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Achievements of Virus Research

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ABSTRACT

The aim of this paper is a brief survey of the achievements in virology as published mainly in the course of 20th century and therafter until recent time. The author believes that in its beginning the virus research has developed in the framework of microbiology and related biological disciplines such as parasitology, pathology, cytology etc. Virologists have payed attention to many biological disciplenes not only for above mentioned reason, but also because they infect not only mammals, but also lower vertebrates including reptiles and/or many avian species as well as plants. To asses the origin of viruses may not be a simple task, it has been anticipated that they had probably sequestrated from cellular structures and then underwent their evolution.

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Virology is a relatively novel experimental scientific discipline, which has brought great amount of actual information to microbiology as well as for related biological disciplines. A simple definition of the expression „virus“ (which original meaning is poison) could be the following: the viral particle (also called virion) is a crystal-like structure which consists of proteins. Two kind of virions can be recognized by electron microscopy: so called „naked“ particles and/or the enveloped ones. The naked virion consists of protein subunits „sticking“ together by keeping the basic principles of symetry. The capsid protects a long string-like molecule (either RNA and/or a DNA) located inside and harboring the genetic information, which is capable to direct the synthesis of virion proteins. In contrast to bacterial and/or animal cells, the virus particle has only a single (one) molecule carrying the genetic information (i.e. either RNA and/or DNA, but never both). An exception are members of the Retrovirus family, which contain two RNA molecules. In case of DNA viruses, the size of the carrier molecule filament varies considerably. Members of the genus Parvovirus posses a short nucleic acid molecule possesing 2 (two) genes only; in contrast, the long DNA of the Poxvirus family members may have nearly 200 genes.

The last century (1901 to 1999), has brought many discoveries, which led to formation of a new branch of microbial research, later on termed ‘‘virology’’. Briefly, in 1930 the yellow fever agent has been isolated in mice (Theiler); this was associated by a substantial achievement which improved the procedures for virus reproduction in culture. Namely, the chick embryo cells have been separated by trypsinization of the embryonal and kept in monolyer. Soon thereafter, electron microscopy has been introduced at several virology departments. This allowed to observe the virus particles directly in infected cells and/or in their extracts. It has been recognized that even though the virus particle (later on called virion) is not a living entity, it is able to modify the susceptible cell. Namely, it blocks the cellular metabolism and forces the proteosynthesis machinery (especially the ribosomes) to produce its own (viral) components. Special purification procedures, such as gradient centrifugation into CsCl, were used for additional virus purificaton. This was a further impetus at „aiming“ to obtain virions in greater amounts. The techniques for virus visualization have been considerably improved by means of labeled antibodies (methods such as immunofluorescence), allowing to demonstrate the antigenic viral components at various intervals post-infection.

Within a relatively short period of (from March 2020 to September 2021), a novel Coronavirus known as Coronavirus 2019 [2019-CoV] has again emerged in China (namely at the city Wuhan), causing coronavirus disease (COVID) also termed COVID-19. The new isolates called coronavirus 2 (CoV-2) have been found to cause a disease already described previously under the name severe acute respiratory syndrome (SARS) [1]. The outbreak of the virus epidemic in question at a different (Hubei) province had an exposure link to the Wuhan’s South China Seafood City (ECSC) market [2]. By the recent CoV-2 outbreak (again beginning in China), have many details remained unclear, except for the fact that the novel virus was transmitted by direct exposure [3]. Noteworthy, the Wuhan fish and wild animal market is still selling live animals such as poultry, bats, marmots and snakes, any of which might be the source of infection [4]. Even though the majority of COVID-19 cases was reported from continental China, also the island of Taiwan has been found involved [5]. Unlike to the previously (in year 2002 and/or 2003) described „classical” coronavirus (cCoV), which had been isolated in a different (Guandong) province, the novel virus (nCOV) quickly spread all over the world. Over 300 million people became infected, a proportion of whom (about 3-4 %) developed signs of a relatively severe lung disease . In association with its aggressive spread, WHO has declared the recent nCOV outbreak for a situation of public health emergency of international concern [6]. There became clear that already accepted and standard health measures may not be sufficient enough to prevent a quick and widespread (possibly worldwide) transmission

of nCOV. Aiming to stop such dangerous outcome, additional measures were implemented, aiming to 1) slow down the spread of illness; 2) provide time (in any state) for better preparation of the health care system, business, educational organization, local health departments as well as the general public for the event if nCOV transmission of disastrous extent would really occur; 3) characterize the COVID-19 in local public health guides [7]. Development and deployment of medical countermeasures, including precise diagnostics, therapeutic recommendations (not excluding the continued efforts for vaccine development) were put into the focus of interest. In this respect, especially the asymptomatic cases should be diagnosed based on positive viral nucleic acid test results. Infections may occur without any COVID-19 symptoms, neither respiratory nor gastrointestinal and without significant abnormalities on the chest radiograph [8]. Transmission of COVID-19 through asymptomatic carriers (i.e. via person-to-person contact) has been observed in several reports [9]. Estimating a large sample, suggested that a median incubation period might be as short as 3 days on one hand but, by other hand, it could be as long as 24 days. Noteworthy, the mean incubation period has calculated for 5.2 days based on a range of 2.1 to 11.1 days [9].

Some patients with SARS, which were defined as laboratory-confirmed COVID-19 cases and had respiratory symptoms, the chest computed tomography (CT) did not reveal signs of pneumonia. Another patients with pneumonia on chest radiograph (and defined as COVID-19 positive cases), had both respiratory symptoms as well as pneumonia. The latter category of patients showed severe pneumonia (a respiratory rate over 30/minute, in addition the SpO_2 at 93% and the $PaO2/FiO_2$ over 300 mmHg) along with a critical clinical condition, which was characterized by shock and respiratory failure requiring mechanical ventilation along with organ failure needing ICU management [10]. Patients with pneumonia were older, with a higher prevalence of smoking history, more underlying diseases, and were more likely to have fever, myalgia/fatigue, dyspnea, headache, and nausea/vomiting compared to patients with ARD (all differences are at $p < 0.05$). In addition, pneumonia cases presented a higher white blood cell count and neutrophil count, but had a reduced leukocyte count compared to ARD cases. These patients received more antibiotics and antiviral therapy and were more likely to require oxygenation therapy, mechanical ventilator, renal replacement, and extracorporeal membrane oxygenation [11].

Person-to-person transmission of 2019-nCoV has been confirmed when asymptomatic individuals have been identified as potential sources of infection. The identification of COVID-19 cases and their contact persons along with immediately recommended measures, such as assessment and monitoring of travelers arriving from the areas with substantial probability of COVID-19 transmission were similar to those introduced by previous influenza virus pandemic. The differences in infectivity of coronaviruses can be attributed to differences in the rigidity of their shells which can be evaluated using computational tools for predicting intrinsic disorder predisposition of the corresponding viral proteins [12].

The estimated reproductive number of 0.3 was obtained from a small number of infected persons with imperfect information in the very early stages of the outbreak. Therefore the reproductive number of 2019-nCoV is likely to be similar to that of the 2002/2003 severe acute respiratory syndrome (SARS) coronavirus during the pre-intervention period (range 2 to 3) and that of the 2009 pandemic A/H1N1 influenza virus in the United States (by a range from 1.3 to 1.7) [4, 12]. Owing to these observations, the current control measures for 2019-nCoV, including the quarantine and an observation period of 14 days for suspected cases, can be considered for appropriate.

As of now, there is no specific antiviral medication available for COVID-19 treatment, and also no vaccine is currently available. Health care providers generally treat the symptoms by using oxygen therapy for patients with severe infection. However, few broad-spectrum antiviral drugs have been evaluated. A potential antiviral treatment of human CoV has been recommended using drugs such as Lopinavir/Ritonavir (400 mg/100 mg), nucleoside analogues, neuraminidase inhibitors, Remdesivir, the peptide EK1, arbidol, RNA synthesis inhibitors (such as TDF, 3TC) and/or certain anti-inflammatory drugs including IFN-alpha (5 million Units/dose). IFN-alpha is a broad spectrum drug, which can be used, for example, to treat hepatitis B [13]. Lopinavir is a protease inhibitor showing anti-CoV activity in vitro. It has been used to treat infection by human immune deficiency virus, together with ritonavir as a booster. For SARS treatment, there was found that in contrast to ribavirin alone, patients treated with lopinavir/ritonavir as well as ribavirin had a lower risk of the so called acute respiratory distress syndrome (ARDS) and/or death [10]. Nevertheless, as shown in mouse experiments using the Middle East Respiratory Syndrome (MERS)-CoV, Remdesivir may have the best CoV treatment potential. Namely, it can effectively reduce the virus titer in infected mice, it even even limits the lung tissue damage. Its effect may be better than that of the treatment using Lopinavir/Ritonavir combined with interferon [14].

China has relied on the use of the anti-viral drug Favilavir to treat the symptoms of COVID-19. This medication was initially developed by Toyama Chemical to treat nose and throat infections. Although the results of the study have not yet been published, it has been assumed that the drug has proven effective (at least in part) in treating symptoms of COVID-19 in a clinical trial of more than 70 patients with minimal side effects. Favilavir is an another new antiviral drug that was approved in Japan in 2014 to treat influenza, but currently also has for treating COVID-19 [15], but not by the U.S. Food and Drug Administration (FDA). Remdesivir (GS-5734) is a broad-based antiviral drug originally designed to target Ebola and was developed by Gilead Sciences. It inhibits viral replication through premature termination of RNA transcription, which disrupts the virus's ability to reproduce. China announced that clinical trials of Remdesivir, have officially started in Wuhan to test its efficacy against COVID-19. Moreover, one clinical trial has also been approved by the FDA in the United States [8]. However, the efficacy and safety of Remdesivir in patients still need further clinical studies.

Chloroquine and Hydroxychloroquine are drugs used to treat malaria, as well as chemoprophylaxis; and certain inflammatory conditions to include rheumatoid arthritis, lupus and the blood disorder *porphyria cutanea tarda*, respectively. They have been approved by the FDA to be tested against COVID-19 [16]. Researchers have found that both drugs have in vitro activity against SARS-CoV and SARS-CoV-2, with hydroxychloroquine having relatively higher potency against SARS-CoV-2. Based on these results, in several countries including USA, chloroquine as well as hydroxychloroquine are currently recommended for treatment of the hospitalized COVID-19 patients. In a Chinese study, when chloroquine was tested on more than 100 patients, the results were superior as compared to a control drug; the exacerbation of pneumonia was inhibited, the lung-imaging

findings improved, and the virus negative conversion promoted along with a shortened course of disease [17]. However, both chloroquine and hydroxychloroquine may cause relatively frequent side effects, such as worsening vision, nausea, digestive disorders not excluding heart failure in more severe cases. A man in Arizona died and his wife was in a critical condition after taking chloroquine just prophylactically, i.e. to prevent SARS-CoV-2 infection only.

Lopinavir/Ritonavir are sold under the name Kaletra by AbbVie and are designed to treat HIV (AIDS). To evaluate the efficacy of lopinavir/ritonavir for SARS-CoV-2 infection, 99 patients with positive infections were treated with lopinavir/ritonavir. No benefit was observed with lopinavir/ritonavir treatment compare to standard care [18]. However, in South Korea, a 54-year-old man was given a combination of these two medications and had a significant and substantial decrease in the levels of the β-coronavirus [19]. According to the WHO, there may be benefits to using lopinavir/ritonavir with other drugs such as interferon-β, oseltamivir or ribavirin [20].

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The structural proteins (and/or glycoproteins) by any Beta-coronavirus (B-CoV) strain are encoded by four regularly present structural genes, namely the spike glycoprotein (S, former E2), the envelope glycoprotein (E, former sM), the membrane glycoprotein (M, former E1) and the nucleocapsid protein (N). The 27.2 kb long single stranded negative sense viral RNA (vRNA) sequence has an untranslated region at its 5´-end (5´- UTR) along with a short leader sequence (LS) which continues into the two relatively long open reading frames (ORF 1a/b) encoding corresponding polyproteins (plp1a and plp1b). These become cleaved by an endogenous peptidase to form at least 10 non-structural viral polypeptides (nsp) involved in vRNA replication. Another four genes encode the structural proteins; their corresponding sequence is interrupted by regions specifying so called accessory proteins (in the case of CoV-2 they are the following: ORF 3, 6, 7a, 7b, 8 and 9b). One of them is located in between S and E sequences (ORF 3), the rest between M and N (with exception of ORF 9b which is positioned directly within the N sequence). The CoV RNA ends by a short untranslated region of the 3´-UTR sequence [11]. When comparing CoV-2 with the earlier isolated CoV strains, a key variation was found in the ORF 3 sequence region.

Taken together, the CoV genome may contain the following genes: **5´-LS--p65/Plp1a/ORF1a/NSP1--Plp1b/ORF1b/NSP2 (poliovirus type protease) NSP3 -- NSP4 --NSP5 -- NSP6 -- NSP7 -- NSP8 -- NSP9 (RNA polymerase) -- NSP10 (helicase/ NTPase) -- NSP11 -- NSP12 -- NSP13 – S – E – M -- N-3´**

The life cycle of SARS-CoV-2 in the susceptible host cells begins by binding of the S protein to a corresponding cellular receptor, namely that for acetylcholinesterase (ACE2). After receptor binding, a conformation change within S protein facilitates the fusion of the virion membrane with the cell membrane, which activates a transportation pathway along the cellular endosomal reticulum (ER). The virus coded polymerase produces a series of sub-genomic mRNAs transcribed from the released vRNA by a process called discontinuous transcription. In the region of cellular ER and Golgi apparatus the set of newly formed transcripts is finally translated into relevant viral proteins. These along with the transcribed novel vRNA are subsequently assembled into new virions, which are via the cytoplasmic vesicles transported back to cell membrane in order to get released out of the cell.

There is no clinically approved antiviral drug or vaccine available to be used against COVID-19. As of now, there is no specific antiviral medication available for COVID-19 treatment, and also no vaccine is currently available. Health care providers generally treat the symptoms by using oxygen therapy for patients with severe infection. However, few broad-spectrum antiviral drugs have been evaluated. A potential antiviral treatment of human CoV has been recommended using drugs such as Lopinavir/ Ritonavir (400 mg/100 mg), nucleoside analogues, neuraminidase inhibitors, Remdesivir, the peptide EK1, arbidol, RNA synthesis inhibitors (such as TDF, 3TC) and/or certain anti-inflammatory drugs including IFN-alpha (5 million Units/dose). IFN-alpha is a broad spectrum drug, which can be used, for example, to treat hepatitis B [13]. Lopinavir is a protease inhibitor showing anti-CoV activity in vitro. It has been used to treat infection by human immune deficiency virus, together with ritonavir as a booster. For SARS treatment, there was found that in contrast to ribavirin alone, patients treated with lopinavir/ritonavir as well as ribavirin had a lower risk of the so called acute respiratory distress syndrome (ARDS) and/or death [10]. Nevertheless, as shown in mouse experiments using the Middle East Respiratory Syndrome (MERS)-CoV, Remdesivir may have the best CoV treatment potential. Namely, it can effectively reduce the virus titer in infected mice, it even improves the lung tissue damage. Its effect may be better than that of the treatment using Lopinavir/ Ritonavir combined with interferon [14].

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The lung involvement was focal but quite prominent at the involved regions; the lesions seen at histological examination were clearly distinct from what could be seen by a standard bacterial bronchopneumonia. Namely, what has occured, was an interstiticial lung disease, by which the interalveolar septi looked widely thickened, yielding a picture later on referred to as usual interstitial pneumonia (UIP). As already mentioned, the cardinal finding at UIP is the thickening of interaleolar septi followed with mononuclear cell infiltration, consisting mainly of lymphocytes. The capillaries of such septi were widened, occasionally accompanied with bleeding into alveolar cavity. The latter phenomenon was interpreted to occur due to probably damaged capillary endothelium lining. In addition, proliferation of type II alveolar pneumocytes could be noted, along with slight hyperplasia of the interalveolar and peribronchial connective tissue. The severity of the lung lesions might vary from mild and/or relatively focal to more conflluent or even extensive, when involving the whole lung lobe. In such cases the clinical course corresponded to the syndrome of acute respiratory distress (ARD). Another patients not only showed pneumonia at roentgenological examination (positive chest computer tomography, CT), but might also develop severe organ failure, a state needing complex therapy as used for shock treatment [18]. Furthermore, the patients suffering of pneumonia might had pathological changes in blood, namely elevated levels of white blood cells, either polynuclear or mononuclear. The patients who developed pneumonia were, as a rule older; many of them were smokers and some of them suffered from an background disease. In such cases, the use of antibiotics and/or antiviral drugs has been obligatory in addition to oxygenation and mechanical ventilation of respiratory tract.

More frequently, the patients developed a mild respiratory disease with slight clinical signs, which state was referred to as Coronavirus Disease (CoViD) [24]. These patients had fever by a probability of 67% to 98% accompanied with a relatively severe cough (statistically ranging from 43% up to 81%) associated with myalgia. Another patients revealed just mild respiratory problems (by a probability of 31% to 55%), which have resembled rather to influenza [25]. Finally, the infection could proceed under non-specific clinical signs (when revealing mild respiratory and/or slight gastrointestinal tract involvement in absence of lesions detectable at roentgenological examinationas as seen by 3% to 11% of patients only (according to other statistical evaluations there wer much more such cases, but their number never exceeded 44% of total) [20, 4]. There should be noted, that cases showing neither clear-cut clinical symptoms nor evident roentgenological lesions (as confirmed at chest rtg examination), could be important from the epidemiological point of view [18]. A person, who feels neither sick, nor is aware of his infectiosity, would not stay at home and would keep any form of prevention.

More data describing the properties of Coronaviruses were obtained by analysing the recently emerged Coronaviruses (in year 2019, again in China, but at another city, called Wuhan and localted in the Hubei province). To distinguish the new CoV isolates (prototype strains OC43, HKU and SARS) from the classical ones (229E and NL63), theirs vRNA molecules have been sequenced. Comparing vRNAs sequences from 14 isolates (i.e. the majority out of the known 16 ones) a total of 129 mutations was detected. Based on these data, the isolated strains were divided into 4 (four) genera called Alpha, Beta, Gamma and Deltacoronavirus [20]. While the former two strains (Alpha- and Betacoronavirus) were coming mainly from bats, the latter ones (Gamma- and Deltacoronavirus) were avian in origin. Interestingly enough, also their geographical distribution differed accordingly, when the former came from Hong-Kong city area, while the letter from Bei-Jin (Peking) and/or Singapur.

Several details associated with the properties of nCoV isolated in China at year 2019 and/or 2020 remained still obscure, just their origin from the food market (at Wuhan) has shown an important analogy. It became clear that the novel strains can also spread from human to human, an important property closely resembling to that of the previously described isolates by nearly 20 year ago. There was quite typical for Chinese conditions that the Wuhan market has still remained in service [EU/EEA − third update; 2020, 7]. This might the reason, along with the more aggressive properties of nCoV, that the letter virus spread very efficiently

and quite soon all around the world, affecting several million people not only at continental China, but also at Taiwan [18] and elsewhere. Later on, the nCoV has „moved" crossing the rest of the world, not even remaining limited to Eastern Asia. In contrast, the nCoV has quickly reached the surrounding states in India and also occured in Australia, Japan, Canada, USA, Russia, on the Arabic peninsula as well as in East and European countries. The average incubation time of nCoV was estimated short (for 3 days), but a few cases could occur even 24 days after estimated (putative) infection. Nevertheless, the average incubation time has been calculated for 5.2 days, by estimated range limits of 2 to 11 days [18].

The man to man transfer of nCoV has been clearly demonstrated; especially persons showing no clinical symptoms could become the most dangerous source of infection. For this reason, air-travellers coming from endemic regions were carefully tested, with emphasis doing so already at airports [15]. The differences among the invasivity properties of individual isolates were attributed to differences in the sequence of virion surface proteins, expecially that of S and M glycoproteins. Computer programmes were also used for testing the affinity of cell surface receptors are [23]. The so called virus reproduction number has been calculated either in association with the above mentioned small epidemic in 2003, but especially at the influenza A/H1N1 pandemic (year 2009 in USA). By latter, the reproduction number ranged in the limits of 1.3 do 1.7. Reflecting these data, the time interval for SARS caranteen has been calculated for 10 upto 14 days, what can be regarded for correct and effective enough.

Local authorities were asked to publish statistical data illustrating the epidemiological situation at their region, namely to tell the number of hospitalized persons, not excluding those sick ones remaining home [24]. Finally, there was delared important to support the coresponding medical personal (including physisians and medical practioners) and provide financial help for their difficult, exhaustive and dangerous work. Neither last but not least, the development of efficient vaccine(s), which would be protective either by preventing disease or at least at mildering of its clinical coarse has been started by several companies. Briefly, a suitable vaccine is not expected not cause any undesired complications, or at least just some low grade side effects only. To achieve these goals, quick diagnostic technics, such as PCR test were introduced to be ready for use.

Until now, no effecient antiviral substance has been identified which would inhibit the replication of nCoV. Patients with severe repiratory symptoms are recomended to receive oxygen by means of a breathing device. Several medicaments have tested including antiviral drugs such as Lopinavir and/or Ritonavir in a dose of 100 mg and even 400 mg daily. In addiition, several neuraminadase inhibitors as well as nucleoside analogues have been tested. Out of letter, Remdesivir, Arbidol and the EK1 peptide have been used by most investigators. Furthermore, vRNA replication inhibitors, such as TDF and/or 3TC have been used for therapeutic purpose. The widely acting IFNalpha, which up to now has been used mostly against B- hepatitis, has been tried as well. There is known that Lopinavirus is an efficient protease inhibitor, but this action has been clearly demonstarted in vitro only, thus its action in the organism should further analysed. Up to now, such treatment has been applied with success in cases of AIDS patients who ahd been positive for HIV (human immuno defficiency virus) infections. In latter patients, Ritonavirus has been found quite helpful. Up to now, there was also shown that a compination of Lopinavir with Ritonavir might be effective for SARS treatment, if a booster of Ribavirin has been added. The latter drug has been shown successful in the experimental treatment of mice, in which the development of pneumonitis could be hindered. Ribavirin has been used with success especially to boost the combined Lopinavir and Ritonavir therapy. In latter case , there was bette to use Ribavirin than IFNalpha [26]. Clearly, Remdesivir has been highly infection in experimental treatment of mice, in which the development of pneumonia could be fully avoided, when the animals had been infected with a closely related Coronavirus causing the disease called MERS (Middle East Respiratory Syndrome). The syndrome named MERS has been recognized among inhavitants of the Arabic Peninsula, where it is endmic in camels. In a comprehensive study, in which 400 camels were infected with MERS, also the corresponding receptor for the latter virus has been identified, namely the enzyme DPP4/8CD23 (dipeptidyl peptidase 4). It should be mentioned finally, in China the substance Filavir (developed by Toyama Chemicals company) has been also used for COVID-19 treatment [12] even though this drug has been yet approved by the U.S. Food and Drug Administration (FDA) office. Nevertheless, the substance Remdesivir/GS-5734 was shown eliciting a wide antiviral effect. It has been developed by the company Gilead Sciences for Ebola virus treatment. Remdesivir inhibits and/or regulates transcrition; furthermore, this substance causes lock-down of vRNA synthesis as such, i.e. it very efficiently inhibits virus replication. In the case of emerging nCoV virus in Wuhan, the latter treatment (using Remdesivir) had been found effective, at according to the judgement of local athority. Based on these data, FDA recently has already approved the use of Remdesivir for human therapy. On the other hand, the affect of Remdesivir would need further careful analysis, as at least stressed by certain critical researchers.

Due to the above discribed quick spread of nCoV in the human population, WHO recently claimed the state of public danger. The attention of individual goverments was drawn to the fact that the hygiene measures acceptable before the emergence of the recent pandemic might not be sufficient enough, since they coud not prevent the nCoV spread. The recommendations to individual goverments were the following: 1. Paying attention to wide control and a unified and general accepted therapy of COVID; 2. Introducing the necessary legislative measures to prevent the virus spread within a reasonably short time interval; 3. To accept and introduce preventive measures for keeping public places (such as supermarkets, cinemas, public transport vehicles etc) safe for example by vearing masks in order to avoid formation of infectious air droplets (at exhalation) as well as to eliminate their inhalation. 4. Introducing additional measures to increase the safety of schools, nurseries, hospital wards etc; to achieve this, the corresponding and responsible local authorities should be asked to cooperate; 5. The population of regions in which nCoV has been found to circulate should be convinced to keep all the special rules helping to avoid and/or interrujpt virus spread. 6. Finally great attention should be payed for getting the inhabitants continuously informed of the nCoV statistics and its possible presence in order to accept the temporarily introduced hygienic measures aiming to limit virus spread. As the transfer of nCoV has been clearly demonstrated, persons showing no clinical symptoms are the most frequent source of infection [24]. For this reason, especially travellers coming from endemic regions should be carefully tested, for example at airports [27]. The differences among properties of the individual isolates were attributed to differences in the sequence of virion surface proteins, expecially that of S and M glycoproteins. Computer programmes used for testing the affinity

of cell surface receptors are suitable for this purpose [20]. The so called virus reproduction number has been calculated either in association with above mentioned small epidemic of SARS in 2003, but especially during the influenza A/H1N1 pandemic in year 2009 at USA. By the latter, the reproduction number ranged iin the limits of 1.3 do 1.7 [23]. Reflecting these data the time for SARS caranteen has been calculated for 10 až 14 dní, what can be regarded for correct.

The RNA molecule of viruses such as influenza, measles and/or infectious parotitis etc is stabilized by the help of several small basic proteins, also called histons and/or nucleoproteins; these not only do protect the nucleic acid, but they are able to stabilize its helical structure and, what is important, to neutralize its negative (acidic) charge. Of course, the number nucleoprotein peptides sticking to the helix is relatively high, in contrast to a single nucleic nucleic acid carrier molecule (with exception of Retroviruses which posses two copies of RNA). Large RNA viruses, such as influenza, measles and/or infectious parotitis, are fomed by a helical filament, which is „stablized" by the help of relatively short basic polypeptides, called histones. These, furthermore, neutralize its acidic charge. Of course, there are several neucleoprotein molecules distributed along a long single twisted (helical) RNA thread. The large RNA viruses, as members of Myxovirus family, are from outside protected by lipid membrane. This outer mambrane may be covered by many glycosylated proteins (also called glycoproteins), which are inserted into outer membrane, which, for example, yields their typical appearance to coronavirus particles. The improved virus detection methods were associated with tremendous discoveries of novel viruses, that time designated newly emerging viruses. There has been clearly shown that the virus not only spreads from man to man but also from lower mammals and poultry to man. This way severe diseases like yellow fever and/or Ebola fever were recognized to have viral in origin. The diapazon of virus diseases, which 2 (two) centuries ago was limited just to the classical small-pox and/or cowpox, has now widened in a dramatic way. It was recognized that apes and also domestic animals not excluding poultry, might become the source of virus disease for human population. It became clear that the so called lymphotropic viruses (not excluding *Retroviruses*) might become activated and then can spread not only within the given body but also from it to whole human population. For example, in 1950 the viral origin of hemorrhagic fever with renal syndrome has been documented, along with its reservoir in small rodents. Alternatively, the Hantaan virus (family *Bunyaviridae*) has been identified which was followed by isolation of a closely related virus causing the Kyanasur Forest fever. Later on in 1958, the arenavírus Junin was isolated in Argentína, then a related arenavírus Machupo was detetced in Bolívia (1966). In 1967 the filovírus Marburg was identified in Uganda, while in 1970 a novel arenavirus causing the Lassa fever has been described in Western Africa. As not enough, in 1976 the filovirus Ebola has appeared in Sudan and in 1976 a filovírus (related to Ebola) was isolated in Zaire. Thereafter, in 1977 the Seul virus has been described and then (in 1989) an additional filovirus called Ebola/Reston was found. Furthermore, in 1993 the hantavirus Sin Nombre has suddenly appeared, while in 1994 the Sabia virus has emerged along with another Ebola-related filovírus at the Ivory Coast area (in Africa). Neither the so called „developed" countries have remained saved from novel virus infections which became unexpectedly widespread. At the end of 70-ties the hepatitis C flavivirus has emerged in Europe and in the US. Then in 1988 the hepatitis E virus, earlier classifed as hepatitis non-A/non B, has been isolated. In 1986 it bacame clear that human herpesvírus 6 (HHV-6), which causes fever and skin exanthema in children (*exanthema subitum*) may also spread in pandemic manner. In 1995 the viral ethiology of Kapossi´s sarcoma has been confirmed along with the identification of a novel human herpesvirus, called Herpevirus 8 (HHV-8). The latter was frequent seen in patients, who showed symptoms of AIDS (acquired immune deficiency syndrome). Then new influenza pandemic has appeared in Russia as well as in China caused by the avian influenza A/H5N1 virus. The latter spread to man probably from wild ducks (which may be regarded for an universal source of naturally occuring viral recombinats), since at Chinese markets poultry might be sold alive.

By the end of year 2002, in the Chinese province Guandong as well as in city of Hong-Kong, several thousand cases of an acute respiratory disease (ARD) have suddenly appeared. Since this disease might occasionally become lethal (when reaching a death rate of up to 9-10 %), it has been later designated, severe acute respiratory syndrome (SARS)'' [13]. The lung involvement in these cases even when focal was quite prominent. At histological examination, the lesions seen were clearly distinct from the standard bacterial bronchopneumonia. Namely, what has occured, was the interstiticial lung disease, in which the interalveolar septi get widely thickened, yielding a picture later on referred to as ''usual interstitial pneumonia (UIP)''. As already mentioned, the cardinal finding by UIP is thickening of the interalveolar septi followed with their mononuclear cell infiltration (consisting mainly of lymphocytes). The capillaries of the thickened septi might be widened, occasionally accompanied with bleeding into alveolar cavity. This may occur from the probably damaged capillary endothelium lining. The severity of lung lesions could vary from mild and/or relatively focal to more conflluent and even extensive (involving the whole lung lobe). In such cases, the clinical course corresponded to a syndrome of acute respiratory distress (ARD). Such patients showed pneumonia not only at roentgenological examination (positive chest computer tomography), but developed a severe organ failure, a state needing complex therapy similar to that used for shock treatment. Furthermore, some patients suffering of pneumonia might had blood changes, namely elevated levels of white blood cells (either polynuclear or mononuclear). Patients developing pneumonia were, as a rule, older; many of them were smokers and some of them suffered from an additioal basic disease which decreased their immune competence. In such cases, the use of antibiotics and/or antiviral drugs has been inevitable, in addition to oxygenation by mechanical ventilation in order to overcome the state of respiratory tract insufficience.

More frequently, the infected patients developed a mild respiratory disease showing slight clinical signs, which state was referred to as Coronavirus Disease (CoViD). Such patients had fever by a probability of 67% to 98%, which had been accompanied with a relatively severe cough (statistically ranging from 43% up to 81%) associated with myalgia. Another patients revealed just mild respiratory problems (by a probability of 31% to 55%), which have resembled rather to influenza. Finally, the infection could proceed under non-specific clinical signs (when revealing mild respiratory and/or slight gastrointestinal tract involvement in absence of lesions detectable at roentgenological examinationas as seen by 3% to 11% of patients only (according to other statistical evaluations there were much more cases like this, but their number never exceeded 44%). There should be noted, that cases showing neither clear-cut clinical symptoms nor evident roentgenological lesions (as confirmed at chest rtg examination), could be important from the epidemiological point of view. If an infected person does not feel sick, he is not aware of his infectiosity; thus, he would not stay at home and/or keep any form of prevention. This might the reason, along with the properties of nCoV that the letter virus

spread very efficiently soon affecting many million people not only at Chinese continent, but also at Taiwan and then crossing the rest of the world; nCov has not remained limited to Eastern Asia, but quickly reached India, Australia, Japan, Canada, USA, Russia, the Arabic peninsula along with near East and European countries. The average incubation time was estimated for 3 days, but cases could occur even following 24 days of putative infection. Nevertheless, the average incubation time has been calculated for 5.2 days, when estimated to range in the limits of 2 to 11 days.

Data describing the properties of Coronaviruses were obtained by analysing of the recently emerged Coronaviruses (appearing in year 2019 again in China, but at another city, namely Wuhan in Hubei province) [15]. To distinguish the new CoV isolates (prototype strains OC43, HKU and SARS) from the classical ones (229E and NL63), theirs vRNA molecules have been sequenced. Comparing vRNAs sequences from 14 isolates (i.e. the majority out of the known 16 ones) a total of 129 mutations was detected. Based on these data, the isolated strains were divided into 4 (four) genera called *Alpha, Beta*, *Gamma* and *Deltacoronavirus*. While the former two strains (*Alpha*- and *Betacoronavirus*) were coming mainly from bats, the latter ones (Gamma- and Deltacoronavirus) were avian in origin. Interestingly enough, also their geographical distribution differed accordingly, when the former came from Hong-Kong city area, while the letter from Bei-Jin (Peking) and/or Singapur. Several details associated with the properties of nCoV isolated in year 2019 and/or 2020 have still remained obscure, just the origin of infection from a food market (at Wuhan) has been an important analogy. It became clear that the novel strains can also spread from human to human, which important property closely resembled to that of previously described isolates nearly 20 year ago. There was quite typical for the Chinese conditions that the Wuhan market has again remained in service [EU/EEA – a third update]. This might the reason, along with the more aggressive properties of nCoV, why the letter virus spread very efficiently all around the world, affecting several million people not only at continental China, but also at Taiwan and additional countries. The nCoV has „moved“ to rest of the world, not remaining limited to Eastern Asia. In contrast, the it has quickly reached the surrounding states. namely India and then Australia, Japan, Canada, USA, Russia, the Arabic peninsula as well as Eastern and Western European countries. The average incubation time of nCoV was estimated for relatively short (for 3 days), but a few cases could occur even 24 days after estimated (putative) infection. Nevertheless, the average incubation time has been calculated for 5.2 days, by estimated range limits of 2 to 11 days. The man to man transfer of nCoV has been clearly demonstrated; especially persons showing no clinical symptoms could become the most dangerous source of infection. For this reason, air-travellers coming from endemic regions were carefully tested, with emphasis doing so already at airports. The differences among the invasivity properties of individual isolates were attributed to differences in the sequence of virion surface proteins, expecially that of S and M glycoproteins. Computer programmes were also used for testing the affinity of cell surface receptors. The so called virus reproduction number has been calculated either in association with the above mentioned small epidemic in 2003, but especially at the influenza A/H1N1 pandemic (year 2009 in USA). By latter, the reproduction number ranged in the limits of 1.3 do 1.7 . Reflecting these data, the time interval for SARS caranteen has been calculated for 10 upto 14 days, what can be regarded for correct and effective enough.

Local authorities were asked to publish statistical data illustrating the epidemiological situation at their region, namely to report the official number of hospitalized persons, not excluding the sick ones who remained at home. For such reason a simple test has been introduced to regonise the agent in question. Finally, there was delared for obligatory to support the coresponding medical personal (including physisians and medical practioners) including to provide additional financial help for their difficult, exhaustive and dangerous work. Neither last but not least, the development of efficient vaccine(s), which would be protective either by preventing disease or at least at mildering of its clinical coarse has been started by several companies. Briefly, a suitable vaccine is not expected not cause any undesired complications, or at least just some low grade side effects only. To achieve these goals, quick diagnostic technics, such as PCR test were introduced to be ready for use.

Data describing the structure of Coronavirus particles were first obtained by analysing the Betacoronavirus virions, which infect cattle. As already known, the nCoV particles are enveloped capsids which vRNA encodes 4 (four) basic proteins, namely the vRNA associated nucleoprotein (N), the two membrane (glyco) proteins called S/E2 (spike glykoprotein) and E/sM (membrane glycoprotein M/E1) and the capsid protein. The above mentioned vRNA is a single strand molecule of positive polarity (26x103 nucleotides) equipped with a preceeding promoter sequence and the 5´-UTR end (UT, untranslated region). The short leader sequence is located in the front of the two open reading frames (ORF1a/ORF1b), which encode the polypeptides Pl1a and Pl1b. The long polypeptides in question are cleaved by an endogenous protease (peptidase). Another important nonstructural polypeptide is the RNA polymerase (RNApoly) which replicates the vRNA molecule. The genes encoding the four structural proteins are interrupted with sequences encoding several accessory proteins (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8a, ORF8b a ORF9). Some of these are located between S and E sequences (ORF3a/ORF3b), but the rest of them is located between genes encoding M a N proteins with exception of ORF9 located in the N-gene sequence. Finally, the vRNA molecule ends with an untranslated 3´-UTR sequence. In comparison with the classical c(CoV) isolates, important variations were found within the ORF3 sequence.

Until now, no effecient antiviral substance has been identified which would inhibit the replication of nCoV. Patients with severe repiratory symptoms are recomended to receive oxygen by means of a breathing device. Several medicaments have tested including antiviral drugs such as Lopinavir and/or Ritonavir in a dose of 100 mg and even 400 mg daily. In addiition, several neuraminadase inhibitors as well as nucleoside analogues have been tested. Out of letter, Remdesivir, Arbidol and the EK1 peptide have been used by most investigators. Furthermore, vRNA replication inhibitors, such as TDF and/or 3TC have been used for therapeutic purpose. The widely acting IFNalpha, which up to now has been used mostly against B- hepatitis, has been tried as well. There is known that Lopinavirus is an efficient protease inhibitor, but this action has been clearly demonstarted in vitro only, thus its action in the organism should further analysed. Up to now, such treatment has been applied with success in cases of AIDS patients who ahd been positive for HIV (human immuno defficiency virus) infections. In latter patients, Ritonavirus has been found quite helpful. Up to now, there was also shown that a compination of Lopinavir with Ritonavir might be effective for SARS treatment, if a booster of Ribavirin has been added. The latter drug has been shown successful in the experimental treatment of mice, in which the development of pneumonitis could be hindered. Ribavirin has been used with success especially to boost the combined Lopinavir and Ritonavir therapy. In latter case , there was better

to use Ribavirin than IFNalpha. Clearly, Remdesivir has been highly efficient by experimental treatment of mice, in which the development of pneumonia could have been fully eliminated. In such experiments, the animals had been infected with a closely related Coronavirus causing called MERS (Middle East Respiratory Syndrome). The MERS has been originally recognized among the inhabitants of the Arabic Peninsula, where it had been found endmic in camels. In a comprehensive study, in which 400 camels were infected with MERS, also the corresponding receptor for the virus in question has been identified, namely an enzyme called DPP4/8CD23 (dipeptidyl peptidase 4). It should be mentioned that in China the substance Filavir (developed by Toyama Chemicals company) has also been used for COVID-19 treatment, even though this drug has been yet approved by the U.S. Food and Drug Administration (FDA) office. Nevertheless, the substance Remdesivir/GS-5734 was shown eliciting a wide antiviral effect [28]. It has been developed by the company Gilead Sciences for Ebola virus treatment. Remdesivir inhibits and/or regulates transcrition; furthermore, this substance causes lock-down of vRNA synthesis as such, i.e. it very efficiently inhibits virus replication. In the case of emerging nCoV virus in Wuhan, the latter treatment (using Remdesivir) had been found effective, at according to the judgement of local athority. Based on these data, FDA recently has already approved the use of Remdesivir for human therapy. On the other hand, the affect of Remdesivir would need further careful analysis, as at least stressed by certain critical researchers .

Due to the above discribed quick spread of nCoV in the human popve individual goverments was drawn to the fact that the hygiene measures acceptable before the emergence of the recent pandemic might not be sufficient enough, since they coud not prevent the nCoV spread. The recommendations to individual goverments were the following: 1. Paying attention to wide control and a unified and general accepted therapy of COVID; 2. Introducing the necessary legislative measures to prevent the virus spread within a reasonably short time interval; 3. To accept and introduce preventive measures for keeping public places (such as supermarkets, cinemas, public transport vehicles etc) safe for example by vearing masks in order to avoid formation of infectious air droplets (at exhalation) as well as to eliminate their inhalation. 4. Introducing additional measures to increase the safety of schools, nurseries, hospital wards etc; to achieve this, the corresponding and responsible local authorities should be asked to cooperate; 5. The population of regions in which nCoV has been found to circulate should be convinced to keep all the special rules helping to avoid and/or interrujpt virus spread. 6. Finally great attention should be payed for getting the inhabitants continuously informed of the nCoV statistics and its possible presence in order to accept the temporarily introduced hygienic measures aiming to limit virus spread. As the transfer of nCoV has been clearly demonstrated, persons showing no clinical symptoms are the most frequent source of infection [29]. For this reason, especially travellers coming from endemic regions should be carefully tested, for example at airports. The differences among properties of the individual isolates were attributed to differences in the sequence of virion surface proteins, expecially that of S and M glycoproteins. Computer programmes used for testing the affinity of cell surface receptors are suitable for this purpose. The so called virus reproduction number has been calculated either in association with above mentioned small epidemic of SARS in 2003, but especially during the influenza A/H1N1 pandemic in year 2009 at USA. By the latter, the reproduction number ranged iin the limits of 1.3 do 1.7. Reflecting these data the time for SARS caranteen has been calculated for 10 až 14 dní, what can be regarded for correct.

As recommended , the local authorities should be asked to publish their statistical data illustrating the epidemiological situation at given region, especially for telling the number of hospitalized persons, stressing the number of heavily sick insdividuals [30]. Finally, there has been calaimed for important to introduce an additional support the medical personal (including physisians and medical practioners) involved in the relevant medical care, namely providing all possible help to their difficult, exhaustive and dangerous work. Neither last not least, more attention should be payed to preparation of an efficient vaccine(s), which would be efficient enough to prevent disease not only to mild the clinical disease. A suitable vaccine is expected not causing undesired complications, except of acceptable low side effects only. Such goals lcan be achieved by means of sensitive diagnostic technics such as PCR.

References

1. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, et al. (2020) Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N Engl J Med 382: 970-971.
2. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, et al. (2020) The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. Mil Med Res 7: 11.
3. Chen N, Zhou M, Dong X, Qu J, Gong F, et al. (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395: 507e13
4. Nishiura H, Linton NM, Akhmetzhanov AR (2020) Initial cluster of novel coronavirus (2019-nCoV) infections in Wuhan, China is consistent with substantial human-to-human transmission. J Clin Med 9: E488.
5. Yu P, Zhu J, Zhang Z, Han Y, Huang L (2020) A familial cluster of infection associated with the 2019 novel coronavirus indicating potential person-to-person transmission during the incubation period. J Infect Dis. 221: 1757-1761.
6. Liu YC, Liao CH, Chang CF, Chou CC, Lin YR (2020) A locally transmitted case of SARS-CoV-2 infection in Taiwan. N Engl J Med 382: 1070-1072.
7. Wong TW, Tam W (2004) Estimating SARS incubation period. Emerg Infect Dis 10: 1503-1504.
8. Bai Y, Yao L, Wei T, Tian F, Jih DY, et al. (2020) Presumed asymptomatic carrier transmission of COVID-19. J Am Med Assoc 323: 1406-1407.
9. The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team (2020) The epidemiological characteristics of an outbreak of 2019 novel coronavirus disease (COVID-19) in China. China CDC Weekly 2: 113-122.
10. European Centre for Disease Prevention and Control. Risk assessment: outbreak of acute respiratory syndrome associated with a novel coronavirus, China: first local transmission in the EU/EEA − third update https://www.ecdc.europa.eu/en/publications-data/risk-assessment-outbreak-acute-respiratory-syndrome-associated-novel-1.
11. Wu A, Peng Y, Huang B, Ding X, Wang X, et al. (2020) Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe 27: 325-328
12. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR (2020) Severe

acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. Int J Antimicrob Agents. https: //doi.org/10.1016/j.ijantimicag.2020.105924.

13. European Centre for Disease Prevention and Control. Risk assessment: outbreak of acute respiratory syndrome associated with a novel coronavirus, China: first local transmission in the EU/EEA − third update https://www.ecdc.europa.eu/en/publications-data/risk-assessment-outbreak-acute-respiratory-syndrome-associated-novel-1.
14. Wong TW, Tam W (2004) Estimating SARS incubation period. Emerg Infect Dis 10: 1503-1504.
15. Guan WJ, Ni ZY, Hu Y, Laing WH, Ou CQ, et al. (2020) Clinical characteristics of 2019 novel coronavirus infection in China. medRxiv https: //doi.org/10.1101/2020.02.06.20020974
16. Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, et al. (2020) Severe acute respiratory syndrome-related coronavirus: the species and its viruses, a statement of the Coronavirus Study Group. https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1.full.pdf+html
17. Lu R, Zhao X, Li J, Niu P, Yang B, et al. (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395: 565-574.
18. Chan JF, Yuan S, Kok KH, To KK, Chu H, et al. (2020) A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 395: 514-523.
19. Liu YC, Liao CH, Chang CF, Chou CC, Lin YR (2020) A locally transmitted case of SARS-CoV-2 infection in Taiwan. N Engl J Med 382: 1070-1072.
20. Cavanagh D (1997) Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch Virol 142: 629-633.
21. Shereen, Muhammad Adnan, Suliman Khan, Abeer Kazmi, Nadia Bashir Rabeea Siddique (2020) COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. J Advanced Res 24: 91-98.
22. Ryu, Sukhyun, Byung Chul Chun (2020) An interim review of the epidemiological characteristics of 2019 novel coronavirus. Epidem and Health 42: e2020006.
23. Goh, Gerard Kian-Meng, A Keith Dunker, James A (2020) Foster and Vladimir N. Uversky. Rigidity of the Outer Shell Predicted by a Protein Intrinsic Disorder Model Sheds Light on the COVID-19 (Wuhan-2019-nCoV) Infectivity. Biomolecules 10: 331.
24. VanGorp HW, VanBreedam P L, Delputte, H J Nauwynck (2008) Sialoadhesin and CD163 join forces during entry of the porcine reproductive and respiratory syndrome virus. J. Gen. Virol 89: 2943-2953.
25. Elfiky Abdo A (2020) Anti-HCV, nucleotide inhibitors repurposing against COVID-19. Author links open overlay pane. Life Sciences 248: 117477.
26. Zhu N, Zhang D, Wang W, Li X, Yang B, et al. (2020) A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 382: 727-733.
27. Lee PI, Hsueh PR (2020) Emerging threats from zoonotic coronaviruses-from SARS and MERS to 2019-nCoV. J Microbiol Immunol Infect 53: 365e7.
28. Nishiura H, Linton NM, Akhmetzhanov AR (2020) Initial cluster of novel coronavirus (2019-nCoV) infections in Wuhan, China is consistent with substantial human-to-human transmission. J Clin Med 9: E488.
29. Yang Y, Sugimoto JD, Halloran ME, Basta NE, Chao DL, et al. (2009) The transmissibility and control of pandemic influenza A (H1N1) virus. Science 326: 729-733.
30. Yu P, Zhu J, Zhang Z, Han Y, Huang L (2020) A familial cluster of infection associated with the 2019 novel coronavirus indicating potential person-to-person transmission during the incubation period. J Infect Dis. 221: 1757-1761.