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THE STUDY OF THE PROPERTIES OF THE NOVEL VIRUCIDAL DISINFECTANT

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Prevention measures are crucial in actual production, whereas the outbreak of a disease requires immediate detection and elimination of the source of infection as well as complex veterinary and sanitary measures. Here the critical role is attributed to disinfection, which breaks the epizootic chain due to the elimination of pathogenic microorganisms on the objects under veterinary surveillance and involves the application of various disinfectants. **Aim.** The study of virucidal properties of the novel disinfectant DZPT-2. **Methods**. The culture technique was used at the first stage of studies to determine the embryotoxic effect of the preparation on chicken embryos and the cytotoxic effect of the disinfectant on BHK-21 cell culture. The second stage of experiments envisaged the study of virucidal effect of the disinfectant using both the suspension method, test-objects (batiste, wood, glazed tile, metal, glass) and the bioburden. **Results.** The experiments using the agent of bovine viral diarrhea demonstrated that the disinfectant DZPT-2 in the concentration of 1.0 % of the active ingredient (AI) when exposed for at least 30 min and in the concentration of 1.5 % of AI when exposed for 15–60 min disinfects all the test-objects, contaminated by the virus, completely. When exposed for up to 15 min, the disinfectant DZPT-2 does not demonstrate its virucidal effect on Newcastle disease virus, but it disinfects all the test-objects, contaminated by the mentioned virus, when used in the concentration of 0.5–1.0 % of AI and exposed for at least 30 min. **Conclusions.** It was determined that the preparation DZPT-2 demonstrates its virucidal properties in the concentration of 0.5 % of AI (Newcastle disease virus) and 1.0 % of AI (agent of bovine viral diarrhea) when exposed for 30 min. The new disinfectant DZPT-2 is a promising preparation to be used in practical veterinary medicine to prevent and fight viral diseases of farm livestock and poultry.

Keywords: disinfectant, virus, virucidal properties, cell culture, chicken embryos, concentration, exposition.

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INTRODUCTION

Infectious diseases of viral etiology, capable of massive contamination of livestock and rapid distribution, are of remarkable danger for commercial production of livestock and poultry. Newcastle disease (ND) of poultry and bovine viral diarrhea (BVD) are among the most economically significant and dangerous diseases. Regardless of current availability of efficient vaccines against the mentioned diseases, the latter are among the most urgent and severe veterinary problems [1, 2].

Although Newcastle disease does not present a great threat for human health, it has high epizootic relevance for poultry production. Despite the fact that this disease has been known for a long time, the main specificities of its agent and pathogenesis have been studied in a fine detail, and the measures of diagnostics and specific prevention have been elaborated, different countries of the world still register the outbreaks of this disease, which result in considerable economic loss [3].

BVD is an infectious disease, caused by a heterogeneous group of bovine viral diarrhea viruses (BVDV), *Pestivirus* genus, *Flaviviridae* family [4]. The most dangerous situation for the population is the occurrence of agents of a persisting infection, which, in most severe cases, amount to 1–2 % of the livestock, and 60–

80 % of animals are seropositive. Thus, BVDV group viruses are economically relevant pathogens for cattle, which cause considerable losses in animal breeding worldwide [5].

Non-specific prevention of infectious diseases of livestock and poultry is based on a complex of organizational, economic, veterinary, and sanitary measures [6]. The significance of disinfection is highlighted in many references; however a much smaller volume of data has been published regarding the study of the spectrum of antimicrobial properties of new disinfectants.

The specialists of NSC "Institute of Experimental and Clinical Veterinary Medicine" have developed a novel disinfecting aldehyde-based preparation, DZPT-2, intended for preventative and emergency disinfection in farms and complexes of animal breeding. Numerous scientific experiments have confirmed that this disinfectant has a wide spectrum of antimicrobial [7], fungicidal [8] and disinvasion [9] properties.

The work was aimed at studying the virucidal properties of the novel disinfectant DZPT-2.

MATERIALS AND METHODS

The innovative import-substituting disinfecting preparation DZPT-2, elaborated by NSC IECVM, was used in experiments.

The determination of virucidal activity of the disinfectant was performed in two stages. The first stage envisaged the detection of embryotoxic effect of the preparation on chicken embryos and the cytotoxic effect of the disinfectant on BHK-21 cell culture, and the second one – that of the virucidal activity of the disinfectant.

To detect the embryotoxic effect of disinfectant solutions, they were introduced into the allantois cavity of 9–11-day-old chicken embryos in the dose of 0.2 cc. Control chicken embryos were injected sterile water.

The estimation of the cytotoxic effect of the disinfectant involved the preparation of cultural vials with two-day-old monolayer of BHK-21 cell culture, where the growth medium was replaced by maintenance medium. Then consecutive working dilutions of the preparation were prepared in the medium 199. The concentration of the preparation and the obtained dilutions were introduced to the vials with the cell culture in the volume of 0.5 cc. The cytotoxic effect was defined using the scale from 0 to 4. The vials of BHK-21 cell culture, where the growth medium was replaced by maintenance medium, were used as the control.

The virucidal effect of the disinfectant was defined by the suspension method by mixing equal volumes of virus-containing liquid and corresponding concentrations of the preparation with further exposition for a specific time period, then the residual infectivity of viruses was determined by titering them on chicken embryos (ND virus) and cell culture (BVD). To neutralize the disinfectant, its mixture with test-viruses was diluted in 1:10 ratio using phosphate-buffer physiological solution (PBPS).

After obtaining preliminary positive results, the virucidal effect of the preparation on test-objects (batiste, wood, glazed tile, metal, glass) was defined using the bioburden. The test-objects, contaminated with viruses, were treated with working solutions of the disinfectant and exposed for a specific time period. Then the smears of the test-objects were taken, and the liquid obtained was centrifuged at 1,000 rpm for 10–20 min. The supernatant obtained was used to isolate viruses in the living systems (chicken embryos, cell culture) with preliminary 10-fold dilutions of the viral material.

The experiments were accompanied with control of viruses and living systems (chicken embryos, cell culture).

The absence of any changes in the living systems on days 4–5 of the observations testified to positive virucidal effect of the disinfectant. Repeated consecutive 2–3 passages of the material confirmed the virucidal effect of the disinfectant with no changes in the living systems.

The following viruses were used in the experiments:

– ND virus (LaSota strain), *Paramyxoviridae* family, *Avulavirus* genus, RNA-containing, vaccinal, kept in frozen-dried state. The virus was cultivated in 9–10-day-old chicken embryos. The changes in chicken embryos occurred for 80–96 h after the infection and were accompanied with extensive hemorrhagic damages of envelopes and vessels. The titer of the virus was 10^6 lg EID_{50}/cc. To determine the virus in chorioallantoic liquid objectively, the hemagglutination reaction was conducted with 1 % concentration of cock erythrocytes using the common method;

− BVD virus (strain BK-1), *Togaviridae* family, *Pestivirus* genus, RNA-containing, industrial. The virus was cultivated in primary and passaged cell cultures of kidneys of calves and sheep, bovine trachea, lungs of bovine embryo, coronary vessels of calves, and baby hamster kidney (BHK-21). The titer of the virus was 6.75 lg TCD_{50}/cc, the cytopathic effect was observed

in 36–48 h. The virus is sensitive to ether, chloroform, trypsin. The culture of BHK-21 cells, incubated for two days in cultural vials of 250 ml at 37.0 ± 0.5 °C, was used as a biological system for the indication of BD virus. The culture medium was the mixture of media Igla and 199 with the introduction of 10 % of bovine serum; the maintenance medium was medium 199.

RESULTS AND DISCUSSION

At the preliminary stage the embryotoxicity of DZPT-2 preparation was studied *in vitro* using chicken embryos (Table 1) and cell cultures (Table 2).

The results, presented in Table 1, demonstrate the absence of any deviations in the development of embryos, introduced DZPT-2 preparation in the concentration of 0.5 % of AI, for four days of observations. At the same time the toxic effect on embryos was noted with the introduction of the preparation in higher concentrations.

The data, presented in Table 2, demonstrate that the introduction of disinfectant DZPT-2 in the concentrated form results in the death of 30–90 % of cells in the monolayer and the change in the morphostructure of 100 % of living cells. The introduction of the disinfectant in the concentration of 2.0 % of AI results in partial destruction of the monolayer of cells and the change in the morphostructure of 50 % of living cells, whereas the disinfectant in the concentration of 1.5 % of AI results in the modification of the morphostructure of 20–40 % of cells. The introduction of the disinfectant in the concentration of 1.0 % of AI does not change the morphostructure of cells, but terminates their reproduction.

When summarizing the results obtained, it was determined that the cytotoxicity of the preparation DZPT-2 on the scale was 2 points.

The following stage of studies was the determination of virucidal properties of the disinfectant DZPT-2 regarding the test-virus of ND agent using suspension methods. The results of the studies are presented in Table 3.

While studying the virucidal properties of the disinfectant regarding the test-virus of ND agent (Table

Table 1. The effect of the disinfectant DZPT-2 on chicken embryos, $n = 3$

DZPT-2 concentration of AI, %	Number of dead embryos				Number of living embryos
	1st day	2nd day	3rd day	4th day	
0.5	–	–	–	–	4
1.0	1	–	–	–	3
1.5	2	1	–	–	1
2.0	4	–	–	–	0
Control (sterile water)	–	–	–	–	4

Note. "–" – no dead embryos. The number of embryos in each experiments – 4.

Table 2. The effect of the disinfectant DZPT-2 on BHK-21 cell culture, $n = 3$

DZPT-2 concentration, %	The condition of cell monolayer in		
	24 h	48 h	72 h
Concentrated product	Detachment of about 60 % of cells, 40 % of the cells, remaining on the glass, have changed morphostructure	Detachment of about 80 % of cells, 20 % of the cells, remaining on the glass, have changed morphostructure	Detachment of about 90 % of cells, 10 % of the cells, remaining on the glass, have changed morphostructure
2.0	Detachment of about 20 % of cells, 80 % of the cells, remaining on the glass, have changed morphostructure	Detachment of about 30 % of cells, 70 % of the cells, remaining on the glass, have changed morphostructure	Detachment of about 30 % of cells, 70 % of the cells, remaining on the glass, have changed morphostructure
1.5	Monolayer of cells was formed in 90 %, 30 % of which have changed morphostructure	Monolayer of cells was formed for 90 %, 40 % of which have changed morphostructure	Monolayer of cells was formed for 90 %, 40 % of which have changed morphostructure
1.0	Monolayer of cells was formed for 80–90 %	Monolayer of cells was formed for 80–90 %	Monolayer of cells was formed for 80–90 %
Control	Monolayer of 100 %	Monolayer of 100 %	Monolayer of 100 %

3), it was determined that there was no destruction of virus, when the preparation DZPT-2 was used in the concentration of 0.5–1.0 % of AI for 15 min of exposition. This was confirmed by the hemagglutination reaction. However, when the preparation DZPT-2 affected the test-virus in the concentration of 0.5–1.0 % of AI (30–60 min) and in the concentration of 1.5–2.0 % of AI (15–60 min), ND agent was inactivated completely.

The results of the studies in determining the virucidal properties of the disinfectant DZPT-2 regarding the BVD agent were presented in Table 4.

The data, presented in Table 4, demonstrate that the preparation DZPT-2 in the concentration of 0.5 % of AI when exposed for 15–60 min and in the concentration of 1.0 % of AI when exposed for 15 min had only virostatic effect on the BVD agent. When the virus-containing suspension was treated with the disinfectant, the cytotoxic effect of these preparations was observed without any signs of viral CPE, i.e. the preparation DZPT-2 in the concentration of 1.0 % of AI when exposed for 30–60 min and in the concentration of 1.5–2.0 % of AI when exposed for 15–60 min inactivated the BVD agent.

To confirm the results obtained, the virucidal effect of the disinfectant was determined regarding the agents of viral diseases, applied to the test-objects (batiste, wood, glazed tile, metal, glass) with the consideration of the bioburden (bovine serum). The results of the studies are presented in Tables 5 and 6.

The results of the experiment demonstrated (Table 5) that when exposed for up to 15 min, the disinfectant did not have any virucidal effect on ND virus on any test-object.

The prolongation of the duration of the preparation activity had the corresponding effect. Thus, the analysis of the experiment results demonstrated that the preparation DZPT-2 disinfected all the test-objects, contaminated with ND virus, when used in the concentration of 0.5–1.0 % of AI for 30 min.

The experiments with the BVD agent (Table 6) demonstrated that the disinfectant DZPT-2 in the concentration of 1.0 % of AI when exposed for at least 30 min and in the concentration of 1.5 % of AI when exposed for 15–60 min disinfected all the test-objects, contaminated by the virus, completely.

The virucidal effect of the disinfectant DZPT-2 was also confirmed in the experiments using living biological systems.

Table 3. The virucidal activity of DZPT-2 preparation regarding ND virus, $n = 3$

DZPT-2 concentration of AI, %	Exposition, min		
	15	30	60
0.5	+ (1:16≥)	–	–
1.0	+ (1:16≥)	–	–
1.5	–	–	–
2.0	–	–	–
Control			
ND virus	+		
Intact embryos	–		

Note. "+" – positive hemagglutination reaction; "–" – negative hemagglutination reaction.

Table 4. The virucidal activity of DZPT-2 preparation regarding the BVD virus, $n = 3$

DZPT-2 concentration of AI, %	Registration in					
	36 h			48 h		
	Exposition, min					
	15	30	60	15	30	60
0.5	CPE+++	CPE+++	CPE++	CPE++	CPE++	CPE++
1.0	CPE++	–	–	CPE++	–	–
1.5	–	–	–	–	–	–
2.0	–	–	–	–	–	–
Control						
ND virus	CPE++++					
Cell culture	The monolayer of cells was preserved (100 %)					

Note. "–" – the cytopathic effect (CPE) of the virus was absent.

Table 5. The virucidal activity of DZPT-2 preparation regarding ND virus on the test-objects, $n = 3$

DZPT-2 concentration of AI, %	Test-object	Exposition, min		
		15	30	60
0.5	Batiste	+ (1:16≥)	–	–
	Wood	+ (1:16≥)	–	–
	Glazed tile	+ (1:16≥)	–	–
	Metal	+ (1:16≥)	–	–
	Glass	+ (1:16≥)	–	–
1.0	Batiste	+ (1:16≥)	–	–
	Wood	+ (1:16≥)	–	–
	Glazed tile	+ (1:16≥)	–	–
	Metal	+ (1:16≥)	–	–
	Glass	+ (1:16≥)	–	–
Control				
ND virus Intact embryos		CPE++++ The monolayer of cells was preserved (100 %)		

Note: "+" – positive hemagglutination reaction; "–" – negative hemagglutination reaction.

Table 6. The virucidal activity of DZPT-2 preparation regarding BVD virus on the test-objects, $n = 3$

DZPT-2 concentration of AI, %	Test-object	Exposition, min		
		15	30	60
1.0	Batiste	CPE++	–	–
	Wood	CPE++	–	–
	Glazed tile	CPE+	–	–
	Metal	CPE+	–	–
	Glass	CPE+	–	–
1.5	Batiste	–	–	–
	Wood	–	–	–
	Glazed tile	–	–	–
	Metal	–	–	–
	Glass	–	–	–
Control				
ND virus Cell culture		CPE++++ The monolayer of cells was preserved (100 %)		

Note. "–" – the CPE of the virus was absent.

Summarizing the results obtained, it should be noted that the novel disinfectant DZPT-2 has virucidal properties regarding the agents of ND of poultry and BVD.

CONCLUSIONS

It was determined that the preparation DZPT-2 demonstrated its virucidal properties in the concentration of 0.5 % of AI (Newcastle disease virus) and 1.0 % of AI (agent of bovine viral diarrhea) when exposed for 30 min. The new disinfectant DZPT-2 is a promising preparation to be used in practical veterinary medicine to prevent and fight viral diseases of farm livestock and poultry.

Вивчення віруліцидних властивостей новітнього дезінфікуючого препарату

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Безпосередньо на виробництві провідного значення набувають профілактика, а при спалаху хвороби − виявлення та ліквідація джерела збудника інфекції і проведення

комплексних ветеринарно-санітарних заходів. Важливу роль при цьому відіграє дезінфекція, яка забезпечує розрив епізоотичного ланцюга за рахунок знищення патогенних мікроорганізмів на об'єктах ветеринарного нагляду та передбачає застосування різноманітних дезінфектантів. **Мета.** Вивчення віруліцидних властивостей нового дезінфікуючого препарату ДЗПТ-2. **Методи.** На першому етапі досліджень культуральним методом визначали ембріотоксичну дію препарату на курячі ембріони та цитотоксичний вплив деззасобу для культури клітин ВНК-21. Другий етап експериментів передбачав вивчення віруліцидної дії дезінфектанту як за допомогою суспензійного методу, так із застосуванням тест-об'єктів (батист, дерево, кахель, метал, скло) та використанням біологічного навантаження. **Результати.** У дослідах із збудником вірусної діареї великої рогатої худоби встановлено, що дезінфектант ДЗПТ-2 у концентрації 1,0 % за діючою речовиною (ДР) при експозиції від 30 хв та в концентрації 1,5 % за ДР при експозиції 15–60 хв повністю знезаражує всі контаміновані вірусом тест-об'єкти. Дезінфектант ДЗПТ-2 за експозиції до 15 хв не виявляє віруліцидної дії щодо вірусу ньюкаслської хвороби, при цьому знезаражує всі тест-об'єкти, контаміновані даним збудником, при застосуванні в концентрації 0,5–1,0 % за ДР при експозиції від 30 хв. **Висновки**. Встановлено, що віруліцидні властивості препарат ДЗПТ-2 проявляє в концентрації 0,5 % за ДР (вірус ньюкаслської хвороби) та 1,0 % за ДР (збудник вірусної діареї великої рогатої худоби) при експозиції 30 хв. Новий дезінфікуючий препарат ДЗПТ-2 є перспективним при застосування у практиці ветеринарної медицини для профілактики і боротьби з вірусними захворюваннями сільськогосподарських тварин та птиці.

Ключові слова: дезінфектант, вірус, віруліцидні властивості, культура клітин, курячі ембріони, концентрація, експозиція.

Изучение вирулицидных свойств нового дезинфицирующего препарата

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Непосредственно на производстве ведущее значение приобретают профилактика, а при вспышке болезни − выявление и ликвидация источника возбудителя инфекции и проведение комплексных ветеринарно-санитарных мероприятий. Важную роль при этом играет дезинфекция, которая обеспечивает разрыв эпизоотической цепи за счет уничтожения патогенных микроорганизмов на объектах ветеринарного надзора и предусматривает применение различных дезинфектантов. **Цель.** Изучение вирулицидных свойств нового дезинфицирующего препарата ДЗПТ-2. **Методы.** На первом этапе исследований культуральным методом определяли эмбриотоксическое действие препарата на куриные эмбрионы и цитотоксическое влияние дезсредства для культуры клеток ВНК-21. Второй этап экспериментов предусматривал изучение вирулицидного действия дезинфектанта как с помощью суспензионного метода, так и с применением тест-объектов (батист, дерево, кафель, металл, стекло) и использованием биологической нагрузки. **Результаты.** В опытах с возбудителем вирусной диареи крупного рогатого скота установлено, что дезинфектант ДЗПТ-2 в концентрации 1,0 % по действующему веществу (ДВ) при экспозиции от 30 мин и в концентрации 1,5 % по ДВ при экспозиции 15−60 мин полностью обеззараживает все контаминированные вирусом тест-объекты. Дезинфектант ДЗПТ-2 при экспозиции действия на протяжении до 15 мин не проявляет вирулицидного эффекта в отношении вируса ньюкаслской болезни, а обеззараживает все тест-объекты, контаминированные данным возбудителем, при применении в концентрации 0,5−1,0 % по ДВ при экспозиции от 30 мин. **Выводы.** Установлено, что вирулицидные свойства препарат ДЗПТ-2 проявляет в концентрации 0,5 % по ДВ (вирус ньюкаслской болезни) и 1,0 % по ДВ (возбудитель вирусной диареи крупного рогатого скота) при экспозиции действия 30 мин. Новый дезинфицирующей препарат ДЗПТ-2 является перспективным при использовании в практике ветеринарной медицины для профилактики и борьбы с вирусными заболеваниями сельскохозяйственных животных и птицы.

Ключевые слова: дезинфектант, вирус, вирулицидные свойства, культура клеток, куриные эмбрионы, концентрация, экспозиция.

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