

Mental Disorders Are Persistent Infections Caused by an Influenza Virus

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Abstract: Neurasthenia, neurosis, depression and the like are persistent infections caused by a low-pathogenic influenza virus. Since this virus was first isolated in August 2016 up to the present (June 2017), it has consistently been isolated from myself and from others with the same symptoms, while it cannot be isolated from healthy controls. The isolation frequency is once every 10 to 20 days, and at the time of isolation there must be no obvious cold symptoms such as fever. The isolation method and characteristics of this influenza virus differ greatly from those of influenza A and B viruses; it is most likely an influenza C virus, although the possibility that it is an entirely new influenza virus that has never been isolated before cannot be ruled out.

This virus may have been with us throughout human history, and it will not simply disappear because politicians and scientists choose to ignore it. The right thing to do is to face it head-on and unite the world's efforts to defeat it.

Keywords: Neurosis; Depression; Influenza virus; Foreign-body sensation in the throat; Infection; Chest pain; Back pain

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0 Introduction

A series of disorders dominated by insomnia and sleep disturbance and accompanied by pain symptoms—neurasthenia, neurosis, depression and the like—are persistent infections caused by a low-pathogenic influenza virus. Since I first isolated this virus from my own body in August 2016 up to now (October 2019), I have consistently been able to isolate it from myself and from others with the same symptoms, whereas it cannot be isolated from healthy controls. Before June 2017, the isolation frequency was once every 10 to 20 days, and at the time of isolation there could be no obvious cold symptoms such as fever. The isolation method and characteristics of this influenza virus differ greatly from those of influenza A and B viruses; it is most likely an influenza C virus, although the possibility that it is an entirely new virus that has never been isolated before cannot be ruled out. I will describe the isolation and identification methods below.

First, let me explain why I set out to find the cause of diseases that mainstream medicine has classified as mental disorders. I fell ill at the end of 1999. At the time I was treated all along for neurasthenia, with no effect at all; because of the unbearable pain all over my body and the insomnia, I thought of suicide every day. It was not until May 2000, when one dose of a Chinese herbal decoction for treating typhoid fever greatly improved my condition, that I was inspired to realize that this is an infectious disease. However, because the composition of Chinese herbal medicine is complex, I could not determine which type of infection it was, and for more than ten years I kept trying to isolate the pathogen through experiments. It was not until August 2016, when I isolated a special influenza virus, that I understood that the pain symptoms of neurasthenia, neurosis, depression and the like are in fact also the symptoms of influenza. Mainstream medicine has long regarded neurasthenia, neurosis, depression and the like as mental disorders, artificially downplaying the pain symptoms and emphasizing psychological factors in papers and in diagnosis, leading the true cause of these diseases astray. The pain symptoms of neurosis, depression and the like are headache, chest pain, back pain, sore throat, and a dry throat or a constant foreign-body sensation; in the early stage or at the most severe stage of the disease, blood tests show hypokalemia, an accelerated erythrocyte sedimentation rate, elevated myocardial enzymes, and low-grade fever—all of which are in fact also symptoms of influenza, although the pain symptoms vary slightly from person to person because of differences in constitution. In severe neurosis and depression, blood tests in the early stage of the disease show a slightly accelerated erythrocyte sedimentation rate, abnormal white blood cells, low-grade fever and other findings that clearly indicate infection, but mainstream medicine ignores these as well. In fact, as early as the 1950s, scientists abroad suggested that depression might be related to influenza^[1] ^[2], but no one has ever isolated the virus. The reason is that the isolation of this influenza virus is somewhat different from that of previous influenza viruses, and moreover, at that time influenza viruses were isolated using chicken embryos. Once this influenza virus becomes a chicken-embryo-adapted strain, it loses the ability to agglutinate red blood cells, and once a chicken-embryo-adapted strain infects a person, it does not recover the ability to agglutinate red blood cells for a very long time.

1 Virus Isolation Method, Gene Sequencing and Electron Microscopy

1. Virus Isolation Method

Laboratory equipment: constant-temperature incubator, microscope, 1 ml disposable syringes, centrifuge, centrifuge tubes, 0.22 μ m hydrophilic syringe filters, 2% levofloxacin injection, fertilized eggs, refrigerator, egg candler, cotton swabs, 10 ml colorless glass bottles, autoclave, 1% formalin, V-bottom 96-well microplates, glass test tubes, awl, pencil, tweezers, surgical scissors, mini incubator, iodine tincture, mineral oil, sprayer, micropipette, candles, adhesive tape, peristaltic pump, chromatography column, Sepharose 4FF agarose gel microspheres, a UV detector, and a 50,000-molecular-weight-cutoff ultrafiltration membrane cassette.

Below I describe the isolation method for this virus, and I also hope to share my findings so that more laboratories and scientists can verify them. Because the symptoms of this disease are not exactly the same in every patient, sampled patients should be selected whose pain symptoms match those described above as closely as possible; in particular, since the sampling site is the throat, it is best for the sampled patient to have a foreign-body sensation in the throat. I also used chicken embryos for virus isolation; ordinary commercial chicken embryos are sufficient and work the same as SPF chicken embryos. The isolation was divided into three groups: the experimental group, the negative control group, and the blank control group. The experimental group was inoculated with samples from people with psychiatric symptoms, the negative control group was inoculated with samples from people without psychiatric symptoms, and the blank control group was inoculated with 2% levofloxacin injection without any sample added.

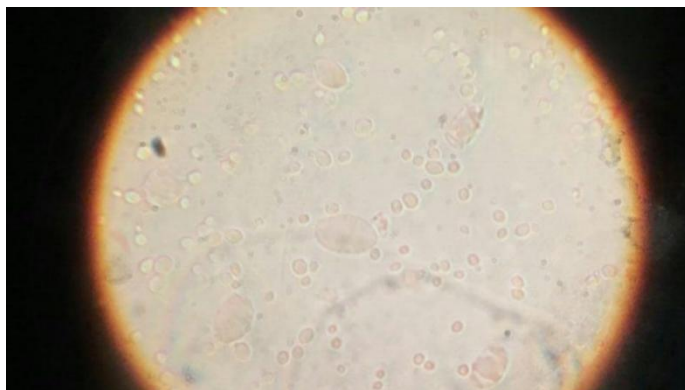
1. Place the required fertilized eggs in a mini incubator or a constant-temperature incubator at 38 ° C and 60% humidity; if using a constant-temperature incubator, place a bowl of water inside it. Turn the eggs at least 3 times a day and incubate until day 8.
2. Sterilize cotton swabs, 10 ml colorless glass bottles, 96-well microplates, glass test tubes, awls, tweezers, and surgical scissors in an autoclave for later use, and spray the workroom with 1% formalin for disinfection one day in advance.
3. Select well-developed 8-day-old chicken embryos for use. Collect samples from the throat with cotton swabs; if vomiting occurs during sampling, wait a few hours before sampling again. Place the collected sample into 2% levofloxacin injection, swirl it a few times, and then place it in the refrigerator compartment to act for 30 minutes. The sample liquid may be centrifuged to take the supernatant, or it may be used directly. Label the sample bottles as the positive group and the negative control group.
4. For the first isolation, the virus must be inoculated into the amniotic cavity of chicken embryos; all subsequent passages are inoculated into the allantoic cavity.

Chicken embryos incubated for about 8 days are the most convenient for amniotic cavity inoculation. Disinfect the eggshell with iodine tincture first, open a small hole over the air cell with tweezers, place a drop of heat-sterilized and cooled mineral oil on the egg membrane, and under the egg candler inject about 0.3 ml of sample liquid into the amniotic cavity toward the embryo with a 1 ml disposable syringe. Then seal the egg with adhesive tape. From passage 2 onward, inoculate the allantoic cavity: at the air-cell end, drill a small hole with an awl in the shell behind the embryo; dilute the viral allantoic fluid 100-fold with 2% levofloxacin injection; inject 0.2 ml into the allantoic cavity with a 1 ml disposable syringe; and seal the small hole with candle wax melted over an alcohol lamp. Mark the positive group, the negative control group, and the inoculation time on the eggshell with a pencil.

5. After inoculation, incubate the eggs upright without turning. The optimal incubation temperature is 32 to 33 °C. The incubation time is 48 to 72 hours; candle the eggs 3 times a day and remove dead embryos. After 72 hours, take out the embryos and place them in the refrigerator compartment for more than 4 hours before harvesting the allantoic fluid. The allantoic fluid of the first three passages can agglutinate chicken, guinea pig, and human type O red blood cells, with a titer of 3 wells measured on a 96-well microplate. Because I had not expected to isolate a virus at that time, the passage-1 seed virus was not preserved, and passages 2 and 3 were combined. Using allantoic fluid diluted into a 4-unit working antigen, the antibody titer of my own previously preserved serum was measured as 3 wells, while no antibody was found in chicken serum. After influenza virus infection, an antibody titer of 8 wells is needed to clear the virus from the body; 3 wells is nearly one hundred times lower than 8 wells, which may be the reason for the persistent infection.
6. After passage 3, the virus lost its hemagglutinating ability, but the allantoic fluid could still darken the color of chicken red blood cells. From passage 2 to 15 there was essentially no embryo mortality. The phenomenon of this influenza virus losing its red-cell-agglutinating ability during chicken-embryo passage is similar to what happens when influenza A and B viruses are isolated and passaged in mammalian cells such as Vero cells: they also lose hemagglutinating activity around the third passage, and after several blind passages they recover the ability and become Vero-cell-adapted strains. This phenomenon indicates that the original strains of influenza A and B viruses are highly adapted to avian cells, whereas the strain I isolated, like influenza C virus, is highly adapted to human or mammalian cells; passage in poorly adapted chicken embryos also causes it to lose its red-cell-agglutinating ability, and adapting it to chicken embryos requires 406 passages[3][4][5][6][7]
7. From passage 3 onward, after the virus lost its red-cell-agglutinating ability, the allantoic fluid remained relatively clear, and its ability to darken red blood cells waxed and waned. Around passage 15 of blind passage there was a peak of embryo deaths, with a mortality rate of over 70%, the deaths being concentrated

at about 36 hours after inoculation. At this point the allantoic fluid began to turn turbid; at first this was thought to be bacterial contamination, but microscopic examination revealed large numbers of apoptotic allantoic membrane cells. The cells were irregularly round, with the cell membrane closely apposed to the nucleus, about 1/10 the diameter of a chicken red blood cell but clearly larger than bacteria; large numbers of apoptotic allantoic membrane cells could be seen at 400x magnification. Their sedimentation rate was about half that of red blood cells, though they were clearly larger than bacteria. At this stage the allantoic fluid can be drawn directly with a syringe without refrigeration; for embryos refrigerated for more than 4 hours, the fluid should be gently pipetted a few times before drawing, because the apoptotic cells sediment relatively quickly and after 4 hours they settle to the bottom, leaving the surface allantoic fluid very clear.

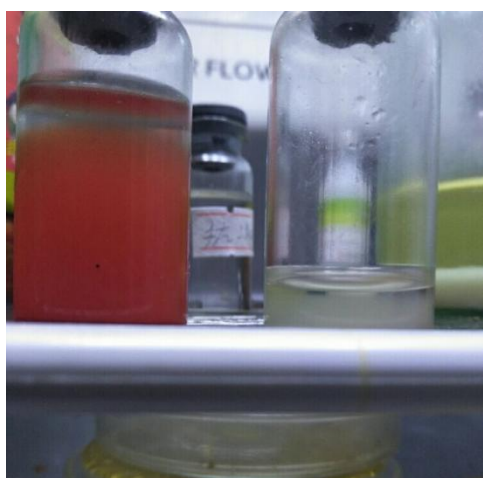
8. At this stage, the allantoic fluid can also accelerate red-cell hemolysis at about 33-37 °C, with hemolysis occurring several times faster than in the blank control: hemolysis generally occurs at about 10 hours, whereas the blank control takes more than 2 to 3 days. This hemolytic ability also varies from passage to passage.
9. Please consult the relevant literature on your own for the hemagglutination test and the hemagglutination inhibition test.
10. The seed virus was centrifuged at 5,000 rpm and the supernatant taken, filtered through a 0.22 um hydrophilic syringe filter, and treated with various antibiotics to suppress bacteria; none of these treatments affected successful inoculation.
11. After each experiment, the workroom was sprayed with 1% formalin for disinfection, and the utensils were autoclaved.



The figure above shows apoptotic allantoic membrane cells at 400x magnification; the largest cells are chicken red blood cells.



The figure above shows the reactions of positive allantoic fluid of different passages with chicken red blood cells. 33 ° C, 1 day: upper left, red blood cells darkened in color; upper right, slight hemolysis of red blood cells; lower left, negative control; lower right, red blood cells darkened in color.



The figure above shows a comparison of the sedimentation rates of apoptotic cells in passage-6 allantoic fluid and chicken red blood cells over a period of 1 hour.

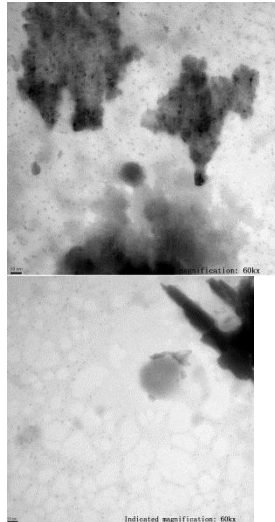
2. Gene Sequencing and Electron Microscopy

For various reasons, I was unable to carry out further experiments for a long time. It was not until 2021 that, thanks to an introduction by Ms. Yu of Tanpu Biotech and the help of Teacher Wu of Jiangxi Agricultural University, the samples were sent to BGI for gene sequencing; however, no viral genes were detected. Every aspect of my experiments indicated that a virus should be present, so I then used samples prepared from the same batch of seed virus to contact several electron microscopy facilities; fortunately, Central South University agreed to accept them, and the electron microscopy results confirmed the presence of large numbers of viruses in the samples.

1. Sample collection for gene sequencing was the same as in Section 1, Virus Isolation Method
2. Because gene sequencing did not detect the target virus, I immediately passaged the same seed virus again and collected the allantoic fluid. The allantoic fluid was first concentrated more than tenfold through a 50,000-molecular-weight-cutoff ultrafiltration membrane cassette and centrifuged to take the supernatant; the supernatant was purified with Sepharose 4FF agarose gel microspheres, and the first protein fraction detected by the UV monitor was collected as the electron microscopy sample.

Electron microscopy showed large numbers of viruses in the sample.

The viruses are round, with an average diameter of 60–70 nm; the vast majority have smooth surfaces and aggregate into clusters, while a few have incomplete spike structures on the surface and are often scattered. Because the virus has lost its ability to agglutinate red blood cells—that is, it may have lost the spike structure, the HEF protein—its diameter is 20–30 nm smaller.



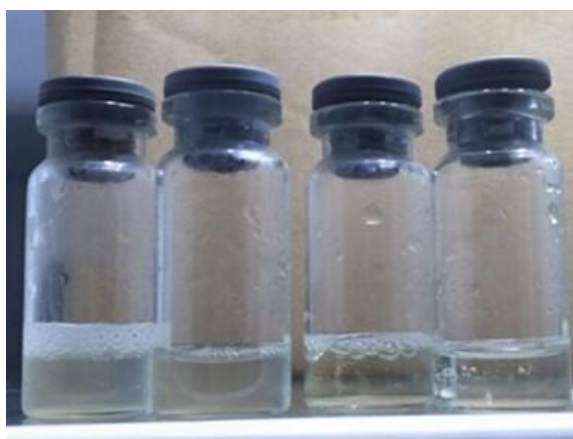
2 Analysis of the Virus

From around passage 15, while continuing the passages I also collected new samples in an attempt to isolate a virus with red-cell-agglutinating ability, and I found that the virus in my own body had unexpectedly also become a virus without red-cell-agglutinating ability. This may be because, after the virus passaged in chicken-embryo allantoic fluid lost its agglutinating ability, I assumed that I had not isolated any virus and became lax about disinfection during handling, so that this mutated influenza virus without agglutinating ability infected me and replaced the original agglutination-capable virus. From then on, all the viruses I isolated—whether from myself or from other patients, even from people thousands of kilometers away—were influenza viruses without red-cell-agglutinating ability. This may be why, after someone proposed in the 1950s that depression might be related to influenza, the virus was never isolated: at that time influenza viruses were all isolated by the chicken-embryo method, but this influenza virus differs from influenza A and B viruses in that it mutates during isolation and culture in chicken embryos, and it takes a very long time for the virus to return to its original agglutination-capable state. The reference [Influenza C Virus] mentions that 406 passages are needed to restore the hexagonal reticular structure. Traditional influenza virus isolation involves only 3 passages, with hemagglutination appearing in the allantoic fluid as the criterion. Using the previous methods for isolating and identifying influenza viruses to isolate this influenza virus would yield a negative result.

I passaged the virus up to passage 70, and the allantoic fluid never regained the ability to agglutinate red blood cells; however, before passage 30 a few passages occasionally showed semi-agglutination when the proportion of allantoic fluid was increased. After passage 30, the

ability to induce apoptosis generally weakened; the strongest apoptosis-inducing ability occurred at passages 15 to 30. Because all my later re-sampling isolations were passaged only 3 to 5 times, with a 100% positive rate, but each isolation differed—apoptosis could be seen within 5 passages in most cases, while a few required more passages—it is best to identify a positive result using the three criteria together: cell apoptosis, darkening of red blood cell color, and accelerated hemolysis. In synchronized control inoculations of chicken embryos with samples from healthy people, all three criteria were negative.

During the year and a half of searching for cases to isolate, I found that the later the isolation, the more passages were needed for the apoptosis phenomenon to appear: at the beginning, apoptosis appeared at passages 2 to 3. The isolation results at the end of 2017 were: the passage-1 allantoic fluid was clear; from passage 2 the allantoic fluid became slightly turbid, but apoptotic cells were hard to find under the microscope and only a small amount of cell debris was visible; at passage 5, large numbers of apoptotic cells appeared again. The isolation results of March 2018 showed no large numbers of apoptotic cells even at passage 5, with slightly turbid allantoic fluid from passage 2 onward. This indicates that the virus is slowly mutating back toward its original state.



The figure above shows the isolation results for the newly added cases in March 2018; both the positive group and the negative control group are at passage 5. The two tubes on the left are the positive group, with turbid allantoic fluid, and the two on the right are the negative control group, with clear allantoic fluid.

For the 5 cases added in October 2019, all the inoculated chicken embryos died within 36 hours; the allantoic fluid had no red-cell-agglutinating ability but could discolor chicken red blood cells, and no passaging was performed. Judging from the high embryo mortality, the virus is getting closer and closer to recovering its red-cell-agglutinating ability.

3 Basic Information of the 16 Cases

Although this disease is very common, so far only 16 patients have volunteered to provide samples. The virus was isolated from all 16 cases, giving a current verification rate of 100%.

The table below shows the basic information of the cases.

No.	Gender	Address	Symptoms	Time of Onset	Result
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1	Male	Jingzhou County, Huaihua, Hunan	Insomnia, sleep disturbance, headache, back pain, chest pain, foreign-body sensation in the throat, dry throat.	Onset in 1999, ongoing.	Positive
2	Female	Huai'an City, Jiangsu	Insomnia, sleep disturbance, headache, back pain, chest pain, foreign-body sensation in the throat, dry throat.	Onset in 2007, ongoing.	Positive
3	Male	Jingzhou County, Huaihua, Hunan	Insomnia, sleep disturbance, tinnitus; no body pain symptoms.	Onset in April 2017, ongoing.	Positive
4	Female	Outuan Township, Jingzhou County, Huaihua, Hunan	Insomnia, sleep disturbance, headache, back pain, chest pain, foreign-body sensation in the throat, dry throat.	Onset in 2010, ongoing.	Positive
5	Female	Jingzhou County, Huaihua, Hunan	Insomnia, sleep disturbance, headache, back pain, chest pain, foreign-body sensation in the throat, dry throat. The symptoms are now very mild.	Now 41 years old; onset in her teens; can no longer recall the time of onset.	Positive
6	Female	Outuan Township, Jingzhou County, Huaihua, Hunan	Insomnia, sleep disturbance, headache, back pain, dry throat.	Now 49 years old; onset in her twenties; can no longer recall the time of onset.	Positive
7	Female	Jingzhou County, Huaihua, Hunan	Insomnia, sleep disturbance, dry throat; no body pain symptoms.	Onset in 2000, ongoing.	Positive
8	Female	Jingzhou County, Huaihua, Hunan	Insomnia, sleep disturbance, headache, chest pain, foreign-body sensation in the throat, dry throat; occasional joint pain.	Onset in 2014, ongoing.	Positive
9	Female	Jingzhou County, Huaihua, Hunan	Insomnia, sleep disturbance, headache, dry throat.	Onset in 2017, ongoing.	Positive

10	Female	Jingzhou County, Huaihua, Hunan	Insomnia, sleep disturbance, headache, back pain, chest pain, foreign-body sensation in the throat, dry throat.	Now 70 years old; the elderly patient can no longer recall the time of onset.	Positive
11	Male	Jingzhou County, Huaihua, Hunan	Reports no insomnia, sleep disturbance, or body pain, but often has headache, edema, early awakening, high uric acid with gout, chronic pharyngitis, and pneumonia, and was once diagnosed with pulmonary heart disease. Cerebral infarction; CT showed brain atrophy.	Too long ago, with no typical identifiable symptoms; the time of onset is difficult to determine.	Positive
12	Male	Jingzhou County, Huaihua, Hunan	Very occasional insomnia; very mild sleep disturbance; cannot recall the starting year and month; no other body pain; has gout and high uric acid.	Sleep disturbance is very mild; cannot recall the starting year and month.	Positive
13	Male	Jingzhou County, Huaihua, Hunan	Insomnia, dry throat; no other body pain.	Six months ago.	Positive
14	Female	Jingzhou County, Huaihua, Hunan	Very mild insomnia; does not consider it insomnia, believing that she sometimes simply thinks too much to fall asleep; no other body pain; type 2 diabetes.	Insomnia is very mild; cannot recall the starting year and month.	Positive
15	Male	Jingzhou County, Huaihua, Hunan	Early awakening, cerebral infarction, hemiplegia.	Cannot recall when the early-awakening symptom began.	Positive
16	Male	Jingzhou County, Huaihua, Hunan	Insomnia, dry throat, sore throat, headache, back pain, chest pain, cerebral infarction, myocardial infarction, high uric acid with gout, type 2 diabetes.	Onset in 2013.	Positive

Acute influenza virus infection can cause not only inflammation of the respiratory tract but also encephalitis, enteritis, hepatobiliary inflammation, and inflammation of the heart, spleen, kidneys, and pancreas. Therefore, for cases after 2018, I paid more attention to people with

diabetes, gout, gastroenteritis, kidney problems, cardiovascular and cerebrovascular thrombosis, and hypertension.

Judging from the above cases, insomnia and sleep disturbance are the primary symptoms. The severity of the primary symptom is not proportional to the overall severity of the disease: some patients with diabetes, gout, or cardiovascular and cerebrovascular thrombosis have only very mild insomnia and sleep disturbance. Moreover, in general, the proportion of persistent infection increases with age.

It is worth mentioning that there were 5 special negative cases: 3 were cases of systemic lupus erythematosus and rheumatoid arthritis, and 2 were recovered cases that had previously had insomnia and pain symptoms. When I first isolated this virus, I wondered whether systemic lupus erythematosus and rheumatoid arthritis might also be caused by this virus; experiments proved that they are not related to this virus, and 5 blind passages all gave negative results. The details of the 5 cases are shown in the table below:

No.	Gender	Address	Symptoms	Result
1	Female	Huitong County, Huaihua City, Hunan	Skin erythema; diagnosed by a hospital as systemic lupus erythematosus. No psychiatric symptoms such as insomnia.	Negative
2	Female	Huitong County, Huaihua City, Hunan	Skin erythema; diagnosed by a hospital as systemic lupus erythematosus. No psychiatric symptoms such as insomnia.	Negative
3	Female	Jingzhou County, Huaihua, Hunan	Joint pain and deformity; typical rheumatoid arthritis. No psychiatric symptoms such as insomnia.	Negative
4	Female	Jingzhou County, Huaihua, Hunan	Previously had insomnia, sleep disturbance, headache, back pain, chest pain, foreign-body sensation in the throat, dry throat. Course of disease about 30 years; all symptoms disappeared 5 years ago.	Negative
5	Female	Jingzhou County, Huaihua, Hunan	Previously had insomnia, sleep disturbance, headache, back pain, chest pain, dry throat. Relapsing course; all symptoms disappeared six months ago.	Negative

Positive cases are easy to confirm — obvious insomnia and sleep disturbance are sufficient. I dared to include cases with only mild sleep disturbance because I have been ill for a long time and can tell the difference; for verification, please select obvious cases. Negative cases, on the other hand, should be people with absolutely no insomnia or sleep disturbance, preferably those aged 20 to 30.

4 Conclusion

A series of diseases once thought to be caused by neurological or psychiatric factors—neurasthenia, neurosis, depression and the like—are in fact merely persistent infections caused by a specific influenza virus. Depression is also called a "cold of the soul"; from this one can see how similar depression is to the common cold—depression and the like are in fact persistent infections caused by an influenza virus. As is well known, some orthomyxoviruses and paramyxoviruses can cause psychiatric symptoms in animals when they infect them. In recent decades, when scientists have tried to isolate pathogens from patients with these diseases, they have generally used blood and cerebrospinal fluid as samples; during infection with a low-pathogenic influenza virus, the virus is almost never present in blood or cerebrospinal fluid, so naturally no pathogen can be isolated. Because of my limited funds, my lack of formal research credentials, and my inability to transport samples, and because my appeals to the government went unanswered, I could not conduct more comprehensive experiments. This virus is most likely an influenza C virus, but the possibility that it is another influenza virus that has never been isolated before, or another kind of virus altogether, cannot be ruled out. If you conduct verification experiments, please use mammalian cell culture as much as possible; if you must use chicken embryos, please be extremely careful in handling and do not spread the virus. I have been waiting for the virus to mutate back, at which point I can make it into a vaccine for further experiments.

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