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ORIGINAL ARTICLE

Review: Molecular evolution and the feasibility of an avian influenza virus becoming a pandemic strain—a conceptual shift

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Abstract During recent years, a conceptual shift took place with respect to the genetic dynamics of influenza A viruses. In difference of the widely accepted approach that avian viral strains have the capacity to infect man only after undergoing genetic reassortment within pigs, it is now contended that direct transfection of man by intact avian-harbored viral genotypes is an actual, recurrent move, which may bring bout the generation of a new pandemic strain. This cardinal conceptual shift has been propelled by the appearance in 1997 of the zoonotic avian influenza H5N1 virus—a virulent, not yet contagious strain for humans—and ostensibly followed a genuine, unprecedented path within the evolutionary paradigm of Influenza A virus. This paper suggests that direct avian-human genetic interface is a pristine fundamental within the natural history of this protean pathogen, points at earlier as well as corroborative findings leading to such postulation, and regards the course of the H5N1 virus (and alike), as a readily detectable and traceable one, presently, rather then a novel development. It further examines the general feasibility of various components of that interface at large, such that give rise—whether gradually or abruptly—to pandemic genotypes, in terms of infectivity, pathogenicity and contagiousness. Within that context, the anticipated involvement of certain human-adapted antigenic subtypes is referred to, extrapolatively. Connectedly, the significance of natural ice as plausible regenerator of influenza A viruses, and its possible contribution to the emergence and reemergence of pandemic strains are accentuated.

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An evolutionary transition?

Influenza type A virus is a common respiratory pathogen of man and few other mammalian species. Its affinity to mammals is evolutionarily but secondary, though, and pertains to only a minority of its inventory of antigenic subtypes. This member of the genus Orthomyxovirus is foremost an asymptomatic enteric virus of numerous avian species. Waterfowls probably constitute the archaic source of all variants of influenza A virus in all other host species, and still serve as the prime, usually clinically unaffected reservoir of this essentially aquatic virus [1]. As a respiratory pathogen, the impact on man, however, is enormous, even if but lateral, on the whole.

The very initial histotropism marking influenza A virus formed, probably, towards the intestinal epithelium of feral ducks. Basically, ducks are tolerant of infection, and they silently harbor in nature a very wide variety of influenza A strains, including virulent epidemic and pandemic ones. This tolerance is the outcome of lasting co-evolution, and but rarely are ducks diseased by this virus [2]. Virus proliferation, mostly lacking cytopathic effect, commonly takes place in the intestinal epithelium. The respiratory epithelium is less affected, yet at times clinically more sensitive. Virus circulation and contraction are mainly fecal–oral, and secondarily air-borne. Latent infection and vertical transmission are very uncommon. Horizontal

transmission to other waterfowls, terrestrial birds, and poultry is frequent [3].

An immeasurable and unparalleled genetic pool of multiple virus strains is currently being sustained within feral waterfowl populations, and is further amplified due to the segmentary alignment of influenza A virus genome [4]. Hence, the remarkable dynamics fueling this genetic pool occasionally gives rise to genotypes endowed, potentially, with the ability to infect pigs, horses, humans and few other mammals. The in effect expression of that potential—namely avian–mammalian direct transfection, both natural and experimental—has chiefly been evidenced within pigs [5, 6].

For many years, yet, direct transfection of influenza A virus from avian species to man was generally regarded unfeasible. Since 1978, it was believed that avian influenza strains undergo genetic changes, mostly reassortment, in pigs, before having the capacity to infect man [7] This cardinal approach, though widely accepted, has substantially altered, and it is now held that avian–human transfection is feasible, and, moreover, may generate a new influenza pandemic [8]. Certainly, those two modes of transfection (swine-dependent and swine-independent) are not in contrast with each other, and may concurrently occur. Still, this conceptual shift provides a paramount case study, in that it ostensibly followed a recent evolutionary course taking place within the parasite–host interface of this protean virus [9]. In reality, direct avian to human transmission could all equally mirror an old, lasting, stable interface, which just manifested itself clinically, increasingly and traceably during recent years. The conceptual shift thus formed is meaningful, then, both scientifically and practically [10]. It plainly means that certain intact avian genotypes—whether ancient, recent or currently forming—bear the capacity to infect man. Of those, occasional gene constellations underlie the emergence of pandemic strains.

In contrast to the prevailing swine-dependent transfection consensus [11], the lately recognized swine-independent apparatus has primarily been suggested in 1993 already. It was then initially hypothesized—with regard to influenza A viruses at large—that ducks perpetually constitute the colossal, direct, yet unnoticed supplier of evolving human-adapted strains, chiefly in China [12]. As opposed to the ostensible necessity of pigs as a connecting link enabling virus gene reassortment, it has thereby been postulated that ''Ducks, mainly domestic but also feral, can thus serve as reassortment media for human and avian strains, so as to enhance reciprocal virus migration between ducks and humans, followed by intra-species transmission. Inversely, strains of primarily avian origin can infect humans, but the hosts are dead ends in such cases, unless productive reassortment with a colocated native human strain takes place in a doubly infected tissue... Hence, the Chinese duck-pond ecosystem can be regarded as a habitat in which influenza A virus genes regularly migrate, directly or indirectly, from ducks to humans, and vise versa'' [12].

This conceptual shift, which reflects, most likely, a pristine genetic fundamental marking this virus, turned out to realize itself throughout the following decade. In 1997, the H5N1 virus was for the first time contracted by a human being, bringing about his death, and designated A/Hong Kong/156/97 [13]. It virulently proliferated, mainly in poultry—hence commonly known as the Avian Flu virus—concomitantly infecting, sporadically, some 130 humans with about 50 % mortality rate from 1997 to 2005 [14]. Gene sequencing showed that except for the H5 gene, all other genes of this semi-anthropophilic virulent strain are almost identical to those of an ordinary collocated strain sub-clinically harbored by a feral migrating duck—A/teal/Hong-Kong/W312/97 (H6N1) [15]. One year earlier, in 1996, the antigenic phenotype H5N1 was for the first time isolated in south China from mildly diseased domestic geese, thereafter contributing its HA gene to a H5N1 reassortant virus derived from the teal virus [9, 16]. Probably, this reassortant virus infected—if not formed within—domestic ducks that could readily facilitate its proliferation, belonging to the same host genus as the teal: Anas sp. Further genetic changes—the in effect dynamics of which is not adequately clear—taking place within domestic duck populations gave rise to H5N1 strains with amplified pathogenicity towards ducks, chickens and humans [9, 16]. Comparisons of all eight RNA segments from those virus classes revealed greater than 99% sequence identity between them [17].

Gene migration between Siberia and South East Asia

Notably, the provenance of the genes of the Hong-Kong highly virulent H5N1 virus is chiefly, if not entirely, Siberian, in all likelihood. This geographical course probably relies on teals and other migrating waterfowls, mainly ducks and geese that prevalently breed in Siberian lakes. They asymptomatically sustain a vast variety of virus genotypes that are regularly conveyed to China and the rest of South East Asia [18]. A/teal/Hong Kong/W312/97 (H6N1) is but one instance that happened to be the progenitor of a remarkably aggressive virus, bearing the phenotype

H5N1. Actually, its buds were observed in feral ducks at Siberian lakes since 1996 [8]. Yet its fate, in terms of attaining human contagiousness and pandemic proliferation is still an enigma, particularly that the specific genetic alterations needed for such attainment to take place are still unknown. Not even whether a mutation, recombination or reassortment move should be anticipated, eventually. No less meaningful is the issue of whether the immeasurable viral genetic core prevailing in Asia makes this continent the only one apt to create the next pandemic genotype, or may the latter form elsewhere.

At any rate, it has been accentuated that, ''acting as a silent reservoir for the H5N1 virus, domestic ducks may have acquired an important new role in the transmission of this virus to other poultry and, possibly, to humans as well'' [19]. Actually, the role of ducks as a grand genetic melting pot and prime direct transmitters of influenza viruses to man seems to be the most significant factor—yet certainly not a new one, as ostensibly observed—within the tangled molecular evolution featuring this multi-face pathogen. It has previously been demonstrated, indeed, that a strain bearing another HA gene, in that case H7, ''may have been transmitted directly from ducks to humans'' [20]. Further, based on the genetic sequence of nearly 500 influenza viruses from Chinese poultry markets collected in 2000–2001, another study concluded that the avian H9N2 influenza virus ''could leap straight from ducks to people'' [21].

Moreover, referring to both H5N1 and the seemingly 'human' H2N2 strain, Webby and Webster [22] noted that ''Counterparts of the 1957 H2N2 pandemic virus continue to circulate in wild and domestic duck reservoirs. Under the right conditions—which are still not completely understood—H2N2 viruses could again be transmitted to and spread among humans''; also (referring to influenza A viruses, in general), ''Transmission from aquatic birds to humans was hypothesized to require infection of an intermediate host, such as the pig'' (serving as an ostensibly necessary, prerequisite genetic 'mixing vessel'), whereas the outstanding epizootic ''1997 H5N1 event demonstrated that domestic poultry species may also act as intermediate hosts'', and the resulting ''observations suggest that ducks and possibly other avian species in mainland China are a reservoir of H5N1''. The swine-dependent concept indeed lost its validity. The interface is, as suggested in 1993, essentially avian–human.

Being, apparently, the primordial, perfectly established host of influenza A virus—and largely represented synanthropically by the domestic duck in China—this very same feral species (the mallard—Anas platyrhynchos) seems to constitute the axis of the viral genetic apparatus needed for the genesis and direct transfection of human-adaptable strains, either by reassortment, recombination and mutations [23]. This may equally pertain, indeed, to many other areas in south-east Asia, especially those regularly visited by sub-clinically infected wintering wild ducks—mallards, teals, and alike—that breeds each summer in northern sub-arctic and arctic aquatic biotopes, mostly Siberian [24]. Over the southern areas, a dynamic interface takes place each winter between domestic ducks—which are, notably, very abundant in south east Asia, in particular—and those feral duck populations, prevalently the very same species, the mallard, thereupon fueling intensive influx of multiple virus genotypes harbored by them into many duck and chicken farms, primarily duck ponds [25, 26]. A critical mass consolidating those quantitative and qualitative factors altogether is thus formed within those cardinal Asiatic avian populations; therefore, given that predispositional critical mass, the rest of the mechanisms propelling the rise of novel mammalian-adapted strains—including pandemic ones—even if extremely complicated, would sensibly rely on merely random genetic events, apparently marginal ones, panoramically. Such a predispositional critical mass cannot be found elsewhere, nonetheless.

Europe served, however, to demonstrate the same principles on a minor scale, concurrently (Netherlands, 2003), subsequent to one isolated, confined epizootic episode of avian influenza in chickens, involving disseminated infections. During that episode, which lasted for but several weeks, the same antigenic subtype virus—H7N7, closely related, genetically, to low pathogenic virus strains obtained much earlier from wild ducks—was detected in 89 diseased humans who handled affected poultry, and in three of their family members [27].

Notably, the permissiveness of different host tissues towards influenza type A viruses in nature is extremely variable [28]. Typical fowl plague virus, for instance A/H7N7, would profoundly infect varitype organs in chickens, while exhibiting but limited histotropism in man, as was here the case. Outstandingly, of these 89 patients, 78 presented with conjunctivitis, 5 presented with conjunctivitis and influenza-like illness, 2 presented with influenza-like illness, and 4 did not fit the case definitions [27]. Though immensely significant, this episode reached a dead end, and the concerned viral genotype disappeared. The Asiatic, H5N1 virus, is incomparably more invasive and durable. But once again, the genetic course and final transfection are avian–human.

Initiation of pandemic course

Those two prominent (Asian and European) avian–human interfaces—though appreciably dissimilar—seem both to imply that in its full course, the generation of a new pandemic strain may progress through three successive stages; each accounts for acquiring:

a. infectivity to man (while circulating within poultry);
b. virulence (while circulating within poultry or humans);
c. contagiousness (while circulating within humans).

Any of those stages may evolve by means of genetic changes: mutations, recombinations, and, typical of influenza virus, reassortment with a different collocated virus. Notably, the first stage necessarily occurs within an animal host, and resultant infection of humans is barely traceable, as long as not clinically manifested. Such phenomenon—asymptomatic infection in man—has been observed with respect to influenza [29], and may reflect, initial adaptation to human host. This phase may take place due to absence of cytopathic effect, or considerable restriction of primary infection, and is detectable serologically or virologically [30]. Thereafter, the established virus may or may not undergo the second, and then the third stage, while the order of probabilities, allover, may descend in the following manner: stage A (alone, without there being any succession); A and B (without succession by C); A and B at once (while still infecting the animal host; without succession); A, B and C (successively); A and B at once (while in the animal host), followed by C (while in the human host); A followed by B and C at once (whether within the animal or human host); A, B and C at once (while still infecting the animal host). Though largely theoretical for now, this paradigm covers the entire avian–human interface underlying, presumably, the formation of a new pandemic strain.

The genes coding for the surface antigens—hemagglutinin (HA) and neuraminidase (NA)—constitute paramount elements within that paradigm. Overall, influenza type A viruses include 16 HA plus 9 NA antigenic subtypes, principally embodying any possible combination within avian hosts [31]. To this vast interchangeability—which is enabled by permanent gene segmentation—should be added the corresponding mobility of the other remaining six gene segments—though relatively stable, genetically—appearing in conjunction [32]. A much more complex, multi-factorial genetic mechanism allows for the formation of viral bridges from avian onto mammalian species at large, usually bringing about notable, and at times profound, clinical manifestations (mostly respiratory) in the infected mammalian host. Those bridges readily form towards pigs; they are long established, though appreciably narrower, towards horses; they occasionally form towards seals; and they have but once—as far as known—formed towards minks, cats, bats, deers, squirrels and whales. In all those cases, apparently, the mammalian virus strains thus given birth to became intra-species contagious. Infectivity towards man has formed, for now, with regard to the avian-originated antigenic subtypes H1N1, H2N2, H3N2, H5N1, H7N2, H7N3, H7N7 and H9N2; the first three became fully contagious and created pandemics [33]. H1N2 (A/Wisconsin/2001) and H3N1 (A/Memphis/1971)—yet not H2N1—were also isolated from humans, independently, as well as H10N7 (A/Egypt/2004). All that can be observed, then, is that any antigenic subtype already showing infectivity towards humans, tentatively has somewhat higher chance to be the progenitor of the next pandemic virus, as compared to other subtypes. Still, taking into account the very present herd immunity status worldwide, it is rather less likely, apparently, that a new pandemic strain will bear one (let alone two) of the genes coding for: H1, H3, N1 and N2. Hence, H2, H5, H9, H10, N3 and N7 are in that sense more foreseeable. Of those, the phenotypes H7N3, H7N7 and H10N7 have already formed and infected humans, whereas H2N3, H2N7, H5N3, H5N7, H9N3, H9N7 and H10N3 have not, as far as known; not yet. Assuming that the current H5N1 virus is prone to originate the next randemic strain, the emergence of H5N3 or H5N7 is more feasible. On the other hand, it was the H3N2 pandemic strain that replaced (in 1968) the H2N2 one, thereby depreciating the presence of herd immunity against N2; or, possibly, against the NA antigen at large. If so, then H5N2—or a antagious variant of H5N1—might as well be expected.

Plausibility of ice borne genes

This complexity is further ramified by an additional factor—genetic drift deceleration or cheesing—which occasionally marks influenza A virus genomes. Current mutational genetic drift, involving both adaptive and neutral mutations, is a well established, prevailing, persisting phenomenon in viruses. It has been quantitatively gauged quite meticulously within influenza type A virus genes, which resemble, thus, a fairly reliable evolutionary biomolecular clock, the rate of this drift being steady and remarkably high [34, 35]. Contrastingly, there are cases of evident reemergence of delayed or dormant influenza A virus genes and

genomes, representing an opposite phenomenon, namely multi-year preservation, which takes place through some abiotic mechanism [12]. A most salient example of this antagonistic phenomenon is the unexplained reappearance of the 27-years-preserved A/USSR/1977 H1N1 pandemic strain [36]. Only 5 of 51 expected nucleotide substitutions took place from 1950 (the year when the progenitor of strain A/USSR/1977 was isolated) until 1977 within the NS gene of this virus [37]. This, alongside with various parallel examples, illustrates a notable phenomenon, which is often described as 'frozen evolution' or 'frozen replication', without pointing at any underlying apparatus. Nevertheless, all genetic, ecological and epidemiological data substantiate the concept that influenza A virus has an inherent capacity for perennial endurance within, and subsequent reemergence from ice [12].

The mechanisms accounting for those unusual viral genetic dormancies are obscure. It has been suggested, though, that such gene preservation is a regular event, and occurs whenever perennial ice is formed from lake or sea water containing influenza A virions in various arctic and sub-arctic environments. Whenever thawing takes place—the following summer or thousand or more years later—viable fractions of the frozen viral material may readily reinfect nearby waterfowls [12]. Preliminary findings supporting, in general, that actuality were recently reached in Siberia and Japan [8, 38].

Northern lake water constitutes, indeed, an optimal abiotic homogenizing reservoir and long term preserver for varitype, aquatic, genetically interchangeable virus like influenza A. This being the case, because northern lakes serve to spatially concentrate a wide rang of genomes (harbored by multiple congregating waterfowls) whenever thawing takes place; temporally sustain the virions deposited by the waterfowls and bearing those genomes (virion durability in liquid water nearly equals the length of waterfowl breeding season, until freezing begins); and thereby locally diffuse those virions in the water (physically) [39–41]. Feces-borne virions are regularly shed, thus, by waterfowls into Siberian lake water that freezes and melts seasonally. When this periodicity is annual, the duration of freezing is meaningless, apart from year-to-year virus survival and reemergence, which are clearly significant in themselves [12]. But considering arctic Siberian lakes in particular, they are at times frozen much longer, thereupon perpetuating the immured cryophilic influenza viruses for lengthy intervals. Upon thawing, they are recurrently visited by the migrating waterfowls, allowing for renewed virus contraction (while drinking water) and then introduction into the above described predispositional critical mass. This apparatus would retain a broad spectrum of chronologically stratified—including various epidemic and pandemic—virus strains that are prone, potentially, to recirculate undetected, reappear in their preserved epidemic or pandemic form, or lend genetic contribution (one gene or more) to a currently evolving pandemic reasortant virus [42].

Suppose, just as an illustrative example, the present zoonotic H5N1 virus—which is for now non-contagious within humans—may acquire one gene specifically contained within the genome of the pandemic 1918 H1N1 'Spanish Influenza' virus, so as to become contagious, hence pandemic. In case this H1N1-related genotype—or this individual gene, within another, reassorted genome—is preserved in perennial lake ice and reemerges, it could bring about the acquisition of this particular gene by the H5N1 virus, transforming the latter into a new pandemic virus. The distance between such hypothetical scenario and reality is both conceivably and practically unclear, for the time being. Preliminary results in the laboratory of S.O. Rogers indicate that influenza virus is present within Siberian arctic lakes ice (Zhang G. et al, in preparation). They have found a high concentrations of influenza (as compared to glacial ice), in Siberian lakes that are visited by migratory birds. The sequences so far obtained are closest to the H1N1, H1N2, and H1N5 influenza A subtypes. Those primary findings were achieved by means of the PCR technique, and are about being elaborated to include more genes, and recovery of infectious virus, as well.

Taking into account the challenging phenomenon of perennial gene conservation revealed within influenza A viruses, it has been further contended, thus, that ''Ultimately, when single genes reemerge as dormant or delayed genes, they should be regarded as parts of genomes that underwent reassortment in any host included in the cycle, before freezing or afterwards. When entire genomes reemerge as dormant or delayed genomes, they should be considered as intact migrants, or, possibly, as occasional reverse reassortants'' [12]. The essential chain of virus or gene transmission would include, thus, reciprocally: ice < —>water < —>feral ducks < —>domestic ducks/poultry < —>man.

Basically, then, the mechanisms presumably underlying avian–human viral transfection are largely understood, though not yet entirely substantiated in terms of empirical data. The range of empirical data is at any rate very limited with respect to the circulation and evolution of many pathogens worldwide—especially those common to humans and animals, and particularly in areas like China—as long as they don't manifest themselves virulently. Nonetheless, the feasibility is

there that at this very moment—or, equally, centauries ago—a mild, unnoticed mutated (and/or reassorted) influenza A virus strain (H5N1, H7N7 or another antigenic phenotype) is being (or has been) transmitted from a healthy duck to a man in a typical duck-farm in China, Thailand or Vietnam, bringing about a subclinical, merely transient infection. It may be traced, probably, only in case it further mutates, very specifically, and/or hybridizes, by sheer coincidence, with a colocated, already established, human strain concomitantly harbored by some countryman, so as to form an epidemic, aggressive variant. The resultant pathogen could consequently generate a virulent pandemic, indeed. Such crucial occurrences pertain, broadly speaking, to a wide variety of zoonotic viruses, some of which—including influenza—are reckoned as salient menaces to man.

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