Appendix Two to Rebuttal of Health Feedback Review of Rosemary Frei and Patrick Corbett's July 2 *Off-Guardian* Article on Dr. Stoian Alexov's Bombshell Revelations

Rosemary Frei

Details supporting information in Error #4. The Health Feedback article includes a statement from ESP officials to try to show that we're wrong.

The statement includes this sentence: "Coronavirus images as observed by pathologists in human tissues may be seen in the articles by M. Ackerman et al. (NEJM 2020)[6], I. Colmenero et al. (Brit J Dermatol 2020)[7], V.G. Puelles et al. (NEJM 2020)[8] and Z. Varga et al. (Lancet 2020)[9], among others."

In this appendix, I show that none of those four papers cam be objectively shown to truly image the novel coronavirus.

Reference 6 - published July 9, 2020 in the *New England Journal of Medicine* – Ackermann M et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19.

The paper had 14 authors from Germany, Belgium, Switzerland, the UK and the US, but only examined seven lungs of people said to have succumbed to COVID-19. Also, the link to the disclosure form – i.e., the form in which the authors disclosed their conflicts of interest – is broken and my search for an unbroken link on Archive.org yielded nothing.

There are only two sentences in the whole paper relating to imaging the novel coronavirus, as follows: "Transmission electron microscopy of the Covid-19 endothelium showed ultrastructural damage to the endothelium, as well as the presence of intracellular SARS-CoV-2 (Figure 3D). The virus could also be identified in the extracellular space." The description of Figure 3D is as follows: "Panel D is a transmission electron micrograph showing ultrastructural features of endothelial cell destruction and SARS-CoV-2 visible within the cell membrane (arrowheads) (the scale bar corresponds to 5 μm). RC denotes red cell."

However, there is no information in the methods section of paper about how the authors went about verifying that these were truly images of the novel coronavirus.

Furthermore, the link to an appendix with details of the methods is broken. Fortunately, there is one functioning link to the methods appendix on Archive.org. Page 5 of that appendix describes the electron microscopy technique. That description mentions the use of four stains and/or contrast agents: osmium tetroxide, tolouidine blue [sic – it's toluidine blue], uranyl acetate and lead citrate.

However, none of those chemicals/techniques can verify the presence of viruses, never mind particular viruses such as the novel coronavirus. (For example, this overview of contrast agents in electron microscopy discusses uranyl acetate and lead citrate, but it doesn't mention viruses at all.)

Therefore, from the available information about this paper, the images could be of a SARS virus, another coronavirus or something else entirely. And, as I noted in the section in the main article

on Error #4, Dr. Moch himself has said it's controversial whether the images in scientific papers said to be of the novel coronavirus actually are showing the novel coronavirus.

Reference 7 – published in the *British Journal of Dermatology* on June 20, 2020 -- Colmenero I et al. SARS-CoV-2 endothelial infection causes COVID-19 chilbains: histopathological, immunohistochemical and ultraestructural [sic] study of 7 paediatric cases.

The full paper is here.

The authors claim to have found the novel coronavirus in the foot tissue of seven children living in Madrid -- six of who had been PCR tested for the virus and were negative -- whose feet or toes had "skin lesions clinically diagnosed as COVID-19 related chilblains" (AKA 'COVID toes' – i.e., toes that are red, itchy, blistered and/or inflamed).

The only sentences relating to how the authors identified the novel coronavirus in the foot tissue are: "We used a monoclonal antibody (1A9, dilution 1:200, GeneTex Inc., Irvine, CA, USA) against the spike protein of SARS-CoV / SARS-CoV-2." And "The antibody was previously optimized using sections of COVID-19 lungs from autopsies as positive controls and different inflammatory skin conditions as negative controls."

First, as I've noted throughout my article, monoclonal antibodies for the spike protein do not appear to be specific to SARS CoV-2 -- and they likely aren't even specific to the family of SARS viruses.

(Note also that there is no independent verification being done of the contents and specificity of the anti-novel-coronavirus antibodies that are being commerically produced, including the antibody used in this study. And as I also note in the main text of this article, the antibody undoubtedly didn't undergo rigorous verification including proving that it's specific to SARS-CoV-2.)

Second, the assertion that they used lungs from people who died of COVID-19 as positive controls is meaningless. That's because they don't provide any information that would allow objective assessment of whether those lungs in fact harboured the novel coronavirus.

Third they said that, using the GeneTex monoclonal antibody, they made the following observations: "Cytoplasmic granular positivity for SARS-CoV-2 spike protein was mainly demonstrated in endothelial cells of the capillary and post-capillary venules of the upper dermis and also in epithelial cells of the secretory portion of eccrine units in all cases (Figure 4)."

Yet in the absence of independent verification that the the antibody used to detect the novel-coronavirus spike protein is specific to the novel coronavirus of these immunohistochemistry results, it's impossible to know how to interpret this result.

Fourth, using electron microscopy in tthe toe tissues of only one of the patients, they said they found "the presence of round membrane-bound structures within the cytoplasm of endothelial cells showing an electro-lucent centre, and surrounded by tiny spikes, giving them a halo-like appearance. Their mean diameter was 92.26 nm (80.76-109.76 nm), and the mean thickness of the spikes was 13.18 nm (12.36-13.88 nm). Based on previous descriptions in the literature, these

structures were interpreted as coronavirus-like particles 8-14 (Figure 4). Tubulo-reticular inclusions (TRI) were also found within the endothelial cells, similarly to other descriptions of SARS-CoV-2 and SARS-CoV infections 8,15."

So they're saying that they found round structures surrounded by spikes that fit with other papers' descriptions of 'coronavirus-like particles.' They're also asserting that there were 'tubulo-reticular inclusions' in the endothelial cells and that these fit others' descriptions of SARS and the novel coronavirus.

But they made no mention of how they independently verified this is the novel coronavirus. And see quotes from Dr. Zsuzsanna Varga in the section on Reference 9, below, on how electron microscopy doesn't reliably show whether viral particles are present.

Note that the paper also lacks any analysis of what other pathogens or other factors could have caused the foot condition in these young people.

Therefore I don't find any of this to convincingly show that any of these children had a novel-coronavirus infection and that it caused their foot condition.

Reference 8 - A 23-author letter published May 13, 2020, in the *New England Journal of Medicine* – Puelles VG et al. Multiorgan and Renal Tropism of SARS-CoV-2.

The paper's appendix is here.

Note that at 10:45 in Dr. Varga's presentation during the June 25 ESP webinar she cites this paper as supporting quantitative PCR being "highly sensitive" for detecting the novel coronavirus in autopsy tissue.

But she makes the same (deliberate?) mistake as public-health officials: she indicates at 10:45 in the video that this high sensitivity refers to the very small quantity of RNA that PCR can amplify.

But, in fact, by definition sensitivity refers to the rate of true positives that a test produces -- and a**s Dr. Varga herself says, RT-PCR yields both false negatives (i.e., a result that's negative when in fact the person being tested is positive) and false positives (i.e., a result that's positive when it should be negative). (And independent journalists have demonstrated that PCR is highly inaccurate.)**

Dr. Varga also notes at 12:27 in the video of her ESP-webinar presentation, **with respect to the the in-situ hybridization methods used in the paper (see descriptions of these methods below),** that "**in our institution we did not have that much luck; we['ve] had until now no positivity to detect any virus in the specimens that we made."**

And she said at 13:19 in the video, with respect to the immunochemistry methods used in the paper – which depend for their accuracy/specificity (highly accurate/specific tests have low rates of false positives) on the presence of monoclonal antibodies specific to the novel coronavirus -- that "**The problem that I see at the moment is that many pathology institutions face unspecific background stain and unspecific stains.... We tried several clones [i.e., monoclonal antibodies] to get such nice and reliable signals [as they showed in this paper, but] we are at the moment not at that step where we can say we have a good antibody and we have reliable signals."**

(And by the way, while the authors listed 10 of the patients as having pre-existing kidney disease they said urinalysis results were unavailable for 24 of the patients. They also did not provide creatinine levels for any of the patients, another key test of kidney disease. The paper also did not mention the patients' ages either.)

They used the finicky techniques of in-situ hybridization and indirect immunofluorescence to produce images they claim showed the presence of novel-coronavirus RNA and protein, respectively, in kidney tissue in people who tested positive for COVID-19. (In-situ hybridization for RNA viruses involves taking a short strand of messenger RNA or DNA that's complementary to part of the virus's RNA sequence [called a 'probe'] and that also is 'labelled' with [i.e., attached to] a radioactive or fluorescent molecule. Then slides with autopsy or biopsy tissue on it are coated with that labelled complementary RNA or DNA. It's assumed that wherever radioactivity or fluorescence then is detected on the slide that's where the virus's RNA is present.)(Immunofluorescence is somewhat similar – it uses an antibody for the virus that is labelled with a molecule that fluoresces.)

But I wouldn't consider images showing bits of novel-coronavirus RNA or protein to be the 'coronavirus images' that Dr. Moch and his ESP-leadership colleagues claim that this and the other three papers in this appendix show.

And also, for the in-situ hybridization the authors of the paper used a probe said to be for the genetic sequence of the novel coronavirus's spike protein. But as I've said several times in this appendix and Appendix One, the spike protein is not specific for the novel coronavirus.

And for the immunofluorescence they used two antibodies. One antibody is specific for a component of the SARS spike protein – and it says right on the company's web page that sells that antibody that it reacts to the 'human coronavirus' – in other words, it isn't specific for the novel coronavirus. And the PDF with the details of the other antibody says, "This antibody detects both SARS-CoV spike and SARS-CoV-2 spike proteins [S2 subunit]. Our internal testing indicates no cross-reactivity with MERS-CoV spike protein."

So these antibodies aren't specific to the novel coronavirus. And anyway, how much do you have trust the second antibody manufacturer's 'internal testing'?

Reference 9 – a short letter/correspondence by Dr. Varga, Dr. Moch and eight others published May 2, 2020, in *The Lancet* – Varga Z et al. Endothelial cell infection and endotheliitis in COVID-19.
They report pathology findings from three people. In only one of the deceased do the authors claim to have detected the novel coronavirus – they report that electron microscopic imaging of a kidney that had been transplanted into the 71-year-old "revealed viral inclusion structures in the endothelial cells."

They also display images of "electron microscopy of kidney tissue" (while they don't say whether that kidney tissue is from the 71-year-old patient, I assume it is) showing "aggregates of viral particles," a "peritubular space consistent with capillary containing viral particles" and "a viral particle."

But they give no information or citations to prove that those 'viral particles' are the novel coronavirus.

And as I noted in the section on Error #4 in the main article, Dr. Moch himself has said there's

controversy about whether such images truly show viral particles.

Also, Dr. Varga said at 16:34 in her presentation in the June 25 ESP webinar that the use of electron microscopy to detect the virus is:

"demanding, time consuming, and searching for virus takes sometimes several hours. **It also needs good expertise, not just [?][in] ultrastructure [**which as she shows in her accompanying PPT presentation can mimic viruses**] -- most cases are autolytic [i.e., the tissues and structures have eroded because of the degradation of tissues that begins right at death], have fixation artefacts, we have to deal with these fixation artefacts, also with the fact that due to these artefacts sometimes there are no viral particles at all in the cases. We have to avoid interpretation errors. And especially we have to recognize mimics in ultrastructure: vesicles can mimic viral particles, so we have to be sure what we are seeing. And it is not always easy to see[k ?] in our autopsy tissue."**

She concludes at 17:59 that: "*These [viral] particles that were described in different papers have similarities, but also dissimilarities. They vary in the size, also in the form. So to say definitely which are specific for a SARS-CoV-2 infection, and which particles are just seen within this infection, needs further studies, and also immune electron microscopy on the same [tissue] section[s is needed to verify the presence of the virus].*

So overall, Dr. Varga, Dr. Moch and the other authors provide very slim support for what they say is evidence that the novel coronavirus is present in the autopsy tissue.

Despite this, they suggest that the virus helps induce endotheliitis in several organs and an overall inflammatory response, and thus leads to the overall clinical sequelae of infection seen with this virus.

They conclude that their findings provide a rationale for tackling these effects with "anti-inflammatory anti-cytokine drugs, ACE [angiotensin-converting enzyme] inhibitors and statins."

Three of the authors (Drs. Andreas Flammer, Mandeep Mehra and Frank Ruschitzka) disclose at the end of the letter that they've been paid to do work for a range of companies such as AstraZeneca, Bayer, Novartis, Pfizer and Roche.