Such modifications might for instance occur when avian viruses infect pigs. As swine tracheal epithelial cells contain the two types of sialic acid molecules described above, pigs are susceptible to both human and avian viruses. Bringing these viruses from diverse origins into contact in pigs increase chances of reassortment between

avian and human viruses. This is why pigs may "serve as an intermediate host acting as a 'mixing vessel'" (*Ibid*., p.14). Investigations of molecular determinants of virus-host interactions are then not only helpful to target molecular factors of virus transmissibility and host adaptation, but they also help uncovering potential routes, reservoirs and vectors of viral transmission. In this case, molecular determinants of viral surface proteins help identifying pigs as a potential transmission route between birds and humans.

Following the so-called "bird-flu" of 1997, the necessity of pigs, and more generally mammalian hosts, as an intermediate host between humans and birds was however questioned. Doubts raised about the necessity of a mammalian intermediate host between birds and humans. Molecular studies of the 1997 H5N1 strain (Hatta & Kawaoka 2002) showed that this virus *directly* jumped from birds to humans, thus identifying a novel possible ecological route of viral interspecies transmission. As virologist Richard Webby noticed, "we have learned a lot in the past decade. The H5N1 strain of influenza A virus – the bird flu that emerged in Asia in the late 1990s – taught us that viruses can also use domestic poultry as the intermediate host" (Interview of Richard Webby by Rebecca Kessler, December 2011, p.4).

Such results led to reinforce the molecular studies of genetic determinants of avian influenza viruses (notable the H5N1 strain) adaptation to mammals. Some of these molecular studies remain almost completely separated from field investigations and ecological questions. In 2012, David M. Morens, Kanta Subbarao and Jeffrey K. Taubenberger from the National Institute of Allergy and Infectious Diseases (National Institute of Health, USA) conducted a study aiming at genetically engineering mutant strains of avian influenza viruses. Specific genetic mutations were engineered into naturally occurring avian influenza viruses. In turn, the resulting characteristics of the engineered strains were to be investigated in model animals (ferrets). Such studies aimed at increasing the transmissibility of the avian viruses in mammals to identify the viral genetic determinants associated with infectivity, cell tropism, viral replication, pathogenicity and transmissibility. The ultimate goal was to determine a "genetic basis" for the adaptation of this highly pathogenic virus in humans. As the authors emphasized, these types of experiments may provide clues about whether and how a virus might adapt to humans, and how to anticipate or control the possibly resulting emergence of the virus in human populations (Morens et al. 2012, p.335).

However, the project to uncover a genetic basis for H5N1 adaptation to humans raised and still raises some important critics, not only because they were associated with controversies about potential 'dual-use' research implications (e.g. Rappert 2007). The authors themselves acknowledge that it was highly improbable to associate transmissibility or pathogenicity with one single mutation because "phenotypic properties such as replication, pathogenicity and transmissibility are likely to be polygenic traits driven by mutations that are independent and possibly competing" (Morens et al. 2012 p.337; see also Taubenberger & Kash 2010). Cooperative as well as competitive interactions between mutations challenge attempts to identify simple causal associations between mutations, transmissibility and adaptation. Moreover, H5N1 viruses, which are highly pathogenic avian

influenza viruses having only occasionally infected humans and other mammals, have a low transmissibility in mammals in nature. In other words, their pandemic potential is, in nature, weak. As the investigations were conducted on model animals in the laboratory, results obtained in ferrets may be hardly transferrable to mammals in nature, and *a fortiori* to humans. Ecological factors surrounding transmissibility, adaptation and emergence in nature make it hard to use such results.

Despite the fact that these studies do not deny the existence of ecological factors (e.g. Influenza A host range, host reservoirs), their ecological relevance is not obvious. The very high rate of evolution in influenza viruses may sometimes lead to strongly focus on molecular and genetic studies of this evolution, a fact that, in turn, makes it difficult to integrate molecular and ecological approaches.

Philosopher and historian of health sciences Pierre-Olivier Méthot and evolutionary biologist Samuel Alizon recently addressed this issue (Méthot & Alizon 2014). In their paper, they focus on human-to-human transmission and describe how ecological and molecular approaches are recruited to understand the exceptional virulence of the 1918-19 influenza pandemic. What made the 1918-19 influenza virus pandemic? Ecological and molecular approaches seek to explain the evolution of the virulence and transmissibility of the 1918-19 influenza virus. However, they do this using distinct methods and concepts. Molecular approaches are looking for tracks (e.g. mutations) of evolution leading to increased transmissibility and try to identify particular genes for pathogenesis, while ecological approaches insist on the role population density, within and between host competition, as well as different selective pressures play in favoring transmissibility and viral adaptation. (Méthot & Alison 2014, p.97). Their work underlines a strong contrast between ecological and molecular approaches and a global lack of communication and integration between them. Indeed, molecular explanations of the virulence and transmissibility of the 1918-19 strain are sometimes considered to be sufficient, despite the fact that no single mutation has been associated with such virulence and transmissibility. On the other hand, ecological accounts of the virulence tend to emphasize the predominance of ecological factors over molecular ones.

Similarly, some studies of species jump and emergence – sometimes in epidemic form –of influenza viruses also sometimes strongly emphasize the important of molecular or ecological factors over each other. As described above, engineering mutant strains in order to identify one mutation or a set of mutations associated with viral adaptation relies on the assumption that adaption may simply be understood in genetic terms. However, numerous recent studies precisely aim at reinforcing the integration between ecological and molecular approaches of potentially epidemic or pandemic influenza A viruses.

2.2.2. Reconstructing the global circulation of influenza viruses with genetic and antigenetic mapping

Ecological surveillance of circulating influenza viruses was already described as a “model” of an effective global surveillance network on emerging infectious diseases in 1992 (Lederberg et al. 1992, p.6). One of the major technical roles of the WHO Global Influenza Surveillance Network (GISN) relies in the detection of “isolates of new influenza viruses infecting humans, especially those with pandemic potential” (http://www.influenzacentre.org/centre_GISN.htm). Collecting data from very diverse geographical areas collected at different times allow the construction of genetic and antigenic maps (Smith et al. 2004) that contribute to better understand the evolutionary history of influenza viruses.

Notably, the geographic mapping of viral genetic diversity led to the identification of “hotspots” of virus activity. These hotspots may first correspond to geographic areas where high prevalence of influenza in one or different host species is recurrent, making it possible to study the complex evolution and ecology of influenza viruses, as well as the ecology of their reservoir hosts, within a given environment (Kessler 2011, p.4).

Hotspots may also refer to important “nodes” in the global network of influenza transmission in human populations. In 2010, researchers from the USA (Department of Ecology and Evolutionary Biology, University of Michigan; Howard Hughes Medical Institute, University of Michigan; Department of Scientific Computing, Florida State University) investigated the “Global Migration Dynamics” underlying the evolution and persistence of Human Influenza A (H3N2) (Bedford et al. 2010). The reconstruction of the genetic history of human influenza A (H3N2) viruses led Bedford and colleagues to precise the role of East and Southeast Asia in global transmission of influenza viruses: “whereas previous hypotheses propose a source-sink model of viral evolution, in which a network of populations in East and Southeast Asia seed annual epidemics in temperate latitudes, we find that strains of influenza often circulate outside Asia, sustained by complex migration dynamics” (Bedford et al. 2010, p.1). The source-sink dynamics is an ecological model where populations circulate between two “patches” or kinds of habitats, a high quality one – “source” – and a low quality one – “sink”. This model enables the study of population dynamics between habitats, and not only inside a given habitat. Inferring global migration patterns of influenza with the use of genetic data and tools, these researchers underline the need to articulate molecular data and tools with ecological methods and concepts. From their articulation, an ecological hypothesis – the applicability of a source-sink model to global influenza migration patterns – was revised, for the benefit of another global pattern of migrations. Such an articulation leads to better understand the global dynamic of influenza viruses, the interplay between migration and persistence of influenza, as well as the potential geographical sources of epidemics, therefore guiding antiviral use and vaccination strategies.

As in the case of rabies viruses, the epidemiology of influenza viruses relies on the integration of molecular data and techniques (e.g. genetic mapping) with ecological concepts and methods (e.g. migration dynamics) inside a coherent ecological framework.

2.2.3. A integrative framework for the understanding of interspecies transmission

The understanding of interspecies transmission and emergence of influenza viruses relies on the integration of molecular and ecological factors (2.2.1), as well as on the integration of molecular data and techniques inside a general ecological framework (2.2.2). Recently, researchers from the Netherlands (Department of Virology, Erasmus Medical Center), from the USA (Center for Infectious Disease Dynamics, Department of Biology, Pennsylvania State University; Fogarty International Center, National Institute of Health) and from the UK (Institute for Animal Health, Compton Laboratory) proposed an integrative framework to articulate various factors involved in interspecies transmission of influenza viruses. In a 2006 paper, Thijs Kuiken and colleagues centered such a framework on the concept of "host species barrier." This framework aims at explaining "why some pathogens [here, Influenza A viruses] become capable of crossing host species barriers" (Kuiken et al. 2006, p.394). In this paper, Kuiken and colleagues neither focus on molecular factors nor on ecological ones but rather "review the interaction of factors that *collectively* limit the transmission of an infection from a donor host species to a recipient species and that constitute the host species barriers" (*Ibid*., p.394, emphasis added).

Significantly, molecular and ecological factors are described as being equally important to understand successful interspecies transmission. The possibility of transmission and replication of the virus in a new host species requires both "sufficient contact" and "enough compatibility" (Ibid., p.394). Framing molecular factors in terms of compatibility, and ecological ones in terms of contact, the authors proposed a general classification of the factors involved in the crossing of host species barrier, thereby defining a research agenda for the epidemiology of influenza A viruses.

The classification of the factors involved relies in the type of interaction. Interactions between donor and recipient species mostly involve migration patterns, trade, differences in habitat use, environmental barriers, host behaviors, structure and density of agricultural sites. Interactions between virus and host necessitates the understanding of interactions between cell receptors and viral molecules, replication and spread of the virus inside the host, factors leading to systemic infections, key mutations and complex epistatic interactions involved in virus evolution and adaptation. Finally, interactions between individuals of the recipient species – "host's contact network" (*Ibid*., p.396) – requires the analysis of spatial distribution and mixing, preexisting and/or long-lasting immunity, long/short infectious period, as well as the roles and different kinds of superspreaders. Superspreader individuals may either be more infective per contact individuals ("higher per-contact transmission" rate) or have many more contacts. In both cases, the rate of spread of the disease is greatly multiply (Kuiken et al. 2006, p.397).

In this general framework, each factor is articulated to other factors inside a given interaction type. Understanding why, for instance, avian influenza viruses may cross the "host species barrier" and be transmitted to mammals or humans requires to articulate a

great variety of factors as well as the various types of interaction. Here, the integration is both an integration of factors and of types of interaction.

Notably, the authors do not *a priori* determine the relative weight of each factor or type of interaction, nor do they specify the precise way of integrating factors and interactions. Again, integration is essentially a matter of context and also pragmatically relies on current state of knowledge, concepts, tools and methods. Significantly, this leads to question the possibility of comparing two influenza interspecies transmission or epidemic events. In many instances, the factors involved, their impact as well as the tools and concepts required for the understanding of particular influenza emergence events are depending on the specific context, time and place. As a consequence, the explanatory framework centered on the host species barrier concept does not aim at being strictly applied to each emergence or epidemic event. Rather, it is a research agenda or a guide for the epidemiology of influenza viruses.

Given such a research agenda, the authors notice that studies of intra-host viral diversity and ecology "are notable for their rarity" (Ibid., p.397). The work of Ferguson and colleagues (2003) constitutes one of the rare examples of such intra-host investigations, articulating molecular and ecological approaches at the intra-host level. Investigating the "ecological and immunological determinants of influenza evolution" Ferguson and colleagues look for the reasons why, during a global influenza pandemic, existing strains are *replaced* by a new avian influenza A subtype, the one that causes the pandemic. In other words, the outbreak of an influenza pandemic seems to be associated by the replacement of strains circulating before the outbreak by the pandemic strain.

However, such a replacement is problematic. Given the antigenic diversity between – at least some of – these pre-existing strains and the newly introduced epidemic subtype, and given that inter-subtype competition is often described as a result of differential antibody recognition (also called "cross-immunity"), infection with the epidemic strain should not restrict further infections with other pre-existing influenza subtypes, as immune responses that have been activated by this epidemic strain should not target antigenically diverse strains of influenza. Yet, in epidemic contexts, infection with epidemic strains prevents further infections with other influenza subtypes, as if some immunological determinants – other than cross-immunity – were at work.

To answer this "enigma", the authors investigate the intra-host ecology of influenza viruses. They hypothesize the existence of a second immune-response component, distinct from cross-immunity, and able to account for this astonishing "nonspecific competitive interaction between strains" that lead to the replacement of pre-existing strains by the epidemic one. This second immune determinant of inter-subtype interactions would be a short-lived nonspecific immunity "that decays rapidly with time from last exposure and inhibits reinfection by any new strain" (Ferguson et al. 2003, p.430). This hypothesis explains the apparently paradoxical extinction of pre-existing subtypes: "with such immunity, subtype extinction becomes highly probable in the context of the globally synchronized large-scale transient dynamics associated with pandemics" (*Ibid*., p.432).

This example and the associated two-component immune response hypothesis show that a deeper understanding of immunological determinants and of immunity-mediated viral inter-subtypes competition may offer a finest account of the dynamics of influenza populations and influenza transmission in epidemic contexts.

Understanding interspecies transmission and epidemic outbreaks of influenza viruses often rely on different kinds of integration (of factors and explanations, of data and tools). Given the prominent role of genetic evolution of these viruses, studies of the transmissibility and pathogenicity of influenza strains are sometimes moving away from ecological approaches. However, numerous studies emphasize the need to integrate a whole range of factors and interaction types to understand, control and sometimes anticipate influenza epidemics.

3. Integration in the epidemiology of two RNA viruses: goals, issues and future challenges.

In the beginning of the 1990s, the elaboration of the concept of emerging infectious disease was sustained by the need to provide a conceptual and institutional framework for a better integration of molecular and ecological factors involved in infectious disease emergence or re-emergence, sometimes leading to epidemic events. Such an integration was seen as a necessary condition for a better understanding, control and anticipation of actual and potential epidemics. The second section of this paper described integration processes at work in the epidemiology of rabies and influenza viruses. This section analyses the meaning of integration in this context, its purpose and goals, and reviewed some issues and challenges associated with integration processes.

3.1. The meaning of integration

Epidemiologists often characterize their discipline as being "integrative", as in the case of the recent epidemiology textbook coordinated by Jean-François Guégan and Marc Choisy, entitled "Introduction to the integrative epidemiology of infectious and parasitic diseases"[9] (Guégan & Choisy 2009). In other instances, epidemiologists describe a particular study as being "integrative." What does integration mean and what is potentially integrated?

Recently, philosopher Ingo Brigandt argued that it would be impossible and counterproductive to attempt to give an universal account of what integration is, as various kinds of integration exist (Brigandt 2013). However, it seems necessary to distinguish integration from reduction and unification. Reduction, in the case of theory reduction, is the process by which a scientific theory or sets of scientific theories is shown to be logically

[9] Original title: *Introduction à l'épidémiologie intégrative des maladies infectieuses et parasitaires*.

deducible from a more fundamental or lower-level theory (*Ibid*., p.461). Integration is not based on a logical process of deduction, but rather puts together heterogeneous units (concepts, explanations, methods, practices, data, standards, and so on) inside a coherent framework in order to achieve some specific goals. There is no *a priori* or logical link between the units. The link is established through the definition of specific problems and the further establishment of a general framework where the resolution of these problems may occur.

The fact that integration is used in particular contexts to answer specific problems is often considered to be a specific trait that distinguishes integration from unification. At first sight, however, unification may also be characterized as a general process putting together heterogeneous units to answer specific goals. For instance, the formulation of a law unifies a whole range of apparently heterogeneous phenomena, thereby answering the question: what do these phenomena have in common? A theory may also unify or synthesize two previous theories in order to answer problems that previously remained unsolved. However, unification is generally associated with theories or laws, while integration is associated with explanations, models, data, tools, techniques. Moreover, unification (of theories) often results in the understanding of a larger range of phenomena, while integration does not. Rather, integration modifies the ways to study the same phenomenon. Yet, despite these differences between integration and unification, they also share common characteristics, as for instance the fact that they "both involve transformation of what would count as an adequate explanation of said object" (for a discussion on integration and unification, see Plutynski 2013, p.469).

There are many ways to distinguish between distinct kinds of integration (see for instance the special section – section 4 – of the Issue 44 of the journal *Studies in History and Philosophy of the Biological and Biomedical Sciences*, 2013). In a 2013 paper, philosopher of science Maureen O'Malley and systems biologist Orkun Soyer distinguished between three kinds of integration. O'Malley and Soyer are analyzing integration inside the realm of molecular systems biology, using examples going from mathematical cell biology to evolutionary systems biology. However, their analysis of different kinds of integration and their interconnections applies to the case of current epidemiology of influenza and rabies viruses.

Integration of *disciplines* refers to a general process where different disciplines work together on the same object or problem. In the case of epidemiology, disciplinary fields such as ecology, macrogeography, molecular biology, evolutionary biology, mathematics, among others, are integrated to help understanding and controlling epidemics. Yet, we agree with O'Malley and Soyer to say that disciplinary integration is a *context* of integration rather than a kind of integration itself. "Multidisciplinary capacities certainly inform and even guide integration, but are conditions for integration rather than integration itself" (O'Malley & Soyer 2012, p.65).

What may then be integrated, in the context of multi- or transdisciplinarity, are either data, methods or explanations, or, more often, data, methods *and* explanations at the same

time (O'Malley & Soyer 2012). Data integration "is the activity of *making comparable* different data types from a huge variety of potentially inconsistent sources" (*Ibid*., p.61; emphasis added). Methodological integration involves the combination of specific methods, either simultaneously or sequentially, to understand "a particular biological system or research problem in order to gain a multidimensional understanding of how the system works" (*Ibid*., p.60). Explanatory integration, in contrast, refers to the synthesis between different explanations or kinds of explanations. Explanatory integration may also refer to the import of explanations from one field of inquiry into another.

To a large extent, the second section of the present paper described integration processes occurring at the same time at the levels of explanation, data and methods. However, another kind of integration was detailed, the integration of scales and levels (e.g. Mitchell & Dietrich 2006) also play a critical role in the articulation between molecular and ecological approaches in the epidemiology of rabies and flu viruses.

One remaining question is to know what integration is made for. What is the purpose of integration? One important result of the present study is that integration is not generally "made for something." Integration serves specific goals in specific situations (see also Brigandt 2013). Here, in the context of the understanding of viral epidemics, integration of ecological and molecular approaches aims at reinforcing the understanding, control and anticipation of epidemic events. More precisely, the two case studies revealed that the epidemiology of rabies and influenza viruses shared some more specific issues. Yet, they also face distinct challenges.

3.2. Common issues and specific challenges of integration in the epidemiology of rabies and influenza viruses

As we have seen, there is some similarities between integration processes at work in the epidemiology of flu and rabies viruses. Notably, molecular data and tools are integrated with ecological data and methods inside a broader ecological framework. Molecular markers and genetic data are used to quantify ecological factors. On the other hand, molecular factors and ecological factors are also integrated in the sense that both are defined as *causes* of emerging or epidemic events. However, integration is not a mere association of factors, a switch from monocausality to multicausality. Integration addresses the issue of estimating the relative weight of different factors. The "balance of emphasis" (Rosenberg 1992, p.303) between heterogeneous factors is sometimes problematic. This is especially the case of influenza viruses where molecular and genetic determinants of viral evolution seem to play a prominent role, sometimes making hard the articulation of such approaches with molecular ones. The attribution of weight to one or the other factor determines to a large extent the choice of measures to fight the epidemic, as distinct priorities are often associated with molecular approaches (e.g. vaccination campaigns) and ecological ones (e.g. managing host population density). A quantitative difference in the weight of factors may then result in a qualitative discrimination between distinct possible responses to an epidemic.

Rabies and influenza epidemiologies shared a common challenge, as do RNA viruses more generally. As Kuiken and colleagues acknowledged in 2006, intra-host ecological studies of virus-virus interactions – as well as virus-host interactions – are often too rare. However, since 2006, research on RNA viruses and their intra-host ecology is growing. This is partly due to a general reinforcement of intra-host microbial studies. Genomic and post-genomic (metagenomic) studies both contributed to the detection of new – previously undetected – microbes inside the human "microbiome" and provided new insights for the study of the interactions between the immune system and infectious agents (see for instance the lectures of Philippe Sansonetti at the College de France (2013)[10]; Relman 2002). Although they initially focus on bacteria, microbiome studies prompted the development of "virome" studies (e.g. Wommack et al. 2012).

However, and more specific to RNA viruses, the growing work on their intra-host ecology mainly relies on the development of studies of viral quasispecies evolution. Understanding viral quasispecies evolution requires to combine a molecular and an (intra-host) ecological approaches. Genetic differences between viral mutants of a given RNA viral quasispecies are not sufficient to explain the evolution of such a quasispecies – and therefore the potential emergence of an epidemic strain corresponding to a particular mutant inside the viral quasispecies. The viral progeny of RNA viruses is a population of ecologically interacting mutants, as competition as well as cooperativity occur between the viral mutants. "Mutant clouds are not mere aggregates of independently acting mutants. Rather, internal interactions of cooperativity or interference can be established among components of a mutant spectrum, mainly through their expression products" (Domingo et al. 2012, p. 159). Viral intra-host ecology would then become a key element in the design of new antiviral strategies: "Recognition of intraquasispecies interactions has influenced research on an antiviral strategy that aims at extinguishing viruses through intensification of negative intrapopulation interactions, which may contribute to deterioration of viral functions. This new strategy is termed lethal mutagenesis, and it is gradually finding its way toward a clinical application" (*Ibid*, p.160). Although relatively new, this perspective highlights the growing ecological understanding of intra-variants interactions at the molecular level.

The ecological study of virus-virus interactions at the molecular level is a challenge for the epidemiology of both rabies and influenza viruses. However, there is a challenge which is specific to rabies virus epidemiology. The complex ecology of influenza viruses make any attempt to eradicate these viruses very doubtful. On the contrary, eradication is part of the agenda of rabies epidemiology (see for instance Freuling et al. 2013). Despite intense debates surrounding eradication programmes in the second 20th century and in the 21st century, eradication was never completely removed from scientific agendas. Today, as well as during the 1980s and 1990s, eradication programmes still exist and are often largely supported, as shown by the successful case of rinderpest, a viral disease also known as cattle

[10] These lectures are available online: http://www.college-de-france.fr/site/en-philippe-sansonetti/course-2013-2014.htm

plague, whose eradication was confirmed by the World Health Organization in 2011. But it is true that the concept of eradication has been partly reformed and eradication attempts are now largely associated with ecologically informed programmes (e.g. Lloyd-Smith 2013; and a special issue on the elimination of infectious diseases edited by Petra Klepac, C. Jessica E. Metcalf and Katie Hampson – Klepac et al. 2013). Elimination and eradication strategies of rabies exist. However, they are challenged by the complexity of addressing at the same time very diverse ecological, behavioral, cultural as well as economic factors.

Conclusion

This paper traces the historical context surrounding the elaboration of the concept of emerging infectious disease. This productive concept significantly altered the understanding of infectious diseases, highlighting the need to articulate ecological and molecular factors. Integration processes between these two approaches have been described in the epidemiology of rabies and influenza viruses, focusing on the understanding of interspecies transmission – and the possibly resulting epidemic event. The specific meaning, limits and challenges associated with integration in these contexts have been discussed.

One important conclusion is that integration has no *a priori* purpose, nor is there a universally good way to integrate ideas, methods, and so on. Integration is context-dependent and often answers pragmatic decisions about how to solve a specific problem.

Another result is the fact that current epidemiologies of rabies and influenza viruses largely reflect the impact of the concept of emergence on epidemiological thinking. Despite some limitations, ecological and molecular factors are often articulated inside coherent frameworks which are also potential strategic plans.

Finally, I would like to conclude on the meaning of "ecological." In this paper, the term was used in its biological sense(s), referring to the study of the interactions of and between species with their environment. However, numerous epidemiological studies described here also include, inside ecological factors, social and cultural factors. One way to understand such an inclusion is to consider that, what is meaning here is that social and cultural factors have repercussions on more "traditional" ecological factors, such as population density and movements, or even behavior. Therefore, it is necessary to include such factors in the inquiry. Another way to understand this inclusion, however, is to admit that it is sometimes hard to distinguish between biology and culture, between the ecological on the one side, and the social and the cultural on the other side. Are not social relations and cultural behaviors part of our ecological environment? If this is the case, then to what extent does epidemiology need sociology?

In 1992, Lederberg, Shope and Oaks already stressed the importance of public education and behavioral change in preventing epidemics (Lederberg et al. 1992, p.14). The same year, Rosenberg underlined the necessity to understand disease as a biological and social

phenomenon. “We need, that is, an ethnography as well as an ecology to explain the network of interactions underlying the appearance, diminution or recrudescence of particular infectious ills” (Rosenberg 1992, p.303-304). If anthropological and sociological studies of different epidemic contexts exist (e.g. Epelboin et al. 2008, Fintz & Moutou 2010), they are still rare and often used retrospectively. One of the main challenges of present and future epidemiological research may then rely on the generalization of social – and psychological – studies and their integration to these combined molecular-ecological strategies.

“Because people are so important in [viral] traffic, close collaboration between biomedical and social scientists will be indispensable, and interdisciplinary approaches should be encouraged” (Morse 1993, p.24). A better understanding of (culturally-mediated) human behavior in epidemic context is necessary to grasp one very important aspect of viral epidemics: fear.

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