

The Libyan HIV Outbreak How do we find the truth?

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We would like to thank Professor Perrin for his comments regarding the Libyan HIV-1 outbreak case [1,2]. The following is our response to the points made by Professor Perrin. We would like to divide our response in separate sections, for clarification.

Vertical Transmission of HCV

Professor Perrin wrote the following comments [2]; "What we found is that all children were infected by a HIV-1 monophyletic recombinant circulating form from West Africa and 40 % of them were infected with HCV. We excluded vertical transmission for both HIV and HCV on the basis of serology in parents. Thus the assumption on page 2 of the viewpoint mentioning that HCV was acquired through vertical transmission is incorrect since most of the mothers were not infected. The fact that we found clusters of four HCV genotypes reflect simply the fact that HCV is much more common in Libya than HIV-1, thus in the case of improper sterilisation procedures or reuse of contaminated needles it is expected to have different HCV genotypes."..... "In western countries we have more or less controlled the propagation of HIV-1 infection in intravenous drug users but still observe frequently HCV infections in this population suggesting that transmissibility of HCV is higher than that of HIV-1."

If we take what the eminent professor stated in his comments that "transmissibility of HCV is higher than that of HIV-1," then it will be impossible to have 100% of the children HIV-1 positive but only 40% of the same children HCV positive. Obviously, according to the professor's own words the statistics do not make any sense [3-6]. In our opinion, when epidemiological data do not add up the first rule is to look for something that may not be obvious (see below for further comments).

As we stated in our original writing, the numbers do not add up. If we take the nosocomial infection and bad medical practices into account then one has to infect between 200,000 to several million children with contaminated needles (taking CDC's rate of 0.3% -0.009%; [7-8]) for HIV-1 to be able to get 500+ children infected with the same monophyletic strain (see more clarification below: 3-8). Obviously, the numbers do not add up. These numbers exceed the number of children, not only in Benghazi, but in the whole of Libya. In our opinion the low rate of HCV and the more than

2.5 times higher rate of HIV-1 in these children may indicate an intentional inoculation (see below for detail).

Case of Monophyletic Virus

Let us look at this possibility. What is a statistical possibility that 500+ children carry the same monophyletic HIV-1 strain? Bear in mind that, according to all three scientific articles, it is a unique sequence that did not exist previously (CRF02-AG: [4-6]). What is the possibility that all the children carry the same monophyletic strain if the infection was initiated by a drug abuser at El-Fatah children hospital and due to inappropriate infection control the contaminated needles were reused that spread HIV-1, HCV and HBV to more than 500 children? The possibility of this is close to zero and the western point regarding this issue is absolutely incorrect. Here are the reasons:

a) Suppose one drug abuser from Sub-Saharan Africa with high viremia (carrying subtype CRF02-AG) came to El-Fatah children hospital and someone drew his blood for some workup and then used the same needle that was used for drawing blood to inject a child for intravenous liquid containing blood products, antibiotics, nutrition or vitamin, etc. (Of course, one has to imagine a lot, because how a dirty needle from an adult ward of the hospital reached the paediatric general medicine unit is beyond logic, but for the sake of argument we take the scenario to be the case. In addition, if that is what really happened it would be considered a criminal act in any country). Now, assuming that that was possible and the dirty needle was reused to inject one child (our hypothetical index case) then one child would be infected with this one needle stick (even though the chance of it is 0.3%; [7-8]). If the same needle were used for another child, then the second child would be safe from this HIV-1 subtype since the first injection will carry the viral particles to the 1st child's body (intramuscular or intravenous route) and the needle will be clean of HIV after the injection (and that is clearly stated from the CDC risk assessment statement refs [7-8]). Now, one supposes that this child #1 got infected. Now, let us see what happens to this virus if we assume that the very first child did get infected with HIV-1 subtype CRF02-AG. This virus will go through a series of replication events and in 3-6 weeks may develop a serious enough viremia with flu-like symptoms and mild fever (chance of that is also

less than 0.01%; [7-8]). We assume that he was brought in to hospital for treatment of some sort (not for flu-like symptoms because they are generally very mild). But, suppose that this happened and he received injection for treatment and the needle that was used to treat him was also used to treat a second and third and fourth child the very same day. The possibility is 1 in 333 that the second child may get infected but the 3rd and 4th would be safe [7-8]. Now we repeat this same scenario again after 3-6 weeks when child #2 got infected and developed viremia high enough to be infectious by needle stick and a 3rd child got infected when a dirty needle from the child #2 was used to inject a 3rd child. Now in order to get 20 children to be infected in this manner it would have taken about two years (an average of 6 weeks X 20=120 weeks= \sim 2 yrs). The question is: would the virus remain monophyletic? The answer is absolutely not! (unless a cloned virus was used to infect each child).

To elaborate on this we have to see how HIV-1 replicates in the human body. Once inside the human body the virus seeks out CD4+ T cells and then the virus releases its two RNA genomes (it is diploid RNA retrovirus) inside the cytoplasm of the infected host cells [9-10]. This diploid RNA exchanges the genes right at the beginning, creating a new recombinant provirus [10]. During the ensuing replicative cycle, recombination between the two genomes would occur [9-12]. To the extent that the two RNA genomes will be distinct, recombination viral cDNAs coding for genetic information different from that of the two parental diploid RNAs [10-11, 13-14]. Numerous studies performed *in vitro* have documented the occurrence of recombination during HIV-1 replication [15]. Although these studies have found that the rate of recombination can be influenced by the nature of the target cell and that certain genomic regions may be hot spots for recombination, there is general agreement that recombination occurs frequently throughout the genome (2 to 20 events/genome /replicative cycle) and has the capacity to rapidly shuffle genomic segments from parental viruses [10-12, 14-18]. Numerous viral strains derived through recombination between distinct HIV-1 subtypes have been identified in infected individuals, indicating that recombination also occurs *in vivo* [18-20], and studies performed using limiting-dilution-PCR assays suggest that such events occur very frequently [9, 21-22]. So, in the Western case scenario, the first child (the hypothetical index case) once infected with a single viral subtype would be expressing thousands of new recombinant quasispecies [23-

24]. And by the time he goes to the hospital again for whatever reason after 6 weeks he would have different HIVs and multiple subtypes (and not the original parental one; [9-23]). So, the presence of monophyletic virus is one of the keys to the mystery! It is becoming apparent from this discussion that it is very unlikely the virus originated from a single human being and spread to 500+ children in a short time period by the nosocomial infection, regardless of how inappropriate the infection control unit was. One can see this kind of scenario all over the developing nations and, yet, no one has encountered the kind of outbreak that is described in El-Fatah children hospital [3-6]. The alternate explanation is that the children were infected with two cloned HIV-1 viruses (first with an attenuated HIV-1 and then with a pathogenic strain of HIV-1). We will explain this shortly.

b) Now, let us look at the assumption that the African drug abuser who came to El-Fatah children hospital was carrying a single HIV subtype. This assumption is also false. No living human being infected with HIV-1 carries a single HIV-1 subtype. There are hundreds of subtypes in a single human being at all times [20-23].

c) Is it possible that a monophyletic strain can be maintained for a long time *in vivo*? If we accept the scenario that the hypothetical index case maintained a single subtype: CRF02-AG and it then spread to the next child due to nosocomial infection and the next and so forth. However, in reality, the 2nd child cannot maintain the same subtype due to multiple recombination events that are so innate to retroviruses and HIV-1 [9-24]. If we assume for a second that this truly aberrant subtype was able to maintain its integrity through several children it could not do so for so many years and in so many children with genetically different hosts [9-24]. The advantages that are acquired through recombination and the impact of recombination on HIV-1 pathogenesis in individual patients appear to be essential for viral survival [23-24]. The shuffling of polymorphisms found in distinct viral quasispecies plays a crucial role in generating viral diversity and genetic-based resistance for viruses in genetically and immunologically different hosts [9-23]. The ability to maintain extensive diversity is extremely important for viral pathogenesis and survival, because it ensures the availability of viral quasispecies that are able to escape changes in the selective pressures exerted by the immune response or by antiretroviral therapy. Indeed, results obtained with other models support the idea that diversity generated by recombination is necessary for adaptation to changing evolutionary

pressures [21-23] and this is one of the major reasons that we are unable to develop an effective vaccine against HIV-1 (reviewed in [25]).

d) From the above discussion it must be clear that if a single HIV-1 infected child was admitted a number of times within 1-2 years and he or she served as the index case, that individual would not be able to maintain the same viral subtype for that long and may transmit different quasispecies to different children [9-23]. These subtypes will further recombine into the new hosts and therefore, it is highly unlikely that the virus will remain monophyletic [4-6].

e) It is one thing that a group of drug addicts spent 24-48 hours together and exchanged a dirty needle and received a single predominant subtype. It is entirely another thing if 400+ children got infected over a long period of time and showed a monophyletic subtype years after exposure [4-6].

Case of unsafe medical practices and reuse of unsterilized needles

One of the most important points that we have to take under consideration is that maybe the children were infected due to reuse of unsterilized needles. It is possible that due to years of sanctions the Libyan Government was unable to provide sufficient number of sterilized needles and reuse of blood laden needles served as the main source of epidemic [3-6]. However, several credible reports refute this theory (reviewed in [24-25]). A recent report by Priddy et al [24] has shown that such practices do not result in HIV transmission. This group investigated the potential medical transmission of HIV through unsafe medical injections in 16 rural health institutions in Ethiopia (where HIV-1 is an epidemic). Most institutions reported re-using disposable needle/syringes, and 12% of observed injections were given with used, disposable syringes prepared for re-use. Analysis of used needle flushes showed no HIV RNA. These investigators concluded that despite the re-use of disposable needles, medical injection practices are not likely to contribute significantly to HIV transmission in this region. Unsafe needle sticks and intravenous injections from used needles and syringes are very common and they generally result in HCV and HBV transmission but rarely in HIV. This particular question was investigated by the World Health Organization Investigators. Therefore, Hutin et al [25] have described the injection practices worldwide in terms of frequency and safety. The WHO defined the global burden of disease into 14 regions on the basis of geography and mortality patterns. Their data sources included published studies and unpublished WHO reports. Studies

were reviewed by using a standardized decision making algorithm to generate region specific estimates. They included both formal and informal healthcare facilities. They showed that the annual number of injections per person and proportion of injections administered with syringes or needles, or both, are being reused in the absence of sterilization in the majority of the developing nations. The analysis excluded four regions (predominantly affluent, developed nations) where reuse of injection equipment in the absence of sterilization was assumed to be negligible. In the 10 other regions, the annual ratio of injections per person ranged from 1.7 to 11.3. Of these, the proportion administered with equipment reused in the absence of sterilization ranged from 1.2% to 75.0%. Reuse was highest in the South East Asia region (seven countries in South East Asia), the eastern Mediterranean region (nine countries, mostly located in the Middle East crescent), and the western Pacific region (22 countries). No information regarding injection safety was available for Latin America. They concluded that overuse of injections and unsafe practices are still common in developing and transitional countries [25]. Obviously, if reuse of injectable needles or syringes were the main culprits this problem would have been more common in India or China or other poor African nations where the numbers of HIV-1 infected individuals are much higher or where the populations are more dense. Of note, in the case of El-Fatah Children's Hospital the problem was associated with only one unit (General Medical Unit) and not other units like the hematology/ oncology, the renal dialysis, and the neonatal units, which are more likely to have such risk from the nosocomial point of view [3-6].

Western Scientists' Mistakes

As it is summarized in the above scientific discussion, the possibility of the HIV-1 outbreak in the Libyan children is virtually impossible to have occurred by the nosocomial route. Therefore, it was essential to carry out more extensive investigation. As you might have noticed none of the research articles reports a thorough and solid scientific investigation. The first thing that would have occurred to the curious minds would be to sequence the full length HIV-1 instead of carrying out the sequence analyses of a short gag sequence [4-6]. If such an incident had happened in any of the Western countries the world would be up in arms to figure out what is behind the outbreak. The case of a dentist in the USA who apparently infected his patients with HIV-1 is a glaring example of this situation [7]. The ELISA

and Western blot analysis reported by Professor Perrin is simply not sufficient to address the issue.

The second question that would have occurred to us is to entertain the possibility of an intentional infection! We will discuss this below.

Mistake with dating the HIV-1

It is concluded by several investigators that the subtype CRF02-AG in question existed in Africa before and it was dated back to 1994-95. That is incorrect [6]. It is one thing to have a material evidence of one of the patients' blood from 1994-95 that was HIV-1 positive. It is entirely different to calculate such dates from the small fragments of HIV-1 sequences that were isolated several years later by mathematical formulas that are inadequate [4-6]. The dating of HIV-1 by utilizing Bayesian algorithm has been challenged and dates for retrovirus and especially HIV-1 by any currently existing methods based on Bayesian logic are incorrect [28-30]. This type of analysis has been tried for dating the Origin of HIV-1 [29]. Meyerhans et al [29] and many others have reported evidence of "massive" recombination in HIV-1 [9-23, 26]. The rate of recombination in HIV-1 is several orders of magnitudes higher than the rate of "point-substitution" (i.e. normal single base-pair mutation is one in a million). Ignoring recombination, they estimate, would lead to gross overestimation of the age of the HIV-1 by using the phylogenetic dating approach that has been used for Origin of HIV-1. Schierup et al [28] have gone much further than that, for they called the whole basis of phylogenetic dating for HIV-1 by Bayesian method into question [28-30]. In many investigators' opinion it is not valid to use a phylogenetic method to obtain the time estimate for HIV-1. They are further stating that such early recombination [10] would mean that phylogenetic dating theory is inherently flawed, and should not be used to date HIV-1 [28-30]. Therefore, the article that concluded that the OUTBREAK viral subtype existed in 1994-95 is of dubious value due to unsound scientific reasoning [6, 9-23].

What might have occurred and what makes scientific sense

It is time for us to face the scientific facts. From the scientific data described in the preceding sections it makes more sense to explore the possibility that the children were part of some sort of scientific experimentation. We have several unsolved issues that cannot be explained by the nosocomial hypothesis: i) unusually high rate of infection, ii) presence of HCV in 40% and HIV-1 in 100%, presence of four HCV genotypes but only one monophyletic HIV-1, iii) presence of unusual

HBV antibodies, iv) presence of monophyletic subtype [3-6], and v) presence of a unique HIV-1 subtype that is refusing to change its genetic makeup after housing in the bodies of close to 600 children after almost a decade [9-23].

Which hypothesis makes sense?

In the mid to late 1990s, there was a strong conviction amongst the top western scientists that an attenuated live HIV-1 vaccine could be used to prevent HIV-1 spread [reviewed in 25, 30-37]. For example, it was shown that if macaques were exposed to a nef deleted mutant SIV first and then infected with a wild type pathogenic SIV strain they were protected from the pathogenic strain of SIV-1. Similarly, Baba et al [31] have reported that an attenuated SIV, designated SIVdelta3 (a mutant of SIV deleted in the nef and Vpr genes), induced a lethal AIDS-like disease in two of the four macaque neonates infected orally, but the infection remained attenuated in the adult after intravenous infection [31, 33]. Deacon et al [32] reported that the six recipients of blood or blood products from a single HIV-1-infected donor had remained free of HIV-1-related disease after 10-14 years. This HIV-1 isolate from this donor was found to be defective at the nef gene, very similar to SIVdelta3 described above [34, 37]. Therefore, it would be logical to assume that the HIV-vaccinologists were looking for a place where they could carry out a secret attenuated delta nef HIV-1 vaccine trial. We are not implying that the same scientists were involved in any way but someone in the corporate world might have decided to do so.

So why Libya?

Between 1997 and 1999 Libya was one of the few countries that were free from HIV-1 infection. It was isolated from the Western world due to sanctions. It was ridiculed by the West. If a clinical trial was carried out in Sub-Saharan Africa the results would have been doubtful due to high infection rate in this part of the world.

Why children?

As mentioned above, in AIDS models the attenuated live mutant of SIV was protective in adults but not in neonates [33-38]. A triple mutant was constructed in 1997-98 that was shown to be safer in neonate macaques [33]. We strongly suspect that a HIV-1 equivalent of the delta nef attenuated mutant was used to experiment on these children. The underlying assumption would be that if the trial vaccine failed then the Western Media could then blame the incident on the craziness of the Libyan Leader, incompetency of

the infection control unit, and discredit the whole event. That is exactly what is happening now [reviewed in 3]. The Western media are full of comments from top vaccinologists and even Noble Laureates that are seeking justice in Libya. One cannot always believe what a Noble Laureate claims. Since, there is one Noble Laureate who claimed that HIV-1 is not the cause of AIDS [40]. However, it is amazing that no one from the other side is being allowed by the same top journals to speak on behalf of the Libyan children, 56 of whom already have died and the rest may also die from this illness. If it is the result of an experiment it needs urgent attention. Why are Western scientists refusing to even consider the possibility of looking into this matter? It has been almost a decade since the incident was first exposed. Why is it that after a decade the investigation into possible scientific misconduct has not been carried out?

Therefore, we argue, that a team might have been assembled in 1997-98 to try a new live attenuated vaccine in the part of the globe where this kind of human experimentation would go unnoticed. Certainly, the political climate, the total isolation of Libya and the tribal nature of Libyan people in Benghazi might have played important roles. Therefore, if the sinister plan was discovered it would be deniable. So we suggest that maybe what is happening, and that these eminent Italian, Swiss and French scientists may be just used as innocent pawns in this game most likely carried out by some corporate vaccine maker.

Possible Nature of Experimentation

We believe that children were divided into three groups; one as positive control, infected with the cloned pathogenic virus, a second group with the attenuated virus and third (the largest most likely > 200 children) were the real experimental vaccine group. If we look at all the live attenuated vaccine trials in macaques we will see the similar three group pattern. The so-called vaccine was most likely stored in human serum bottles (that may explain HCV and HBV positive infections and unusual antibodies to HBV). Group one received live pathogenic virus (the children that became sick in a few months and showed very high levels of viremias). The second group may exhibit relatively lower viremia or alternatively the attenuated virus may be more pathogenic than was expected. And the third initially received a nef deleted attenuated HIV-1. After six months or so, the same children (the 3rd group) were given a full length pathogenic virus to monitor the potential protective effect of the attenuated putative

vaccine. This largest group would have shown very high viremias if the attenuated vaccine failed. If we are correct then the experiment in reality is still in progress and these children are still experimental subjects as far as the trial goes.

Certainly this would be shocking, and therefore our fellow scientists and colleagues would not wish to believe that this might have happened! Yet, have they ruled out all the possibilities? The answer is no.

Just to give a few examples of the current affair of scientific experimentation. Where are the infamous circumcision trials being conducted? In the poor nations of Africa or Asia! Where was the original live attenuated polio vaccine trials carried out? In Africa! Where are the majority of the current anti-retroviral drug trials being carried out? In Africa or Asia! Why then would it be such a surprise to anyone that such a human experimental trial might have occurred in Libya?

What is the next step? How do we find the truth?

Very simple. If our assessment is correct then all we have to do is to isolate PBMCs (CD4+ T lymphocytes) and separate them into single cells and culture each single cell into separate wells and stimulate them with PHA. If there was an attenuated mutant used for experiments, we should be able to find it in a small fraction of cells (i.e. 1 in 1,000 CD4+) latently infected cells [reviewed in 39]. These latent cells should still carry any experimental attenuated HIV-1 that was used. As we know nef deletion does not often happen naturally, therefore, a full length sequencing of HIV from 300+ children would go far in addressing the issue. A full length sequencing of the stimulated CD4+ cells even from a small group of relatively healthy children with low viral load may prove to be very useful in solving the issue at hand.

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