Below is the translation of Sections “MATERIALS AND METHODS”, “RESEARCH RESULTS” as well as the «CONCLUSIONS» of the Report of the Gromashevsky Institute of epidemiology and infection diseases National Academy of Medical Science of Ukraine on the results of testing the device IOON verses model strain of coronavirus from Ukrainian intoEnglish. The original is available here. In case of ambiguous interpretation, trust the Ukrainian version of the document.

The purpose of the study: to determine the virucidal activity of the IOON substance in a model of coronavirus transmissible gastroenteritis of pigs (TGP).

MATERIALS AND METHODS

Viruses:

TGEV - the etiological agent of transmissible gastroenteritis of pigs (TGP) - a highly contagious intestinal disease of pigs.

Virus strain:

$D_{52\text{-}5}$ (BRE_{79}) - is a highly pathogenic virus for pigs of all ages at the level of 5 passages in transplanted monolayer culture of testicular cells of piglets ST.

The tropism of the virus of the gastrointestinal tract and respiratory tract is shown. The strain was provided by Dr. Hubert Laude from the Laboratory of Molecular Virology and Immunology of the INRA Center for Biotechnology in Jouy-en-Josas (France).

Infection titration:

Titration of infectivity of viral materials on cell cultures was performed by two methods - final dilutions by CPA (Fig. 1), and the titer of infectivity was determined by the method of Kerber-Ashmarin and was determined in TCD50/ml, by the method of negative colonies (S-sign) under 1.35% agar coating (Difco-Bacto) (Fig. 2), and the infectivity titer was determined in PLU/ml. The results were calculated after 120 hours of cultivation at 38 °C.

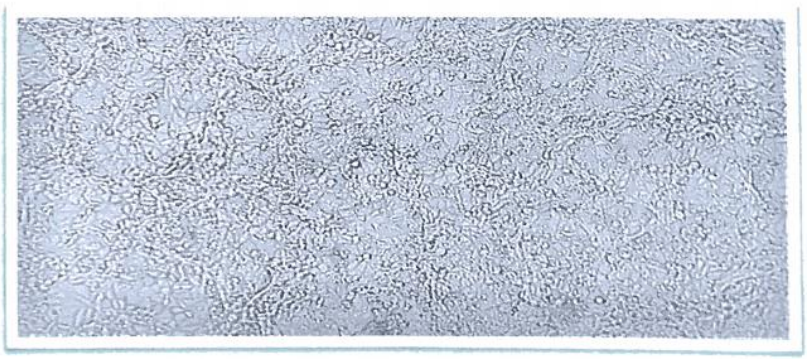

Fig. 1 - Control of CHEB culture
(transplanted culture of pig kidney cells)

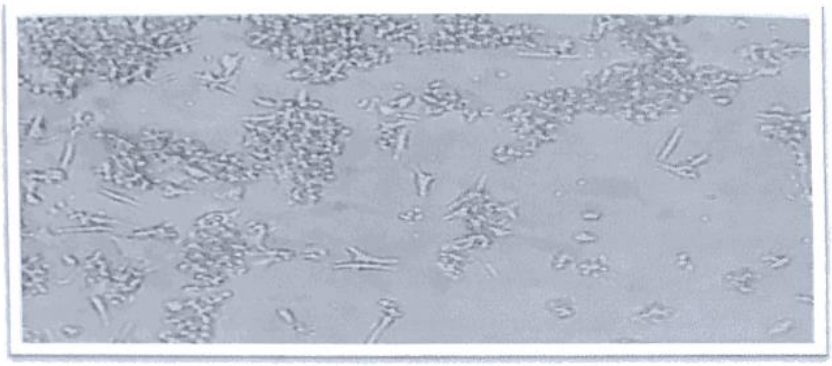

Fig.2 Control of the virus on CHEB
(transplanted culture of porcine kidney cells)
Virus - pigs gastroenteritis virus (PGV) - coronavirus.

Cell culture:
CHEB - is a transplanted culture of kidney cells of a pig embryo,
NSP is a transplanted culture of piglet kidney cells,
ST - transplanted culture of testicular cells of piglets,
CTGP is a transplanted culture of pig thyroid cells.

Substance:
IOON - spray with silver ions generated with IOON device.

The value of cytotoxic drug concentration (CC50)

To determine CC_{50} the effect of the drug used cultures of CHEB cells. The experiments used at least ten rows of alveolus in cells with cell culture for each dilution of the drug in the culture environment. Cell culture plates were incubated at 37 °C with 5% CO_2 for 5 days. Experimental and control samples of cultures were observed daily to establish the presence or absence of cytopathogenic action (ACA). The degree of ACA was determined by changes in cell morphology (rounding, cell shrinkage, rejection from the surface of the alveolus of degenerative altered cells) on the 4+ plus system from + to ++++:

"-" - complete absence of cell degeneration;

"+" - affected no more than 25% (protection of monolayer cells from antiviral drugs by 75%);

"++" - affected no more than 50% of the cell monolayer;

"+++" - affected no more than 75% of the cell monolayer;

"++++" - complete degeneration of the cell monolayer.

The CC_{50} of the drug was taken in the largest amount, which did not cause cell degeneration.

Determination of virucidal action of drugs

The purpose of these studies is to identify the direct destructive effect of IOON substance on the extracellular virus.

The viral suspension at a dose of TCD50/ml was incubated with a lower concentration of CC50 at different exposures, taking samples to determine the infectious titer of the virus as a control used viral suspension which was incubated under the same conditions without the IOON substance. The decrease in the infectious titer of the virus by 1.5-2.0 lgTCD50 compared to the control of the virus indicates the severity of the virucidal effect.

Detection of RNA of transmissible gastroenteritis virus of pigs str.D52 by reverse polymerase chain reaction (RT-PCR)

RNA isolation was performed using a set of "Fish-sorb" in accordance with the manufacturer's instructions (AmpliSens, Russia). The reverse transcription reaction was performed using a kit "RevertAidTM H MinusFirstStrandcDNASynthesisKit" according to the manufacturer's instructions (ThermoScientific, Lithuania) For PCR were used gene-specific nucleoprotein oligonucleotide primers of the following sequence: direct Uni_1 (5'-TGCACTGATCAATGTGCTAG-3) and reverse Uni 2 (5' TGAAAACACTGTGGCACCCTT-3"). A fragment amplified by size 309 P.M. .. M-marker "100 bpPlus DNA Ladder" ("ThermoFisherScientific", Lithuania).

Statistical processing of research results

The digital material presented in the work is processed statistically. Statistical evaluation of the levels of significance of differences in the obtained figures was performed using Student's t-test using Microsoft Excel and MicrocalOrigin. Differences at $p < 0.05$ were considered authentic.

RESEARCH RESULTS

Characteristics of pigs gastroenteritis virus

Coronaviruses of transmissible porcine gastroenteritis (CBT) were passed on to different cultures and were characterized by infectious titer. The results are presented in the table.

Table 1. Infectious titer of TGP virus in cell cultures

Cell culture	**Infectious titer PLU**
ST_5	$3,9x10^7$ PLU/ml
$CHEB_{100}$	$3,1x10^7$ PLU/ml
NSP_{100}	$4,3x10^7$ PLU/ml
$CTGP_{100}$	$1,4x10^8$ PLU/ml

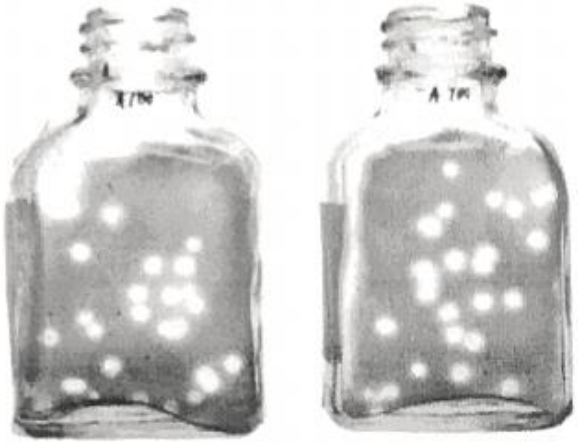

Fig.3 - Negative colonies of porcine gastroenteritis virus str. D_{52-100} CTGP

The virus neutralization reaction was performed in 96-cell plates "Costar" (USA), according to the method of H. Laude, using as a positive control the reference hyperimmune serum N6926, the same author.

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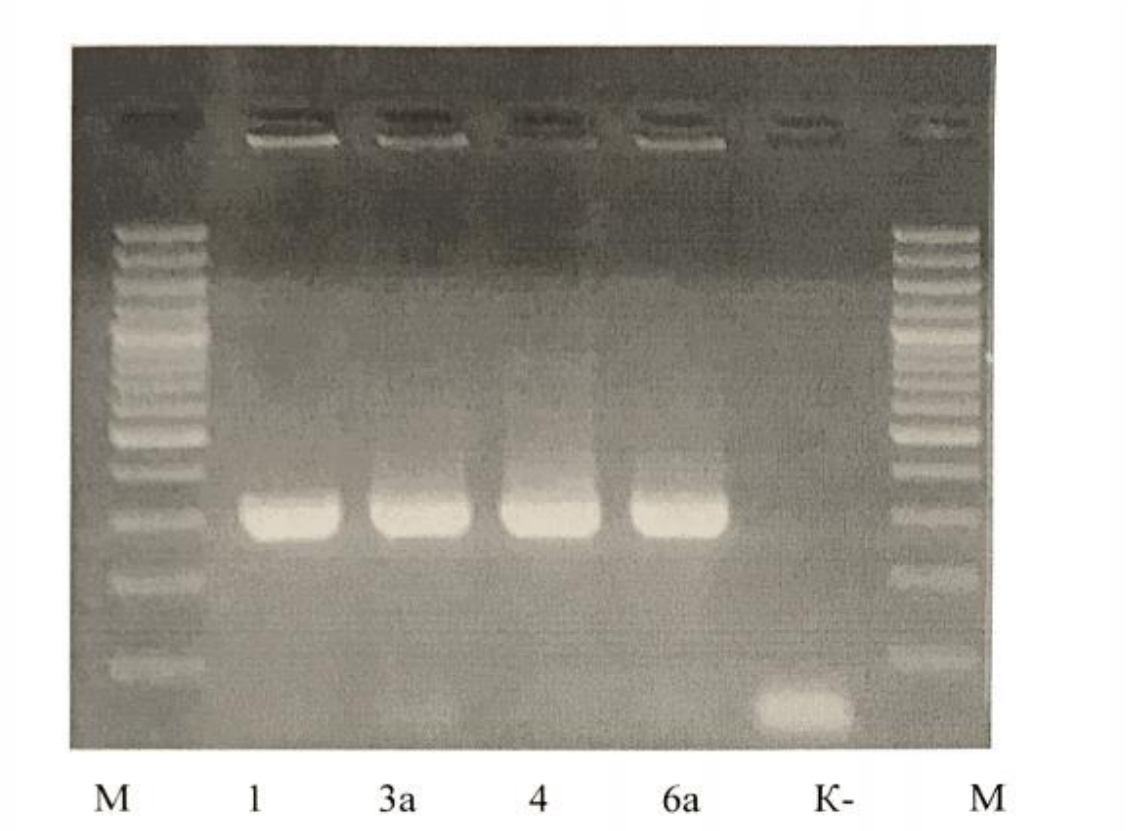

Fig.4 - Electrophoretic analysis of amplification products of porcine gastroenteritis virus with a primer to the nucleoprotein gene of oligonucleotide primers of the following sequence: direct Uni_1 (5'-TGCACTGATCAATGTGCTAG-3) and reverse Uni 2 (5' TGAAAACACTGTGGCACCCTT-3"). A fragment amplified by size 309 P.M. .. M-marker "100 bpPlus DNA Ladder" ("ThermoFisherScientific", Lithuania).

M-marker of the size of RNA fragments
No. 1 - coronavirus strain D52 with NSP
No. 3a - strain of coronavirus D52 with CC CHEB
No. 4 - D52 in cell culture ST-testicles of piglets
No. 6a - D52 in the culture of the thyroid gland of pigs (CTGP)

Determination of cytotoxic effect of IOON substances

To determine the cytotoxic concentration of the test substances used CHEB cells sensitive to transmissible gastroenteritis of pigs (TGP). 96-alveoulus plates in which CHEB cells were grown were used, after 24 hours of cell growth with the monolayer formed, the growth environment in the plates was removed and 100 mcl of pure environment with antibiotics was added to the cells. The IOON test substance was made in dilutions from 1:10 to 1: 100,000 in 3 replicates. After 24 hours, a visual assessment of the effect of the test substances was performed.According to visual observations, the CC50 for drugs was 1: 100.

Study of virucidal activity of the IOON substance on a model of coronavirus of transmissible gastroenteritis of pigs (TGP)

To study the virucidal activity of the IOON substance used transplanted cell culture CHEB. Cells were grown in plates on fetal serum RPMI-1640 + 10% medium at 37 °C in a thermostat with CO2 supply.

A strain of pigs gastroenteritis virus with an infectious titer 8,5 lg ID_{50} was used.

Coronavirus is moderately resistant to chemical factors and retains infectious activity for several years in the lyophilized state at a temperature of 4 °C and in the frozen state - 70 °C.UV radiation inactivates viruses in 15 minutes. Organic solvents and detergents - for a few minutes. Viruses are thermolabile at a temperature of + 37 °C inactivated in 10 hours, at a temperature of 33 °C in 16 hours, at + 56 °C in 10 minutes. For human coronaviruses, the pH range of 7 to 7.5 is optimal. Changing the pH of the environment in any direction is detrimental to them. The action of sunlight causes slow inactivation. Coronaviruses are stored in the aerosol for 8-10 hours, in drinking water for up to 9 days, indoors at a temperature of 18 ° from 4 to 11 10 days.

Determination of the effect of the IOON substance in extracellular coronavirus TGP was investigated thus: to the suspension of coronavirus TGP at a dose of 1000 TCD50/ml was added the IOON substance in diluted 1:1, incubated at room temperature and samples of extracellular virus were taken after 5 minutes and 20 minutes.

The results for determining the effect of the IOON substance on the extracellular coronavirus TGP are presented in the graph.

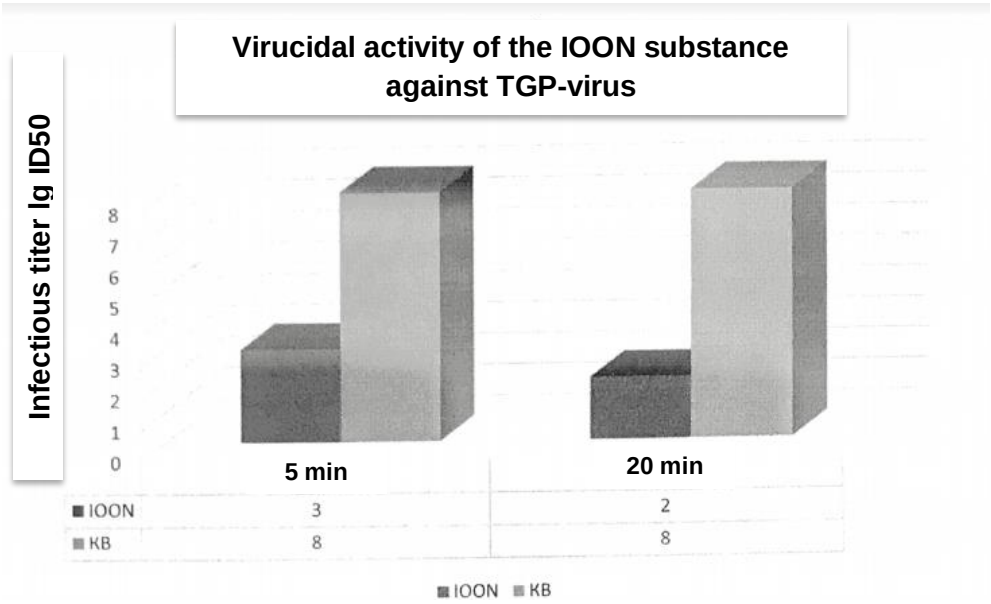

Fig.5 Infectious titer of TGP virus in alveolus treated with the virus TGP 1000 ID50 and the IOON substance

According to the results, it was found that the IOON substance inhibited the reproduction of extracellular coronavirus TGP by 5.0 lg at exposure for 5 min and 6.0 lg at exposure for 20 min**.**

CONCLUSIONS

As a result of research of IOON is determined that the drug is an effective virucidal drug and can be used at exposure for 20 minutes to disinfect the hard surface and the tools for doctors' hands.