Epistemic considerations on research about *Flavivirus* induced fevers.

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Abstract: We analyze the knowledge structures (epistemic frames) used to reach conclusions based upon observations in the field of *flavivirus* induced fevers such as dengue, zika, west nile and yellow fever. By reviewing the current literature, and specially, the surprises produced by the Zika virus pandemic we identify two main trends: the use of a bare form of empiricism, and concurrently, a reduction to a chemical conception of life. We show that such epistemic frame leaves out of our considerations some of the most relevant attributes of living things and often lead to a lack of preparedness for the public health system. We will argue that this epistemic frame relates more closely to technology than to science and it is convenient for the proposing of technological patches but introduces important risks by turning relevant concerns into no-questions.

Keywords: genetic epistemology, mosquitoes, quasi-species, understanding

Abbreviations: **DENV**: Dengue Virus; **ELISA**: Enzyme Linked Immunosorbent Assay**;** **I**mS: Immunological Systems; **RNA**: ribonucleic acid; **UTR**: Untraslating region; **WNV**: West Nile Virus; **YFV**; Yellow Fever Virus; **ZIKV**: Zika virus

1 Introduction:

Research in the natural sciences is about producing understanding, this is to put in relation what reaches our sensory system, the signal originated in the object, with our concepts and mental organization [1]. It follows that knowledge cannot be completely objective in the direct meaning of such expression. If we consider what comes from the subject, the subjetivity, the negation (or opponent) of the objectivity, there seems to be only one path into a transcended objectivity, which is the negation of the subjectivity. This negation is reached by putting

the contributions of our conceptual framework under critical vigilance. Husserl pointed out that, too often, the claim of objectivity of the empirical sciences was only the result of the denial of the participation of the subject in understading, as Morin reminds us in his call for a Science with consciousness [2]. Piaget and García [3] introduce a distinction between what is observed, the observable, and the form in which we apprehend it, the fact. The observable is transformed into facts by the use of an epistemic frame, this is, our preexisting and organized knowledge. But the epistemic frame operates even before the observation, since we design our experiments to make measurements that we expect to be able to interpret, this is, to process them with our epistemic frame. We normally dismiss as no-questions those possible measurements that we do not preconceive as potentially useful. Thus, every experiment is "contaminated" by theory. To make things even worse, much of our epistemological frame is incorporated as habits [4, 5] and accepted forms, even protocols, escaping in such a way to conscious scrutiny.

The original aim of our research was to identify what was behind the stream of surprises given by the Zika epidemic during the years 2015 and 2016. A surprise corresponds to a mismatch with our expectations, hence we must ask, is ZIKV so unusual or, on the contrary, are our expectations wrong? Blaming the virus is the easy and selfindulgent method that advances no knowledge, so we must consider the second possibility first. How were our expectations formed? Reviewing the bibliography we arrive to the conclusion that the epistemic frame most usually employed consists in two steps. First, a biochemical (genomic) classification of *flavivirus* is constructed based upon common chemical features of the virus quasispecies. Thus, for example, dengue, yellow fever, Japanese encephalitis, ZIKV are labels that refer to their chemical (partial) identity. The identity is necessarily partial since diversity is what characterizes virus quasispecies. Second, to establish a function from the label into the information obtained by several forms of experiments and observations. This system has no step of conceptualization but only experience, it is bare empiricism, it organizes information in terms of the chemical labels. If the frame is correct, whenever we identify the chemical label, we are to expect the repetition of the experiences attached to it. We shall call this point of view the chemical frame. It is a very natural frame for inert matter and its manipulation. There is a fixed identity for gold and sulphur, as well as for most (if not all) the things made by inert matter. Then, it makes sense to consider what the object is, removing from its determination what comes from its interaction with the universe.

A contrasting epistemic frame, that we will call the ecological or life frame, was initiated by early ecologists such as AF Thienemann [6]. Living things do not live in isolation but rather in direct relation with the Universe. Notice that we call the environment the complement of the species (or living creature) in the Universe. Life is about change, and this change has an aleatory component. The dynamics of change is regulated by adaptation to the environment and positive adaptation is another name for improved reproductive performance. But the relation with the environment has a dual form, it can be said that the environment conditions the life of the organism as well as that the organism

modifies the environment. The relation is complex since the organism is as well part of the environment of the remaining organisms in its own environment. Such reciprocal relations are reminiscent of dialectic pairs and, indeed, the epistemology of complex systems [7, 8] is based upon a dialectic view. We translate R García [7] "the basic principle of the theory of complex systems ... says that every alteration of a sector propagates in diverse forms through the structure of relations that defines the system, and in critical situations (low resiliency), it generates a complete reorganization. The new relations - and the new emerging structure- imply modification of the elements as well as the total functioning of the system". In the ecological frame, any observation is a situated observation and in order to conceive what would happen if some of the elements in it are changed we would have to achieve some understanding of the operating principles of the relations that are implied.

Let us now focus on Zika virus (ZIKV) as a *flavivirus* member of the *flaviviridae* family. ZIKV was first isolated in a sentinel monkey that had been exposed in a cage with the intention of capturing wild strains of Yellow Fever virus (YFV, another member of the same genus and family) in the forest of Zika, Uganda, in 1947 [9].

The *flavivirus* are RNA-viruses composed of about 11,000 nucleotides [10] and lack replication controls as those associated to DNA [11]. This characteristic produces high variability of the virions, hence, viruses are better considered as quasispecies represented by populations of great diversity [12]. The rate of mutations for *flavivirus* is of about one mutation per copy [13]. A frequent form of reproduction of ZIKV consists in a succession of replications in the tissue of a mammal (say human beings) followed by replications in a mosquito (*Aedes aegypti* and *Aedes albopictus* are the best known vectors of ZIKV). This particular form of life, in perpetual adaptation to two rather different environments (hosts), is common to all the *flavivirus* transmitted by mosquitoes or ticks and, needless to say, operates as a particular selection mechanism [14, 15].

In what follows we will review some (mostly recent) contributions to the knowledge of ZIKV (and other *flavivirus* when necessary). We have selected striking cases where the chemical frame lead to wrong or contradictory conclusions to illustrate the thesis of this article.

2 Not everything is as expected

The Zika pandemics and its Changing Epidemiology The first serious epidemic produced by ZIKV was registered in 2007. Wikan and Smith [9] indicate "Before 2007, virological and immunological evidence suggested that although Zika virus was distributed widely in Africa and Asia, Zika fever was not a disease of substantial concern to human beings because only 14 cases had been documented worldwide, consisting of 13 natural infections and one laboratory-acquired infection."

The Zika epidemic outbreak of 2007 in the Pacific islands was a slowly spreading disease [16, 17] and the associated neuronal damage was not detected timely

[18]. About 90% of ZIKV infections in the human being result in cases that do not require medical assistance, the remaining cases are mostly characterized by a febrile syndrome. Yet, it is important to understand that the epidemiology is changing. We quote again [9] "The early clinical picture of natural human Zika virus infection was of a short duration, self-limiting, mild febrile illness that was accompanied by a maculopapular rash. In the first reported substantial outbreak of Zika virus infections, in Yap State in 2007, the disease was associated with rash, fever, arthralgia, and conjunctivitis, but no hospital admissions or deaths were reported. Similarly, the cases in Cambodia in 2010 and Philippines in 2012 were resolved without any hospital admissions. The cases in Thailand between 2012 and 2014 for which full clinical details were available were all classed as mild, with fever and rash as the main symptoms, and sore throat, muscle and joint pain, and headache as other reported symptoms. ... The outbreak in French Polynesia was associated with about 70 cases of severe presentation including Guillain-Barré syndrome, and other more severe pathological abnormalities have been associated with Zika virus infection, including meningoencephalitis in the Pacific Islands, and myelitis in Guadeloupe."

The epidemiology of ZIKV changed again by 2015 when the virus spread swiftly through the Americas [17] and microcephalia was epidemiologically associated to ZIKV first by pediatric doctors. Microcephaly in new born children exposed to ZIKV as a fetus was first doubted [19] but later confirmed by several studies, for example [20].

ZIKV can also be transmitted by sexual relations [21, 22]. The persistence of the virus in the human depends on the biofluid or tissue in consideration and it goes from 10 days in blood [23] to more than six month in semen [22].

Ability of DENV to invade mammalian cells depends on history of selective pressure. Dengue viruses become unlikely to infect human cells after as little as five consecutive passages through C6/36 (*Ae. albopictus* cells) in the laboratory [15]. The normal life history of DENV alternates replication in *Ae. albopictus* or *Ae. aegypti* cells and mammalian cells (humane for example) each one exerting a different selective pressure. As a consequence of this alternating pressure the virus types selected are viable in both environments and optimal in none of them. According to the result, the ability to cope with the mammal immunological system (ImS) can be rapidly lost, yet the ability of reproducing in C6/36 cells of virus populations selected in Vero cells is not lost in the same degree. Further explanation has been given by observing that most *flavivirus* (with the noticeably exception of the YFV) have some structure duplicated in the untranslating region (UTR) 3', and that the loss of one of these structures decreases the reproductive ability of the virion in mammalian cells while the suppression of both structures implies a reproductive ability below the experimental sensitivity. YFV presents some related mechanisms discussed as well in the review [15].

Observation/Studies	[25]	[26]	[27]	[28]
Virus origin	Asian lineage	Puerto Rico	Rio de Janeiro, Brazil	Samoa
Vector origin	Laboratory	Vero Beach, Fl.	Rio de Janeiro, Brazil	China
Probability	˜0	˜0	˜0	[0.2,1]
Reprod. media	Vero Cells	Vero Cells	Vero Cells	C6/36
Virus fed (log10)	6.83	7.22	6.0	5.5
Virus replica	1	1	2	1

Table 1: *Culex pipiens quinqufasciatus* as vector for ZIKAV studies. The line Virus fed indicates the reported base 10 logarithm of the virus density fed to the mosquitoes. Reproductive media is the media used to replicate the virus.

Can Culex pipiens quinquefasciatus be a vector for ZIKV? In the aftermath of the ZIKV epidemic in Brazil emerged the question on whether *Aedes aegypti* was the only vector of ZIKV or not. And in particular, whether *Culex pipiens quinqufasciatus* could be contributing to the epidemic development [24]. The question can be addressed at two different levels, the first one is to determine if *Culex pipiens quinqufasciatus* has the capability of being a vector of dengue, the second level is to determine if it is actually involved in the epidemic. Shortly after the question was posed, four studies addressed it. The four studies address the first level and emphasize (in their respective abstract and/or conclusions) the geographical origin of the mosquitoes and virus.

All the cases reported in Table 1 emphasize the origin of the mosquito and perform statistical studies using some small set of mosquitoes, yet none of them considers the reproductive media as a factor potentially influencing the results. The virus space is not explored at all while emphasis is put on geographical factors.

Antibody Enhancement of Infection It is well known that ELISA tests cannot distinguish between *flavivirus*, and it has been documented that the ImS cross-reacts when a second, different, *flavivirus* invades the body [29] resulting in an enhancement of the infection. For example the pairs WNV-DENV [30] and DENV-ZIKV [31]. It would appear as the worst scenario, that where the ImS recognizes the new virus but not as well as it would be needed for prevention and makes the results of the second infection worse. However, there is speculation that DENV and ZIKV infections migh cross-protect against YFV [32] (the author does not facilitate the evidence supporting his opinion).

Additionally, *flavivirus* use the same mechanisms to avoid the action of the ImS [33, 34]. This family of results suggests that a wider view, at the level of *flavivirus*, is needed if we intend to understand the problems posed to human health.

Yellow fever vaccine The history of the yellow fever vaccine, interestingly, shows elements of both epistemic frames. The original vaccine was obtained as an attenuated strain derived from wild yellow fever collected in Asibi (Ghana)

in 1927. The process used to attenuate the virus was to develop the virus in a different environment, cell cultivates of rat brain. The resulting vaccine was effective as well as dangerous. Unavoidably, the change in environment selected strains that were neurotropic despite the wild yellow fever having notorious affinity for the liver. A new environmental change was then sought to avoid the neurological side effects, and passages through chick embryo cells was introduced. The result was a vaccine that was believed to be safe for massive usage. Yet, given the opportunity, the neurotropic strain developed and forced a new modification of the vaccine [35], neurotropic cases in infants after vaccination continued to be reported [36]. After new changes in the protocol, the yellow fever vaccines were considered safe again, and this was the situation by 2001 [32] when serious (fatal) cases of yellow fever, clinically indistinguishable from the wild disease emerged after vaccination [37]. By 2007 the safeness of 17DD vaccine was severely questioned [38] since the incidence of acute viscerotropic disease was calculated between 1:200000 and 1:400000 being severe (1:50000) for people older than 60 years. Yet, other authors [39] were still confident in the safeness of the vaccine. Hayes [39] studied all the cases of acute viscerotropic disease reported between 2001 and 2006. After conceding that "the precise genetic determinants of attenuation are not known" he considered three hypothesis: mutations, co-morbility factors and immunologic or genetic susceptibility. Two factors (thymus disease and older age) were identified and it was judged that the virological evidence did not convincingly support the case for mutations. It is important to notice that by 2007 the works [40, 41] already had indicated that the untranslating region (UTR) 3' of the viral RNA was associated with virulence changes. The work [37] indicated precisely that this region of the virus obtained from the victims corresponded with the 17DD vaccine, showing no evidence of mutations. None of the deceased had the risk factors later identified, hence we must conclude that in these cases the difference is in the environment, i.e., the patients [42], or that other regions of the RNA might be as well associated with changes in virulence, a possibility not even discussed. The 2007 papers were published in July and November, yet Nature has other plans. A massive vaccination campaign in Peru from September to October 2009, using the 17DD yellow fever vaccine, produced the first known cluster of viscerotropic decease resulting in five cases and four deaths, all of them related to the same lot of vaccine [43]. The cases and the suspected lot were studied in depth. The consensus sequence of the virus found in the death cases was compared with the 17DD vaccine finding no differences, yet, realizing that consensus sequences might mask mutations, partial sequencing of a series of clones was performed. "Clonal sequencing of the envelope protein gene from nucleotides 1249–2646 was performed at CDC from viral RNA extracted directly from three 17DD vaccine lots (050VFA119Z, 121Z and 123Z)" [43], no difference was found with the 17DD vaccine. On this basis, the authors conclude that mutations can be discarded as a factor.

By 2011 a consensus had been reached that a safer vaccine was needed [44, 45], one produced with dead viruses.

The extrinsic incubation period of dengue For more than 50 years, the extrinsic incubation period of dengue fever has been considered to be something between 8 and 12 days based on a few set of studies. Yet a recent work reprocessing reported cases and experimental studies [46] indicates that the 95% credible interval at 25C is $[5, 33]$ days and moves to $[2.4, 15]$ days at 30C. An explosive epidemic of dengue struck Cairns, Queensland, Australia in 2008-2009 taking by surprise the health authorities [47]. A shorter than expected extrinsic incubation period was indicated to be behind the surprise.

3 Discussion and Conclusion:

Let us discuss, paragraph by paragraph the examples offered in terms on how conclusions are reached.

- In front of an emerging viral disease that is bound to quickly change after changing environment from wild mosquitoes and mammals into domestic mosquitoes and human beings, as well as a substantial change in the number of replicas of several orders of magnitude, can we expect an invariant disease in terms of its pathology? Because it was a mild and slowly developing disease, ZIKV received no attention until it was suspected of being associated to microcephaly in Brazil. The evidence is that the expectations were produced with bare empiricism. It could be resumed in the expression: the future is to be expected to be a slight variation of the past.

- DENV may be likely or very unlikely to reproduce in human cells, depending of the selective pressure in its live history, from the point of view of the ecological frame, this is what we expect. Yet protocols are followed that do not consider how the experimental procedure may modify the outcome of the experiment when manipulating low resilient quasiespecies such as *flavivirus*, as evidenced by the *Culex sp.* experiments.

- In the *Culex sp.* experiments the possible variability of the outcome is considered from the contributions of the individual variability of the mosquitoes, the regional variability of mosquitoes and/or virus, but in no case the intrinsic variability of the quasiespecies was taken into account and one or at most two samples were considered. This is a clear evidence of the application of the rule: the chemical label of the quasiespecies determines the outcome. This belief is so strong that we can indulge ourselves ignoring contradicting evidence.

- We do not know how *flavivirus* evade the ImS. We have evidence that the ImS does not react so distinctly to different *flavivirus*. Instead of realizing that DENV, ZIKV, WNV and the like are chemical labels produced by our chemical approach to the problem (this is, subjective) but not so relevant for the ImS, we insist in narrowing the investigation to our chemical frame.

- We use some indicative numbers for the extrinsic incubation period of dengue without giving consideration that for a very wide distribution such numbers have no reasonable meaning. We insist in descriptions proper of low variability (deterministic) situations contrary to the evidence. Variability and randomness is outside the chemical frame.

- The Yellow Fever vaccine follows a pattern of trial and error, a characteristic pattern of technological development rather than science. And above all, very little knowledge about the relation between the RNA and the ImS has been advanced despite a large number of investigations and economical resources poured into research. Most of the understanding I have been able to identify comes from considering the more general setting of *flavivirus* rather than the specific setting of particular viral quasispecies such as DENV, ZIKV, YFV, etc. Yet, the most telling of all the stories presented here is the one about the vaccine. The ecological frame was used to propose manipulation of the virus to produce a vaccine, but it was not used further. Despite knowing since 1986 [40, 41] of the relevance of the 3'UTR sector (about 565 basis above the ˜10000, see [48] for a relation between sector and number) a decision was made to study the nucleotides of another region, the envelope. It is also well known that the frequencies of changes are not uniform in the genome. There are four, out of eleven, regions where differences between 17D and the Asibi virus are more frequent: envelope protein region (1.0% nucleotide sequence difference), ns2a region (1.6% difference), ns2b region (1.0% difference), and 3'UTR (1.2% difference) [48]. Yet, only one region was studied. Which knowledge allowed for this simplification? The answer is the epistemic frame, which allows us to be ignorant without suffering social consequences, in as much as we exercise a "consensus ignorance", preferably without even knowing about it (otherwise it would be intellectual dishonesty, a charge I am not making). The epistemic frame eventually may even "command" us to ignore research that does not fit in it as in the present case [40, 48, 41] have been consistently ignored until [33, 15]. In such cases, the frame becomes a "reason-proof", blind, ideology. In practice, the facts (interpreted observations) of 1398 nucleotides allow us to make statements for the total of more than 10847 nucleotides, this is, the chemical frame accounts for about 9500 cases that otherwise should be recognized as ignorance. Such recognition would force us to lower our social pretensions but it would put us in the path to have a virtuous (Socratic) ignorance [49].

The examples given above are just a few of those available, they have the distinctive feature that they are simple to present without a lengthier elaboration and, in addition, they have led to clear mistakes. In all of them, the subjective contribution, the chemical frame, plays a mayor role in constructing the facts from the observations. In all of them, the life frame would have been a better guidance. The chemical frame can be observed in most papers in the literature, the ecological frame is not frequently represented, the reason is probably that the chemical frame can be produced by guidance and instruction, it has

no creative step, it is mechanical. The construction of understanding requires a creative step called retroduction (or abductive inference) by Peirce [50], being the only creative step in science.

New technological patches [51, 52] such as dengue vaccines [53], genetically modified mosquitoes [52, 54, 55] and *Wolbachia* infected mosquitoes [56, 57] are being proposed as potential solutions to the dengue epidemic problem. We should be deeply aware that they rest exclusively on the chemical frame and as such they incorporate information as well as ignorance into the proposal. On an epistemological basis these attempts are technological but not scientific. The result is unavoidably gambling with life. Consequently, there is an urgent need to develop the understanding requested by the life (or ecological) frame and to reconsider technological proposals under a true scientific view.

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