

Risky Research

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Abstract

Research on corona viruses is strongly recommended and supported by granting agencies. However, this research can be extremely dangerous as shown by some published examples. The safety requirements need to be improved.

Keywords: Biosafety • Leaky labs • Gain of function mutants • New pandemic viruses • Influenza • Regulations

Introduction

This is a letter of concern. Colleagues from virology write and ask for help to get drugs tested in vivo which they designed in silico against CoV-2. They need proof of concept in cell culture or even animals. Numerous organizations announced programs with enormous amount of funds for research on corona virus. This sounds like a great chance for young investigators. Students are encouraged to apply for grants to study Corona viruses. A young researcher advertised for getting new students on youtube explaining his plans to study viruses in silico, construct mutants, characterize them and test them in vivo. The hope this young fellow even mentioned, was, to find "Gain of Function (GOF)" mutants, dangerous ones, to learn what makes them dangerous. Then one could develop preventions.

Safety Regulations

One can check the official safety regulations issued by WHO and the German Government. The WHO regulations for studying corona viruses were published on 13 May 2020 and prescribe Biosafety Level 3, BSL3, facilities, not the highest one BSL4. "Propagative work (for example virus culture or neutralization assays) should be conducted in a containment laboratory with inward airflow (BSL3). Handling of material with high concentrations of live virus.... should be performed only by properly trained and competent personnel" [1]. In Germany the Committee on Biological Working Compounds, (ABAS) for studying CoV-2, updated on 8.12. 2020 [1]. CoV-2 is classified under BSL3 The argument is, the virus is not very lethal, on average about 1% lethality, most of them with high age and often with vulnerability by other diseases, symptoms are similar to flue, the number of asymptomatic people is high and estimated to be 11 fold higher than the number of positive ones. On the cruise ship Diamond Princess with 3700 passengers, 410/712 infected passengers were asymptomatic. This is mentioned in the document, while the number of 6 deaths is not mentioned.

Furthermore, face masks are explicitly mentioned as available efficient protections. By scientific arguments, death rate, severity of the disease, epidemiology, and clinic, it was decided to classify these studies as risk group 3. The risk group 4 would apply to infectious agents with higher

mortality rate, such as lethality above 30% and would therefore apply to hemorrhagic fever such as Lassa or Junin, then Ebola, Marburg Disease. (MERS is BSL3) [1]. There exist about 50 BSL4 facilities around the world, 8 in the USA, 4 in Germany, 3 in Switzerland, one in China, whereas BSL3 facilities are in almost every University with Microbiology or Virology departments.

The CoV-2 virus, leading to a pandemic and lock downs around the world is not dangerous enough for the highest safety level, not according to the present law [1]. The parameters for classification of dangerous viruses may have to be reconsidered and possibly changed at international levels to include such dangerous pandemic viruses such as corona.

As a virologist and former Director of the Institute for medical Virology, Zurich, Switzerland, including viral diagnostics and potential bioterrorist threats during the World Economic Forum at Davos for several years, I remember a publication from the year 2004, mentioned below [2].

Case reports

A microbiology student, 27 years of age, studied in Singapore the West Nile Virus (WNV) and needed a BSL4 laboratory for a more highly virulent strain. As explicitly stated in the published report he got special permission at the Environmental Health Institute in Singapore, and was instructed for "20 minutes" by an experienced technician how to use this facility. His cultures got contaminated and she helped to set them up again, inoculated them with the virus and he came back only a few times. They concentrated the supernatant by centrifugation, which can be dangerous because of aerosols. The student fell sick, went briefly to the hospital, was dismissed and came back a week later with dry cough, so that a doctor correctly suspected a CoV infection and isolated him. He remembered the pandemic which was already over! All contact peoples were put into quarantine, about 200 of them, the student never traveled and did not go to China.

The search for the cause was difficult, and lead to the Vero cells in the laborator, which produced as expected WNV but high titers of Corona virus as well. In the Institute coronavirus was a research topic [2].

Here a quotation from the Singapore case in 2004 [2]. "The swift

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detection of this case illustrates the importance of continued global vigilance for SARS. This is especially needed in areas that were affected by SARS, and in all institutions that have retained virus isolates and/or diagnostic specimens from SARS patients. It also highlights the importance of strict adherence to appropriate laboratory biosafety procedures and practices for work with communicable agents, such as the SARS coronavirus." [2]. Critical commentaries came from an Australian biosafety expert Anthony Della Porta, the lab had "problems with improper air circulation, poorly located autoclaves and freezers, poor operating procedures, including training and record keeping" [3]. Klaus Stöhr, WHO virologist, who coordinated the SARS epidemic in 2003, pointed out: "We can make recommendations", but are not a "police organization" [3,4].

Consequently the rules in Zürich, Switzerland, for the BSL3 were changed immediately, and only two experienced coworkers were admitted from then on into the BSL3 area.

Two more accidents had happened in the Institute of Viral Disease Control at the Chinese CDC in Beijing, then China's leading SARS Lab. A 26 year old student and a 31 year old postdoc got independently infected there, suggesting a "systematic mistake or procedure", "a failure in applying guidelines" as commented by the WHO representative Robert Dietz in Beijing. 600 people were put into quarantine, the mother of the student died. WHO sent experts to help. A BSL3 was available, but the source of the initial infection was never found [5,6]. "The next SARS epidemic may be more likely to emerge from a research lab than from the presumed animal reservoir. We need surveillance and global control" (Julie Hall, WHO) [6].

Another case was reported in Taiwan. A "medical researcher raised alarms that the lab not the presumed animal reservoir may be the most likely source for reintroduction of the virus".

A 44 years old lieutenant had been screening antiviral drugs at the National Defense University in Taipei. Even though BSL4 conditions were used with gloves attached to the safety hood, corona virus - infected fluid waste material dropped inside, the gloves did not reach far enough, he therefore disinfected it by alcohol for ten minutes, opened the safety cabinet and then disposed the waste material in the autoclave. Hereby he must have contracted the infection. He traveled without knowing but survived [7]. This "should not happen" all lab workers should strictly follow the "safe procedures" said Hiroshi Oshitani, Head of SARS response team in the Manila regional office of WHO [7].

(Diagnostic virology laboratories routinely have quality control (QC) investigations for the safety of patients as e.g. in Zürich).

Recently, a "candidate human pandemic influenza virus" termed G4, was detected in pigs, where it was collected throughout 2011 to 2018 from about 30.000 pigs in slaughterhouses in 10 provinces in China. Of concern was that the swine workers show elevated seroprevalence for the new virus indicating that the virus had acquired increased human infectivity. This poses a serious problem to human health [8].

Gain of Function

Furthermore, GoF mutants are extremely dangerous. They have been produced with Influenza viruses by Kawaoka's and Fouchier's groups in 2012 [9,10]. The investigators wanted to answer scientific questions and learn how many and what kind of mutations would make a virus potentially pandemic. If this is known, one can prevent such risks was the notion. They tested infections from ferrets to ferrets as an animal model for human to human transmission. When they tried to publish the new laboratory variants, one paper got published the other one was rejected as too dangerous with potential for bioterrorism. This is designated as "Dual Use", meaning for both, research and warfare. The second paper got finally also published in a reduced form. Every molecular biologist would have known anyway how to make such GoF mutants.

Also, in 2015 a GoF coronavirus was engineered at Duke University in collaboration with colleagues from other countries including China. They used reverse genetics and infectious clone technology, passaged the engineered virus repeatedly, adapted it to grow on human lung cells, IT became dangerous, more pathogenic, potentially pandemic and was banned by the government authorities (US Funding Pause from 17.10.2014) and not allowed to be further studied in animal models in the USA [11,12]. The authors stated that further studies would have been needed. Do such GoF mutants warrant investigations versus the inherent risks involved?.

Recommendation

A pandemic virus as CoV-2 should be classified as dangerous enough to be classified under BSL4 conditions. For that additional criteria need to be included other than death rates as today. Missing are characteristics of the mode of transmission, such as aerosol or blood etc. The classification needs to include additional or other parameters than death rate.

Researchers and BSL3 and 4 facilities need to undergo training and quality control similar to what is a routine in every diagnostic laboratory. Coronavirus studies should not be granted by funding agencies without access to high containment laboratories and training including a certificate for "virology laboratory experience" (which exists for animal studies since a long time).

GoF cell culture studies and animal studies should be completely forbidden, no grants should be given, funding should be forbidden. Furthermore, publications including such data should not be possible. This is most likely a very effective measure, because scientists need publications as their record for follow up grant applications and funding of future research. Therefore this ban would be highly effective. Even restriction to BSL4 facilities is not safe enough as shown above. The risk is higher than the benefit.

Conclusion

How can one identify dangerous pandemic like viruses? That is very difficult, since the sequence alone would not allow that prediction. Viruses are detected when they already start to spread and have the features of pandemic like viruses. The potential risks should already be made public, even if an outbreak can be controlled. Only then immediate counter actions can be taken. A worldwide independent surveillance system is needed.

Conflict of Interest

We have no conflict of interests to disclose and the manuscript has been read and approved by all named authors. We have no conflict of interests to disclose and the manuscript has been read and approved by all named authors.

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