THE VIRUCID ACTIVITY OF *DECONTAMINOL** BIOCIDE

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Abstract

The purpose of the paper was to investigate the virucide activity of a disinfecting, product based on quaternary amines mixed with glutaraldehyde. The virucide activity was determined against the viruses of the Newcastle disease (ND), aviary influenza (AI), infectious aviary bursitis (IAB) and the infectious aviary bronchitis (IB). Solutions in phosphate buffered saline (PBS), each of 10^{-4} DIE_{50}/ml of the mentioned viruses were put into contact for 10, 30 and 60 minutes with DECONTAMINOL 0.1% dilutions in sterile hard water[4]. After the time for contact ended, the mixtures of virus and DECONTAMINOL have been into SPF embryos, with an age of 9 days. Each embryo received $2x10^{-3}$ DIE_{50}. The alanto-amniotic fluid was examined for hemaglutinant activities (ND, AI), the embryos for the presence of the specific lesions of IAB, IB. DECONTAMINOL 0.1% dilution had an intense virucide activity (100%) against ND, AI and IAB viruses, which were destroyed after 10 minutes of contact. IAB virus was by the 0.1% DECONTAMINOL solution after 60 minutes of contact.

Key words: *quaternary amines, glutaraldehyde, virucide activity, aviary viruses (ND, AI, IAB, IB)*

Introduction

Quaternary amines are ammonium salts in which the organic radicals have been substituted by 4 hydrogen atoms. This substitution allowed important biological and physico-chemical properties, among which we mention the biocide activities, a high electrolytic power and good water solubility. The biocide activity was mentioned in an ample monography, which studied the bactericide, fungicide and bacteriostatic activity of the quaternary amines (14).

Due to these properties, the aminoquaternary are used as antiseptic agents, detergents, disinfecting and sanitation agents, algaecide and emulsifiants (6).

As antiseptic agents they have bacteriostatic and bactericide effect on Gram positive and Gram negative germs and display antifungal activity and fungicide activity on protozoa (13).

The *quaternary amines* have a low toxic activity on the teguments and are not toxic to the environment (6).

Glutaraldehyde is a saturated dialdehyde used as disinfectant and chemical sterilizer due to the wide bactericide, fungicide, tuberculocide, virucide and slow sporicide spectrum (5, 8).

DECONTAMINOL is a disinfecting solution based on quaternary amines (alkyl dimethylphenol o/p-ammonium chloride) mixed with glutaraldehyde. Due to its bactericide and bacteriostatic, antifungal and algaecide activity, the manufacturer recommends the use of DECONTAMINOL for prophylactic and necessary disinfection of various surfaces (flooring and walls) in animal farms, in the food industry (dairy products factories, slaughterhouses, meat processing units, etc.) and to disinfect the tires of the cars and vans hauling animals.

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* *DECONTAMINOL* is a ROMVAC Co. S.A. disinfecting solution prepared from a mixture of quaternary amines 15 % and glutaraldehyde 5 %.

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[4] The hard water contains 300 µl/calcium carbonate.

DECONTAMINOL is used as 1% disinfecting solution after the hydromechanical cleaning of the flooring, walls, working tables, installations, etc.

The working dilution has a low level of aggressiveness on the environment and is not toxic to the persons handling the substances. The mechanism of action of the quaternary amines and glutaraldehyde on the microorganisms is not known.

The literature mentions the high resistance in the environment of the aviary viruses, which cause important economic damages, the viruses of the *Newcastle disease* (ND), *aviary influenza* (AI), *infectious aviary bursitis* (IAB) and the *infectious aviary bronchitis* (IB) (1, 4, 7, 9).

These viruses resist at 56°C for 6 minutes to 6 hours. IAB resists in the environment between 2 months and 2 years, which makes it one of the toughest viruses (2, 9).

The purpose of the paper was to study the virucide activity of *DECONTAMINOL* on ND, AI, IAB and IB viruses.

Material and method

1. **Working solution**, *DECONTAMINOL* diluted in sterile hard water was tested for embryonic toxicity in dilutions of 10%, 1%, 0.1%, and 0.01%. The 0.1% dilution was selected to study the virucide activity; this dilution ensured the survival of all inoculated SPF embryos. The hard water was processed according to SR EN 14675 Standard and it contains 300 μg/l calcium carbonate. After preparation, the hard water was sterilized for 15 minutes at 121°C.

2. SPF[5] embryos age 9 days were inoculated via the intraalantoamniotic route with 0.2 ml of a mixture of equal parts of dilution 10^{-4} DIE_{50}/ml of the virus with 0,1% *DECONTAMINOL* solution, after a contact of 10, 30 and 60 minutes. Each embryo was inoculated with $1 \times 10^{-3} DIE_{50}$ ND, IB, AI and IAB virus.

3. The used viruses are from the strain collection of ROMVAC COMPANY SA, as follows:

a) *Newcastle disease* virus, La Sota strain, with titre $10^{-9,2} DIE_{50}$/ml;

b) *Infectious aviary bronchitis* virus, ***hot*** P.A. strain, with titre $10^{-7,7} DIE_{50}$/ml;

c) *Aviary influenza* virus, ***Avx*** – 7367 strain, with titre $10^{-9} DIE_{50}$/ml;

d) *Infectious aviary bronchitis*, $\boldsymbol{H_{120}}$ strain, with titre $10^{-6,7} DIE_{50}$/ml.

The virus dilutions have been done in saline phosphate buffer with pH 6.8-7.0, at the temperature of 20°C.

Experimental model

Groups of 10 SPF embryos age 9 days were inoculated with 0.2 ml as follows:

- 3 groups of embryos with a mixture of ND virus and *DECONTAMINOL* 0.1%, after a contact of 10, 30 and 60 minutes;
- 3 groups of embryos with a mixture of IAB virus and *DECONTAMINOL* 0.1%, after a contact of 10, 30 and 60 minutes;
- 3 groups of embryos with a mixture of AI virus and *DECONTAMINOL* 0.1%, after a contact of 10, 30 and 60 minutes;
- 3 groups of embryos with a mixture of IB virus and *DECONTAMINOL* 0.1 %, after a contact of 10, 30 and 60 minutes;
- 3 groups with 0.1 ml of ND virus dilution $10^{-4} DIE_{50}$/ml at intervals of 10, 30, 60 min.;

[5] Specific Pathogen Free.

- 3 groups with 0.1 ml of IAB virus dilution $10^{-4}DIE_{50}$/ml at intervals of 10, 30, 60 min.;
- 3 groups with 0.1 ml of AI virus dilution $10^{-4}DIE_{50}$/ml at intervals of 10, 30, 60 min.;
- 3 groups with 0.1 ml of IB virus dilution $10^{-4}DIE_{50}$/ml at intervals of 10, 30, 60 min.;
- 1 control group not inoculated;
- 1 control group inoculated with 0.2 ml PBS/embryo;
- 1 control group inoculated with 0.2 ml hard water/embryo;

During the contact period between the virus and the *DECONTAMINOL* solution, the tubes with virus were kept in the refrigerator, same as for the virus dilutions.

After inoculation, the embryos were evaluated daily by ovoscopy for 7 days. During this interval, the embryos which died within 24 hours after inoculation were regarded as accidents and removed, while the embryos which died after this interval were examined for the hemaglutinant activity of the alanto-amniotic fluid for viruses ND and AI.

Viruses IB and IAB produce embryo lesions, each dead embryo being examined. At the end of the experiment, the live embryos were slaughtered and examined.

The experiment conducted at the refrigeration temperature of + 4-8°C was repeated, this time the suspensions being kept at +20°C.

The alanto-amniotic fluid collected from the slaughtered embryos after 60 minutes of contact, were used to make two blind passages for each category of virus. The SPF embryos age 9 days were inoculated with 0.2 ml sterile alanto-amniotic fluid and evaluated daily by ovoscopy. At the end of the 7 days, the embryos were slaughtered and the alanto-amniotic fluid was used for the third passage.

Results and discussions

Tables 1, 2 and 3 show the experimental results.

Table 1

Testing the embryotoxicity of the *DECONTAMINOL* solutions

Group	DECONTAMINOL solution in hard water	Number of inoculated embryos	Amount of inoculum	Embryo mortality			
				Dead	Survivors	%	Total dead/ total inoculed
1	10%	10	0,2	10	0	100	10/10
2	1%	10	0,2	9	1	90	9/10
3	0,1%	10	0,2	0	10	0	0/10
4	0,01%	10	0,2	0	10	0	0/10

Table 2

Virucide activity of 0.1% *DECONTAMINOL* solution

No.	Inoculum	Period of contact			Virucide activity, %		
		10 minutes Positive*/ Total	30 minutes Positive*/ Total	60 minutes Positive*/ Total	10 minutes	30 minutes	60 minutes
1	ND virus + D	0/9	0/10	0/10	100	100	100
2	IAB virus + D	0/9	0/10	0/9	100	100	100
3	AI virus + D	0/10	0/10	0/10	100	100	100
4	IB virus + D	8/10	2/10	0/10	20	80	100
5	ND virus	10/10	10/10	10/10	-	-	-
6	IAB virus	10/10	9/10	8/10	-	-	-
7	AI virus	8/10	9/10	8/10	-	-	-
8	IB virus	9/9	10/10	8/10	-	-	-
9	Control	0/10	0/10	0/10	-	-	-
10	PBS control	0/9	0/10	0/9	-	-	-
11	Hard water, control	0/10	0/9	0/10	-	-	-

* Positive/Total (with HA activity or with embryo lesions/total inoculated).
D – DECONTAMINOL

Table 3

Results of the blind passages of the alanto-amniotic fluid (a.a.f.) collected from the slaughtered embryos

Group	a.a.f. inoculum virus	Embryo inoculum	First passage			Second passage		
			Positive*	Survived with no lesions	Total positive/ inoc.	Positive*	Survived with no lesions	Total positive/ inoc.
1	BN	20	0	10	0/10	0	10	0/10
2	IA	20	0	10	0/10	0	10	0/10
3	BIA	20	0	10	0/10	0	10	0/10
4	BI	20	0	10	0/10	0	10	0/10

Positive* – embryos with specific embryonic lesions and with hemaglutinant activity.

The results from Table 1 show that the 0.1% *DECONTAMINOL* dilution ensured the survival of all inoculated embryos, which is why this dilution was selected for the experiment. The 0.1% dilution supports the completely atoxic activity of the product.

The virucide activity of Decontaminol was manifested after the first 10 minutes of contact against the viruses of the Newcastle disease, IAB and aviary influenza. The quaternary amines and the glutaraldehyde may act on the

neuraminidases and hemaglutinins encountered on the surfaces of the two viruses and cancelled their infectivity and pathogenicity. The hemaglutinine and neuraminidase receptors are very receptive to the lytic action of the quaternary amines. The quaternary amines and glutaraldehyde, particularly, destroy the viral capside, preventing the infection mechanism of ND, IAM and AI viruses on the erythrocytar cells (10, 11).

In the case of IB virus, even though it also has hemaglutinine on the surface of the capside, the biochemical structure of the hemaglutinine is different from that of the other two viruses. The hemaglutinine of IB virus has 51% proteins, 34% lipids, traces of carbohydrates and RNA (12). It is possible that the lipids and, particularly, the proteins within the viral hemaglutinine make it more resistant to the action of the biocide substance.

In the case of the IAB virus, the action of the quaternary amines in combination with the glutaraldehyde was fast and didn't need a long time of contact with the virus to destroy the viral capside, such it was the case with Catiorom. IAB virus is particularly resistant in the environment (2). The literature shows the virucide and slow sporicide activity of glutaraldehyde, but gives no concrete evidence on its action on different types of viruses, and doesn't mention whether this action is accompanied by the destruction of the viral particle. The blind passages we did after collecting the a.a.f. from the slaughtered embryos, which had a 60 minutes contact with the biocide substance, showed the virucide activity of the two substances against the aviary viruses. This supports the stronger virucide activity of Decontaminol biocide compared to Catiorom (3).

Conclusions

1. Decontaminol biocide, prepared from quaternary amines and glutaraldehyde, when used in 0.1% dilution, didn't produce embryo mortality in SPF embryos.
2. The 0.1% dilution of Deconaminol has 100% virucide activity against ND, IAB and AI viruses after 10 minutes of contact.
3. The 0.1% dilution of Deconaminol has 100% virucide activity against IB virus after 60 minutes of contact.
4. The virucide activity of Decontaminol biocide has been confirmed by two blind passages.

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