Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2021, 11(6), 117-126, doi: 10.15421/2021_233

ORIGINAL ARTICLE

Evaluation of virucidal action of disinfectant against pathogens of infectious rhinotracheitis and viral diarrhea in cattle

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Received: 17.07.2021. Accepted: 31.08.2021.

Prevention and control of viral diseases of farm animals remain an urgent problem despite the success achieved in their eradication. In the general complex of anti-epizootic measures, disinfection measures are of great importance, for which the presence of highly effective disinfectants is necessary. The study aimed to study the virucidal properties of a modern disinfectant based on quaternary ammonium compounds (25.0%), glutaraldehyde (11.0%), isopropyl alcohol, nonionic surfactants. The production strain of bovine infectious rhinotracheitis virus "Moldavian" with an infectious activity of 8.1 lg TCD 50/cm^3 and the production strain of bovine viral diarrhea virus "BK-1" with an infectious activity of 7.7 lg TCD 50/cm^3 were used as test cultures. Studies have shown that the disinfectant neutralizes bovine infectious rhinotracheitis virus when used at a concentration of 0.1% for 20 minutes, and destroys bovine diarrhea virus when used at a concentration of 0.5% for 20 minutes. The disinfectant in these modes of application completely disinfects various surfaces (metal, tile, glass, plastic, wood, cotton) contaminated with pathogens of viral diseases of farm animals. The obtained results expand the range of disinfectants that are promising for use in animal husbandry.

Keywords: Disinfectant, virus, cell culture, concentration, exposure cytotoxic effect, cytopathic effect, test object.

Introduction

Infectious diseases of farm animals cause significant economic damage to the livestock industry, so monitoring the epizootiological welfare of livestock is the main task of veterinarians (Callan & Garry, 2002). Among the large number of diseases of infectious etiology, viral infections occupy a special place. They cause massive lesions of susceptible livestock and spread rapidly to safe areas (Yadav et al., 2019, Nugroho et al., 2020).

Among the existing economically significant infectious diseases of cattle, infectious rhinotracheitis and viral diarrhea deserve attention (Wernicki et al., 2015, Saravanajayam et al., 2015, Dagalp et al., 2020). With the appearance of these diseases in herds, animal productivity significantly reduces, abortions occur, and weakened young are born, which causes significant economic losses (Lanyon et al., 2014, Kane et al., 2015, Pinior et al., 2017, Yitagesu et al., 2021).

The overall individual seropositivity among cattle in central Costa Rica has been reported to be 48% for IRT and 19–27% for VD (Raizman et al., 2011). The seropositivity of productive animal herds in Italy for IRT is 31.89–55.49% (Maresca et al., 2018), and in Estonia, this figure is 22% (Raaperi et al., 2010). In India, 38.0% of cattle, 85.0% of buffaloes, 38.6% of bulls, and 71.1% of yaks were tested positive for antibodies to the IRT virus (Nandi et al., 2011). In Iran, the seropositivity in dairy herds for IRT is 72.2%, and for VD-52.8% (Noaman & Nabinejad, 2020).

The IRT virus has been shown to circulate among cattle in the Naari area of Meru County, Kenya (Kipyego et al., 2020). Given that this studied population is not vaccinated against this disease, to reduce the transmission and impact of the virus on animals, it is recommended to take measures for biosecurity, which provides for the organization of vaccination and disinfection (Muratore et al., 2017, Maresca et al., 2018, Kipyego et al., 2020).

Given the epizootiological significance of these infectious diseases, it is necessary to develop and apply effective schemes for the control and prevention of these infections (Sekiguchi et al., 2018, Ortega et al., 2020). Non-specific measures, such as disinfection of livestock facilities (Bielanski et al., 2009, Traverse & Aceto, 2015, Paliy et al., 2020d), are important. Today, many disinfectants from different groups of chemical compounds are used in animal husbandry. They differ in composition and spectrum of antimicrobial action (Paliy et al., 2018a, Maertens et al., 2019). Despite the rather wide arsenal of proposed disinfectants today, extensive work is being done to improve existing and develop new antimicrobials (Rodríguez Ferri et al., 2010, Kovalenko et al., 2020, Bondarchuk et al., 2020, Rodionova et al., 2021). The main problems in the testing of disinfectants are the mismatch of results obtained in laboratory conditions and field tests (Wales et al., 2021). Along with this, the development of resistance of field isolates of microorganisms to the action of long-term disinfectants has been reported (Kamal et al., 2019).

Materials and Methods

Our work aimed to study the virucidal properties of a disinfectant, the formulation of which consists of a mixture of Quaternary Ammonium Compounds (QAC)-25.0%, glutaraldehyde-11.0%, isopropyl alcohol, nonionic surfactants.

The study of virucidal properties of the disinfectant was carried out in the laboratory of virology and laboratory of veterinary sanitation and parasitology of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (NSC "IECVM") following the guidelines "Methods of quality and safety assessment of disinfectants used during the production, storage, transportation and sale of products of animal origin "(Iacubchak, 2010) and other current methodological approaches (Kovalenko et al., 2014).

The following materials were used for research:

- Continuous cell culture lines: Calf kidney (CK), Sheep Kidney (ShK-2),
- Eagle nutrient medium for culturing cell cultures (DMEM), nutrient medium 199 following current regulations,
- Normal cattle blood serum following current regulations,
- Versene solution following current regulations,
- Trypsin solution 0.25% following current regulations,
- Saline solution with a pH of 7.2 ± 0.2,
- 0.2 M phosphate-salt buffer with a pH of 7.2 ± 0.2,
- Antibacterial drugs: 2.0% solution of gentamicin sulfate and 2.0% solution of tylosin.

Viruses were used as test cultures:

- Production strain of bovine infectious rhinotracheitis virus (bovine IRT) "Moldavian" with an infectious activity of 8.1 lg TCD 50/cm^3 (inv. No. 43 in the depository of the NSC "IECVM", deposited under the No. 591 in the SSCIBSM),
- Production strain of bovine viral diarrhea virus (bovine VD) "BK-1" with an infectious activity of 7.7 lg TCD 50/cm^3 (inv. No. 49 in the depository of the NSC "IECVM", deposited under the No. 594 in the SSCIBSM).

The experiment to determine the virucidal properties of the disinfectant was performed in three stages. The first stage involved the determination of the cytotoxic effect of the drug for cell cultures, the second - the determination of the virucidal effect of the drug by suspension metod, and the third–the determination of the virucidal effect of the drug on test objects.

At the first stage of the research, we determined the cytotoxic effect of the disinfectant on the continuous cell lines of CK and ShK-2 cells, which are sensitive biological systems for the cultivation of bovine viruses. The cytotoxic effect of the disinfectant on cell culture we determined under the GOST ISO 10993-5-2009. For this purpose, working solutions of disinfectant in the concentrations of 0.001%, 0.01%, 0.1%, 0.25% and 0.5% were prepared. 10-fold dilutions we prepared on 0.2 M phosphate-buffered saline (FSB). The experiment used one-day cultures of CK and ShK-2 cells, which were grown in tubes with 2.0 cm^3 of the growth medium, which included 45.0% Eagle medium (DMEM), 45.0% medium 199, 10% normal bovine serum, and antibacterial drugs (gentamicin sulfate and tylosin solution). Before the experiment, the growth medium was removed from the filled with monolayer tubes, replacing it with a support medium consisting of 50.0% Eagle medium (DMEM) and 50.0% medium 199. Five tubes of continuous cultures of CK and ShK-2 cells were used for each dilution. In addition, five tubes of each culture were used as a control of their condition.

For further study, we used the concentration of the disinfectant solution that did not cause cell death in the monolayer of continuous lines and did not cause the development of any morphological changes in the above cells. Based on the studies, the maximum concentration of disinfectant was taken into account, which did not lead to the development of destructive changes in the monolayer, and continuous cell cultures were selected that better withstood the drug and were sensitive to the studied viruses.

After determining non-toxic dose for continuous cell culture, an experiment was performed to determine the virucidal properties of the disinfectant by the suspension method. For this purpose, a working dilution of disinfectant was prepared, which was not toxic to cell culture and did not cause its death. We used viruses with known infectious activity - strain of bovine IRT virus "Moldavian" (8.1 lg TCD 50/cm^3) and strain of bovine VD virus "BK-1" (7.7 lg TCD 50/cm^3). All viral suspensions were standardized by infectious activity-100 TCD 50/cm^3 (infectious units).

A mixture containing 9.0 cm^3 of the corresponding virus and 1.0 cm^3 of disinfectant was prepared (final concentrations of 0.001%, 0.01%, 0.1%, 0.25% and 0.5%). The resulting mixture was incubated for 20, 40 and 60 minutes at different temperature conditions (10 ± 0.5°C, 20 ± 0.5°C and 37 ± 0.5°C) after that the virus-disinfectant mixture in a volume of 0.2 cm^3 was added to the tubes with a corresponding one-day cell culture with 100% formed monolayer and supporting medium. For each dilution of the drug, for each time duration of incubation and its temperature, five tubes of the corresponding continuous cell culture were used. Under the conditions of the experiment the following controls were used:

- Control of cell culture on a supportive medium–5 tubes each,
- Control of 100 TCD 50/cm^3 of each virus used to determine the virucidal properties of the disinfectant (pathogens of infectious rhinotracheitis, viral diarrhea)–5 tubes each.
- Control of the cytotoxic effect of the drug on cell culture used for the main experiment.

We performed observations and accounting of research results daily for four days. We daily took into account the state of the monolayer, its integrity, changes in cell morphology in respective controls, and test tubes containing a mixture of virus and disinfectant and incubated under different regimes. To determine the virucidal action of the disinfectant on the test objects (wood, tile, metal, glass, plastic, cotton) the test objects under study were contaminated with viruses, treated with respective working solution of disinfectant, and kept at temperature of 20 ± 0.5°C for 20, 40, and 60 minutes. The test objects were then flushed, and the resulting liquid was centrifuged at 1000 rpm. for 15 minutes. The resulting supernatant was used to isolate viruses on susceptible cell cultures in tubes (5 tubes for each exposure). As in the study by the suspension method we used a control of the culture of cells and viruses in test tubes (5 tubes). Observations and accounting of research results were performed daily for 4 days. Positive virucidal activity of the drug was considered in the absence of violations of the integrity of the monolayer and changes in cell morphology in sensitive cell cultures on the 4th day.

Results

To assess the cytotoxicity of the disinfectant, the following concentrations on the nutrient medium were prepared: 0.5%, 0.25%, 0.1%, 0.01% and 0.001%. For each dilution used 5 tubes with one-day cell culture. The disinfectant was added to tubes with continuous cultures of CK and ShK-2 cells with 100% formed monolayer of cells in respective concentrations after removal of the growth medium and the introduction of the support medium. The exposure of the drug interactions with cell culture was 60 minutes, after that the disinfectant was drained from the tubes and a supportive nutrient medium was added. The cytotoxic effect was determined by the presence or absence of cytopathic changes in the monolayer of cell cultures, which was evaluated in crosses. As a control, 5 tubes of each cell culture were used, which were planned for the experiment with a 100% formed monolayer, in which the growth medium was replaced by a supporting one (Table 1).

Table 1. The results of determining the cytotoxic properties of the disinfectant on cell cultures CK and ShK-2.

Cell Culture/ No. Test Tube	Cytotoxic Effect Of Disinfectant / Observation Period, Hours / Disinfectant Concentration, %														
	24 hours					48 hours					72 hours				
	0.5	0.25	0.1	0.01	0.001	0.5	0.25	0.1	0.01	0.001	0.5	0.25	0.1	0.01	0.001
CK 1	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	–	–
CK 2	++ ++	+++ +	++ +	–	–	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	–	–
CK 3	++ ++	+++ +	++ ++	+	–	++ ++	+++ +	++ ++	+	–	++ ++	+++ +	++ ++	+	–
CK 4	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	–	–
CK 5	++ ++	+++ +	++ +	–	–	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	+	–
ShK-2 1	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	–	+	++ ++	+++ +	++ ++	–	+
ShK-2 2	++ ++	+++ +	++ ++	+	–	++ ++	+++ +	++ ++	++	–	++ ++	+++ +	++ ++	++	–
ShK-2 3	++ ++	+++ +	++ +	–	–	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	+	+
ShK-2 4	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	–	–
ShK-2 5	++ ++	+++ +	++ +	–	–	++ ++	+++ +	++ ++	–	–	++ ++	+++ +	++ ++	–	+

Note: Accounting for cytotoxic effects: "-" - no cytotoxic effects, "+" - destruction of the monolayer of cells and change in cell morphology not more than 10%, "++" - destruction of the cell monolayer and change in cell morphology up to 50%, "+++" - destruction of the monolayer of cells and change in cell morphology not more than 70%, "++++" - complete destruction of the cell monolayer and change in cell morphology.

When determining the cytotoxic properties of the disinfectant on different cell cultures with contact for 60 minutes, it was found that the drug in dilutions of 0.5%, 0.25% and 0.1% has toxicity to continuous cell cultures CK and ShK-2 cells. The cytotoxic effect of the drug was observed after 24 hours with the destruction of the monolayer of cells at the level of 70% until their complete death. Disinfectant dilution of 0.01% was less toxic to continuous cell cultures, but it should be noted that within 72 hours its toxicity increased slightly and was characterized by the destruction of the monolayer of cells and changes in their morphology by

10-50%. The destruction of the monolayer of cells and the change of their morphology in individual samples at the level not exceeding 10% were observed under the action of the disinfectant on the culture of CK cells. Disinfectant dilution of 0.01% caused the appearance of minor morphological changes in the structure of the monolayer, which were manifested by an increase in the intercellular space without its significant destruction after 48 and 72 hours. In a dilution of 0.001% disinfectant in the culture of CK cells did not show any signs of adverse effects, which would be characterized by morphological changes in the cells or the destruction of the monolayer within 72 hours. As for the effect of disinfectant in the specified concentration on ShK-2 culture, its insignificant toxicity was established, which manifested itself after 24-72 hours and was characterized by a change in morphology of no more than 10% of cells and an increase in intercellular space in the monolayer.

According to the results of observation of continuous cultures of CK and ShK-2 cells, to which no disinfectant was added in any concentration (control, n=5 for each cell culture), it was found that in all samples there was 100% monolayer performance, and cells of continuous lines had the correct characteristic morphology, which corresponded to the passport data.

According to the results of studies of the cytotoxic effect of the disinfectant, it was found that its smallest effect on these cell cultures is manifested in a maximum concentration of 0.001%.

When studying the virucidal activity of the disinfectant using the suspension method for the preparation of the test mixture (virus-disinfectant), appropriate dilutions of disinfectant were prepared to obtain its final concentration in a mixture of 0.001%, 0.01%, 0.1% and 0.25%. For this purpose, appropriate dilutions of the drug were prepared, namely 0.01%, 0.1%, 1.0% and 2.5%, which were added in a volume of 1.0 cm^3 to 9.0 cm^3 of culture viral biomass (n=9 for each concentration). The resulting virus-disinfectant mixture was incubated at different temperature regimes, namely 10 ± 0.1°C, 20 ± 0.5°C and 37 ± 0.5°C and different exposures involving contact for 20 minutes, 40 minutes and 60 minutes. After incubation of the mixture at the appropriate temperature and exposure period, it was added in a volume of 0.2 cm^3 to a one-day culture of CK cells, which were cultured in test tubes on growth medium. At the time of application of the mixture, the growth medium was replaced with a supportive one. For each dilution of disinfectant, for each time exposure and for each temperature, 5 tubes with cell culture were used. In addition, taking into account the cytotoxic effect of the disinfectant on cell culture in concentrations higher than 0.001%, a corresponding dilution of the mixture was carried out, which was incubated under these modes. The above samples were incubated at 37.0 ± 0.5°C for 4 days.

The results of the experiment were recorded after taking into account the results of control samples-with cell culture (preservation of the monolayer should be at 100%), cell culture that was in contact with the disinfectant (preservation of the monolayer should be at least 70%) and evaluation of cytopathogenic effects of viruses in appropriate cell cultures (it must correspond to the infectious activity of viruses in the passport).

The virucidal activity of the disinfectant against these viral pathogens was considered to be its last dilution (concentration), which delayed the manifestation of the cytopathic effect of viruses in sensitive cell cultures (Table 2).

Table 2. The results of the study of virucidal activity of the disinfectant against the causative agent of bovine infectious rhinotracheitis (strain "Moldavian") in the culture of CK cells.

S.No Test Tube	Incubation Temperatur, ± 0.5°C	Cytopathic Effect Of Irt Virus / Incubation Period Of Virus With Disinfectant, Min / Concentration Of Disinfectant, %											
		20 minutes				40 minutes				60 minutes			
		0.25	0.1	0.01	0.001	0.25	0.1	0.01	0.001	0.25	0.1	0.01	0.001
	10	–	–	++	+	–	–	++	++	–	–	+	–
1	20	–	–	+	+	–	–	+	++	–	–	–	++
	37	–	–	–	++	–	–	–	+	–	–	–	++
	10	–	–	+	+	–	–	+	+	–	–	+	–
2	20	–	–	+	++	–	–	–	++	–	–	+	+++
	37	–	–	–	+	–	–	–	+	–	–	–	+
	10	–	–	+	–	–	–	+	–	–	–	–	++
3	20	–	–	+	++	–	–	–	++	–	–	–	–
	37	–	–	–	++	–	–	+	+++	–	–	–	+++
	10	–	–	++	++	–	–	–	–	–	–	–	++
4	20	–	–	–	++	–	–	–	++	–	–	+	++
	37	–	–	+	+	–	–	+	+	–	–	–	+
	10	–	–	+	+	–	–	–	+++	–	–	++	–
5	20	–	–	–	–	–	–	+	–	–	–	–	+++
	37	–	–	+	+	–	–	–	+	–	–	–	+++

Note: Accounting for cytopathic action: "-" - no morphological changes in cells, monolayer at the level of 100%, "+" - destruction of the monolayer of cells and change in cell morphology not more than 10%, "++" - destruction of the cell monolayer and change in cell morphology up to 50%, "+++" - destruction of the monolayer of cells and change in cell morphology more than 70%, "++++" - complete destruction of the cell monolayer and change in cell morphology.

In order to account the Cytopathic Action (CPA) of the causative agent of bovine infectious rhinotracheitis, microscopy of cell cultures was performed using a biological inverted microscope Medline Scientific 3650.0000 CETI Inverso, using 200 times magnification (10 × 20) (Fig. 1).

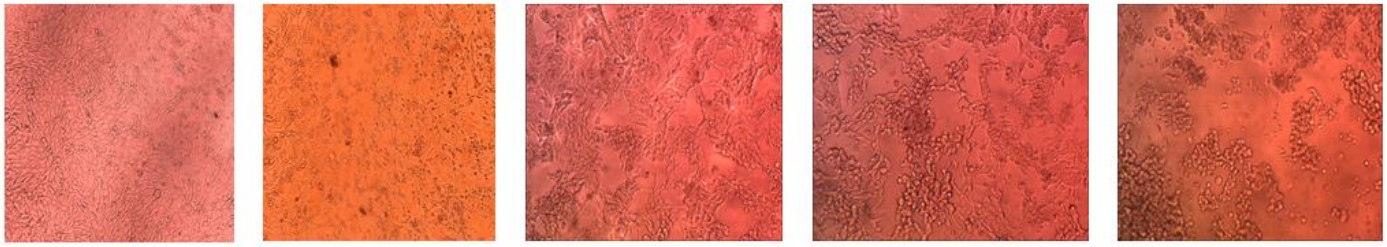

Fig. 1. Accounting for cytopathic action. **a**) morphological changes of cells are absent, monolayer at the level of 100%, **b**) destruction of the cell monolayer and change in cell morphology not more than 10%, **c**) destruction of the cell monolayer and change in cell morphology up to 50%, **d**) destruction of the cell monolayer and change in cell morphology more than 70%, **e**) complete destruction of the cell monolayer and change in cell morphology.

As can be seen from Table 2, the tested disinfectant has virucidal properties against infectious rhinotracheitis virus (completely neutralizes its infectious properties) at a final concentration of not less than 0.1% and exposure of not less than 20 minutes. In addition, it should be noted that the specified drug exhibits virucidal activity at the specified concentration and exposure at a temperature of 10-37 ± 0.5°C. The virucidal activity of the disinfectant against the IRT virus at a concentration of 0.01% was significantly lower, especially when using the drug at a temperature of 10 ± 0.5°C, even with increased exposure to 60 minutes.

Similarly, a study of the virucidal properties of the drug against the pathogen of bovine VD (strain "BK-1") has been carried out (Table 3).

Table 3. The results of the study of virucidal activity of the disinfectant against the causative agent of bovine viral diarrhea (strain "BK-1") in the CK cell culture.

S.No. Test Tube	Incubation Temperatur, ±0.5°C	Cytopathic Effect Of Bovine VD Virus / Incubation Period Of Virus With Disinfectant, Min / Concentration Of Disinfectant, %											
		20 Minutes				40 Minutes				60 Minutes			
		0.5	0.25	0.1	0.01	0.5	0.25	0.1	0.01	0.5	0.25	0.1	0.01
	10	–	–	+++	+++	–	–	+++	+++	–	–	+++	+++
1	20	–	+	+++	++++	–	–	++	++++	–	–	+++	+++
	37	–	+	+++	++++	–	–	++	++++	–	+	++	++++
	10	–	–	++	++++	–	–	++	++++	–	–	++	++++
2	20	–	–	++	++++	–	+	++	++++	–	–	++	+++
	37	–	–	++	++++	–	+	++	+++	–	+	++	++++
	10	–	+	++	+++	–	–	++	++++	–	–	++	+++
3	20	–	–	+++	++++	–	–	+++	++++	–	+	+++	+++
	37	–	+	+++	++++	–	–	++	+++	–	–	+++	+++
	10	–	–	++	++++	–	–	++	++++	–	–	++	+++
4	20	–	–	++	+++	–	+	++	++++	–	–	++	+++
	37	–	++	++	++++	–	–	++	++++	–	–	++	++++
	10	–	+	+++	++++	–	–	+++	+++	–	–	+++	++++
5	20	–	–	+++	++++	–	–	+++	+++	–	–	+++	++++
	37	–	+	+++	++++	–	+	++	++++	–	+	+++	+++

Note: Accounting for cytopathic action: "-" - no morphological changes in cells, monolayer at the level of 100%, "+" - destruction of the monolayer of cells and change in cell morphology not more than 10%, "++" - destruction of the cell monolayer and change in cell morphology up to 50%, "+++" - destruction of the monolayer of cells and change in cell morphology more than 70%, "++++" - complete destruction of the cell monolayer and change in cell morphology.

Cell culture microscopy and accounting for the bovine diarrhea-induced cytopathic effect were performed using a biological inverted microscope Medline Scientific 3650.0000 CETI Inverso with a 100-fold magnification (10 × 10) (Fig. 2).

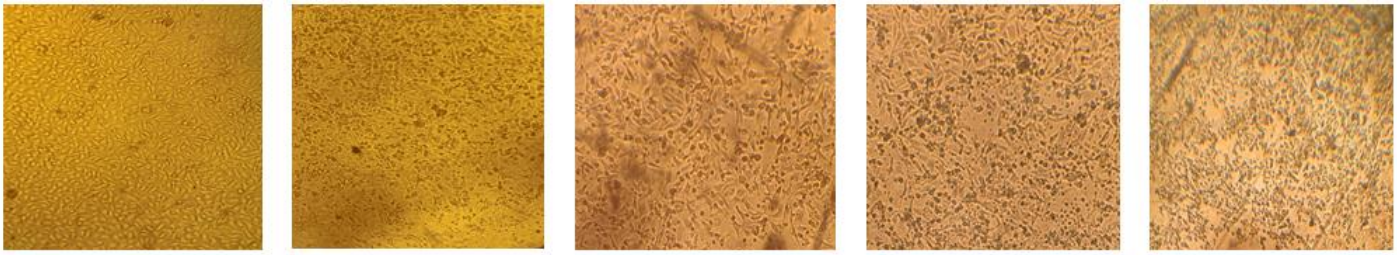

Fig. 2. Accounting for cytopathic action. **a**) morphological changes of cells are absent, monolayer at the level of 100%, **b**) destruction of the cell monolayer and change in cell morphology not more than 10%, **c**) destruction of the cell monolayer and change in cell morphology up to 50%, **d**) destruction of the cell monolayer and change in cell morphology more than 70%, **e**) complete destruction of the cell monolayer and change in cell morphology.

As can be seen from the data in Table 3, the disinfectant has virucidal properties against bovine diarrhea virus (strain "BK-1") at a concentration of not less than 0.5% and exposure of 20 minutes or more. In addition, it should be noted that the specified drug exhibits virucidal activity against the causative agent of bovine VD in the specified concentration and exposure at temperatures from 10 to 37 ± 0.5°C.

Based on the results of research and obtaining positive data on the virucidal properties of the disinfectant against viral pathogens of bovine pneumoenteritis, further studies were performed on test objects using the suspension method. The experiment used the concentration of the drug for the pathogen of bovine IRT–0.1%, and for bovine VD–0.5% at the exposures of 20, 40 and 60 minutes.

After contamination of the test objects (wood, metal, tile, glass, plastic) with the appropriate virus-containing material, they were incubated at room temperature for 1 hour. Sterile pieces of cotton cloth were completely immersed in the virus-containing liquid at the same exposure. After contact between contaminated with agents of IRT and VD test objects and disinfectant in the appropriate concentration and exposure for 20, 40, 60 minutes, the swabs were made. The obtained samples were examined on sensitive cell cultures (Table 4).

Table 4. The results of determining the virucidal properties of disinfectant at a concentration of 0.1% using test objects for bovine IRT virus.

Test Object	Exposure, min	Number of Test Tubes, pcs.	The number of Test Tubes with the Manifestation of CPA, pcs.
Metal	20	5	0
	40	5	0
	60	5	0
Control of the cell culture	CK	5	0
virus control		5	5
Tile	20	5	0
	40	5	0
	60	5	0
Control of the cell culture	CK	5	0
virus control		5	5
Glass	20	5	0
	40	5	0
	60	5	0
Control of the cell culture	CK	5	0
virus control		5	5
Plastic	20	5	0
	40	5	0
	60	5	0
Control of the cell culture	CK	5	0
virus control		5	5
Wood	20	5	0
	40	5	0
	60	5	0
Control of the cell culture	CK	5	0
virus control		5	5
Cotton	20	5	0

40	5	0
60	5	0
Control of the cell culture CK	5	0
Virus control	5	5

According to the results of studies (Table 4) it was found that 0.1% concentration of the disinfectant causes inactivation of infectious rhinotracheitis virus applied to metal, tile, glass, plastic, wood after 20 minutes. Cotton that has been contaminated by immersion to the viral mass of the IRT pathogen, when soaked in 0.1% solution of the drug is disinfected after 20 minutes.

The results of the study of the virucidal effect of the drug in the disinfection of test objects contaminated with bovine diarrhea virus are shown in Table 5.

Table 5. The results of determining the virucidal properties of the disinfectant at a concentration of 0.5% against bovine diarrhea virus using test objects.

Test Object	Exposure, min	Number of Test Tubes, pcs.	The number of Test Tubes with the Manifestation of CPA, pcs.
	20	5	0
Metal	40	5	0
	60	5	0
Control of the cell culture CK		5	0
virus control		5	5
	20	5	0
Tile	40	5	0
	60	5	0
Control of the cell culture CK		5	0
virus control		5	5
	20	5	0
Glass	40	5	0
	60	5	0
Control of the cell culture CK		5	0
virus control		5	5
	20	5	0
Plastic	40	5	0
	60	5	0
Control of the cell culture CK		5	0
virus control		5	5
	20	5	0
Wood	40	5	0
	60	5	0
Control of the cell culture CK		5	0
virus control		5	5
	20	5	0
Cotton	40	5	0
	60	5	0
Control of the cell culture CK		5	0
virus control		5	5

According to Table 5 data it is seen that 0.5% concentration of the drug causes inactivation of the diarrhea virus applied to metal, tile, glass, plastic, wood after 20 minutes. Contaminated with diarrhea virus cotton when soaked in 0.5% solution of the drug is disinfected after 20 minutes.

Discussion

Disinfection of livestock facilities is an integral part of the overall veterinary and sanitary complex, which is carried out on livestock farms and complexes (Hao et al., 2013, Shkromada et al., 2019). This is due to the widespread distribution of pathogenic, opportunistic microflora and exogenous stages of helminth development in animal biocenoses (Tomley & Shirley, 2009, Paliy et al., 2018c, Rahman et al., 2020).

A significant number of agents have been proposed for use in practical veterinary medicine, but the high efficiency of complex disinfectants based on several active substances has been proven (Stegniy et al., 2019, Paliy et al., 2021, Acsa et al., 2021).

To destroy the bovine diarrhea virus, it is recommended to use alcohol-based products with a total alcohol concentration of at least 75% (Kampf et al., 2007).

It is reported that the spectrum of virucidal activity of ethanol in 95% covers most clinically significant viruses, and the addition of acids can significantly increase the virucidal activity of ethanol at lower concentrations (Kampf, 2018). It was found that the drug

"DZPT-2" shows virucidal properties to the causative agent of bovine viral diarrhea at a concentration of 1.0% by the active substance at exposure for 30 minutes (Paliy et al., 2016), and at a concentration of 0.5-1.0% at exposure for 1 hour it kills the African swine fever virus (Paliy et al., 2020a).

In experiments, chlorine dioxide and potassium peroxymonosulfate completely inactivated feline calciviruses and parvoviruses, but the Quaternary ammonium complex was not effective against these test cultures (Eleraky et al., 2002). Chlorine and iodine disinfectants are quite effective against most farm animal viruses, while Quaternary ammonium compounds have low activity (Shirai et al., 2000).

The research results show that the halogen compound, oxidants and a mixture of Quaternary ammonium compounds, alcohol and aldehyde are effective in disinfecting objects contaminated with noroviruses (HuNoV) (Zonta et al., 2016).

Betapropiolactone (BPL) destroys the nucleus of nucleic acids of viruses, but does not damage the capsid. This tool inactivates the bovine IRT virus at a temperature of 4°C for 4, 5 and 12 hours at a concentration of 1:250, 1:500 and 1:1000, respectively, and at a temperature of 37°C it inactivates the virus for 30 minutes at a concentration of 1:250 (Kamaraj et al., 2008).

Disinfection is of paramount importance in the prevention and elimination of parasitic animal diseases (El-Dakhly et al., 2018, Ursache et al., 2019, Paliy et al., 2020b), as well as maintaining high standards of sanitation in the processing plants (Fagerlund et al., 2017, Paliy et al., 2018b). The development of disinfectants for use at low ambient temperatures remains relevant (Jang et al., 2014, Paliy et al., 2020c).

A potential direction is the development of powerful disinfectants from natural compounds, as they can be less toxic, which allows them to be used in the presence of humans and animals, and be environmentally safe for long-term use (Lin et al., 2020).

The organization and adherence to high standards of biosafety in livestock complexes should be combined with the introduction of innovative technological aspects (Manuja et al., 2014, Sinclair et al., 2019).

Conclusion

Disinfectant consisting of a mixture of quaternary ammonium compounds (25.0%), glutaraldehyde (11.0%), isopropyl alcohol, nonionic surfactants has virucidal properties (completely neutralizes the infectious properties of viruses) against pathogens of bovine infectious rhinotracheitis and bovine infectious diarrhea. The use of the drug at a concentration of at least 0.1% neutralizes the virus of infectious rhinotracheitis at an exposure of at least 20 minutes, and at a concentration of at least 0.5% at an exposure of at least 20 minutes inactivates the diarrhea virus at a temperature of 10-37 ± 0.5°C. The test disinfectant decontaminates test objects (metal, tile, glass, plastic, wood, cotton) contaminated with pathogens of infectious rhinotracheitis and bovine viral diarrhea. The disinfectant can be used for current and forced disinfection of livestock premises in livestock farms.

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Citation:

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