فاعلية بعض المطهرات الكيماوية فى بعض العدوى الفيروسية للدواجن
١ ـ فيروس النيوكاسل

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الملخص

(خمسة جداول ورسم بيانى)

فى هذه الدراسة تم اختبار فاعلية بعض المطهرات الكيماوية على فيروس النيوكاسل- وذلك باستخدام الطريقة القياسية المقننة من الجمعية البيطرية الألمانية فى عام ١٩٧٤ والمشروحة بالتفصيل فى هذا البحث .

تم اختبار فاعلية كل من ٢٪ فورمالين ، ٢٪ ليزوفيت ، ٢٪ صوديوم هيبوكلوريت كمطهرات لفيروس النيوكاسل فى التجارب السائلة (Suspension experiments) وعلى الأسطح الملوثة بالميكروب ب (Surface carrier exper) هذا وقد استخدمت أجنة بيض الدجاج لاستبيان مدى فاعلية هذه المطهرات .

وأمكن تلخيص النتائج كالآتى :

١ ـ فى التجارب السائلة أثبت كلا من الفورمالين والليزوفيت فاعليتهما فى القضاء على الفيروس فى خلال ١٥ دقيقة ، حتى مع وجود مواد حامية مثل مصل الأبقار الخالى من الاجسام المناعية .

٢ ـ لم يثبت هيبوكلوريت الصوديوم أى فاعلية فى القضاء على الفيروس مع وجود المواد الحامية له .

٣ ـ فى تجارب تطهير الأسطح الملوثة بالفيروس أمكن تطهير الشاش الملوث فى ١٥ دقيقة ، وقشر البيض الملوث فى مدة ٣٠ دقيقة ، بينما صعب تطهير الخشب الملوث حتى بعد ٦٠ دقيقة .

٤ ـ أمكن استنتاج أن فاعلية المطهرات الكيماوية على الفيروسات تعتمد على نوعيد الفيروس ، المطهر المستخدم ، نوع السطح الحامل ، مدة التعرض للمطهر بالاضافة الى درجة الحرارة أثناء عملية التطهير .

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ANTIVIRAL ACTIVITY OF SOME CHEMICAL DISINFECTANTS AGAINST SOME POULTRY PATHOGENIC VIRUSES

I. NEWCASTLE DISEASE VIRUS (NDV)

(With 5 tables, one figure)

By

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SUMMARY

A standard method for evaluation disinfectant activity developed by the German Veterinary Sociaty (1974), which is also fully described, was used in this investigation. The antiviral activity of 2% formalin, 2% lysovet[R] J-forte and 2% sodium hypochlorite against NDV was studied in suspension and organism carrier experiments. Results obtained are summerised in the following :

1. In suspension experiments, formalin and lysovet [R] J-forte in 2% concentration inactivated NDV within 15 min. exposure even in the presence of cattle serum (protecting substance).
2. 2% sodium hypochlorite failed to show any disinfecting activity on NDV in the presence of the protecting substace.
3. In carrier surfaces disinfection experiments with formalin, NDV gauze-carrier surface was inactivated in 15 min., whereas on egg shell carrier needed 30 min exposure,Wood carrier proved to be difficult to disinfect even after 60min. exposure.
4. It could be concluded that disinfecting activity depends on the virus to be disinfected, disinfectant chosen, kind of carrier surface, exposure time and temperature.

INTRODUCTION

The increasing need for disinfection in the course of general health prophylaxis in intensive animal breeding, which is now a significant feature of poultry production has led to the appearance of numerous disinfectant preparations in the market.

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R= Fa. Schulke and Mayer (W. Germany)

REBER (1973)a defined disinfection as the selective elimintation of certain undesirable micro-organisms inorder to prevent their transmission and is achived by action on their structure or metabolism irrespective of their functional state. SCHLIESSER (1974) a found that this definition fullfills the veterinary interest, while SCHMIDT (1973) and GEISSLER(1974) prefair waiting until it's usefellness is proved by time.

HARMSEN *et. al.* (1955), GOLDMANN (1956), ALBRECHT (1957), HOLZ (1958), SCHRAMM (1962), HARMS (1963), SCHMIDT & GROSSGEBAUER (1963). SCHMIDT (1973), GREUEL (1963), STRAUS (1965), SPROSSIG & MUCKE (1965), ROLLE & MAYR (1966), WEUFFEN (1967), FRIEDEMANN (1967), KIRCHHOFF (1967, 68&69), SAKOMYRDIN (1967), SPICHER (1970), NOSLER (1970), STELLMACHER and SCHWEBS (1970), OSTERTAG (1971), PERKINS & MOSSEL (1971), MEHLHORN & BEER (1972), MUSSGAY *et. al.* (1972), WILLINGER & THIEMANN (1972), REBER (1973 b), STEUER & LUTZDETTINGER (1973), STELLMACHER *et. al.* (1973), HUDEMANN & WEUFFEN (1973), ROJAHN (1973), SCHLIESSER (1974 a & b) and GEISSLER (1974) discussed in details chemical disinfectants. their classification, mode of action, factors influencing their activites (time allowed, temperature, pH and presence of protecting substances ect.) as well as the basic requirement needed for an efficient disinfectant.

The absence of a standard method for evaluation of the disinfecting activity of viricidal preparations has led to contradicting and in some times considerable results. Therefore the German Veterinary Socitey (GVS) in 1974 presented "Guidelines for testing chemical disinfectants".

The aim of this study is to test the antiviral activity of 2% formalin 2% lysovet J-forte and 2% sodium hypochlorite on the virus of Newcastle disease (NDV) according to the GVS-guidelines.

MATERIALS AND METHODS

Virus strain:

The Italia strain, a velogine NDV was used as a model for enveloped lipid-containing virus.

Disinfectants:

2% solution of each of formalin; lysovet[R]J-forte (Iodophor) and sodium hypochlorite in distelled water were used.

Test system:

Fertile chicken eggs obtained from the Specific Pathogen Free (SPF) Farm, at the poultry diseases Institute in Giessen. The numbers of eggs used in each experiment are the given elsewhere.

Cattle serum:

A final concentration of 40% of sterile antibody free inactivated, cattle serum containing 6% total protein was used as protecting substance.

Evaluation of disinfecting activity

1. *General* :

The method developed by GVS-1974 is done in two steps: *Preliminary Testing*: (suspension experiments), This gives a hint about the viricidal activity of a disinfectant under favourable conditions. If the results of this preliminary testing were unsatisfactory further testing is not necessary.

Main Testing: (Germ-carrier experiments.) This is carried out to determine the efficiancy of a disinfectant in practical conditions.

2. *Preliminary Testing*:

2.1 *Determination of pH-Value*:

pH of disinfectant in the applied concentration (DC) chosen in distilled water as well as that of reaction mixture (DC, virus and serum) were determined by an electric pH-meter.

2.2 *Determination of toxic effect of disinfectant on test system*:

The toxicity was determined in the same test system used for virus demonstration i.e. corresponding old chicken embryo or tissue culture A 10^{-1} dilution of the DC. was inoculated in the test system and observed for the same period as the virus control. For virus control phosphate buffer saline (PBS) substituted the disinfectant. solution.

2.3 *Determination of the Viricidal activity of disinfectant in supension*:

2.5 ml-test virus (with at least 10^7E ID_{50}/ml) diluted with 2.0 ml PBS were added to 0.5 ml. of 10-fold (10X) DC. The reaction mixture was allowed to stay at 20-22 C°. 0.1 ml. of the reaction mixture was then pippetted at 15, 30 and 60 minutes (min) and added immediately to 9.9 ml PBS to stop further effect of disinfectant. The virus dilution in this case was 10^{-2}. Using PBS 2 further tenfold dilutions (10^{-3}, 10^{-4}) were then prepared. Five embryos were inoculated with 0.2 ml./dilution/egg. Parallel Virus control was prepared in the same way using PBS instead of disinfectant.

2.4 *Determination of the Viricidal activity in suspension in the presence of protecting substance*:.

2.5 ml test virus and 2.0 ml cattle serum were mixed well and 0.5 ml. DC in 10x-fold DC was then added. The same procedure in (2.3) for further dilution and inoclation. was used. An effective disinfectant concentration was the dilution in which the test virus could not be demonstrated after exposure time of 15, 30 and 60 min.

3. *Main testing*: "*Organism experiments*".

When the preliminary testing proved sucessful the organism carrier experiment is to be done. The main testing represents the adverse condition which could be met with in the practical course of the disinfection Process, but dosnt substitut a field experiment. Gauze, egg-shell and wood were used as carrier surfaces in these experiments. Disinfectant concentration and virus dilution used were as in (2.2 & 2.3(. The experiments were done with cattle serum. The virus suspension was mixed with serum and left on the carrier surface to dry; then the latter was placed in applied DC. This was carried out as follows:

3.1 *Gauze-carrier*:

A sufficient number of guaze pieces each consisting of two layers of 2.0 cm^2) were sterilized by (dry heat) in a pertirdish. 0.1 of ml serum virus suspension was dropped on each carrier sufrace and then allowed to dry for 90 min at 37 C°. The carriers were then placed in petri dish containing the applied DC. for 15, 30 and 60 min. They were then transferred immedialty in 9.9 ml PBS and homoginised by Ultra-Turrax (WZ), centrifuged at 1.600 g/10 min. and finally inoculated in test system.

3.2 *Egg shell*:

A sufficient number of 2 Cm^2 dry sterilized egg shell pieces having no shell membrane. were treated with 0.1 ml. of the serum virus mixture and allowed to dry in the dissicator for 90 min at 37 C°. After 15, 30 and 60 min. exposure to the desired DC. Egg shell was grinded in a sterile morter with 9.9 ml. PBS. The suspension was then treated as under (3.1).

3.3 *Wood carrier*:

A sufficient number of wood pieces (2 Cm^2 X 1mm thick) were autoclaved as the absorption capacity of wood is reduced by dry sterelization. Disinfection procedure and evaluation was carried out as (3.1).

A disinfectant concentration was considerd effective, when infectious virus was not to demonstrable in the test system. Two carrier surface experiement were necessary for final judgmant of the efficacy of a disinfectant preparation.

4. *Virus purification and assay*:

Virus preparations were partially purified with Frigen 113 (ISMALI 1966). Demonstration of virus in the test system was done by the rapid hemagglutination on a glass slide. The EID_{50} of demonstrable virus was calculated after SPERMAN & KARBER (1973).

5. *Titer reduction*:

A completely efficient disinfection was considered to be achieved when infectious virus could not be demonstrated in the tested samples. A reduction of virus titer (experssed as the difference between the end titer of control titration and end titer of disinfestant treated virus) was parallel to the disinfecting activity. A titer reduction udner 90% was considered insingnificant (ALBRECHT & WASIELEWSKI 1958).

6. *Effect of homoginization on carries surface on virus tites*:

Wood carrier was subjected to homoginization for 45 & 90 seconds to determine it's effect on virus titer in relation to rise of temperature occuring during the process.

RESULTS

1. *Disirfectant toxicity to test system and pH-value of* D.C.:

The results of detemination of the pH value and toxic effect of each of 2% solution of formalin, lysovet[R] J-forte and sodium hypochlorite on the test system are summerised in table (1);

TABLE 1. pH of disinfectant concentration and its toxicity to test system

Disinf.	pH	observation period (in days)						
		1	2	3	4	5	6	cont.
formal 0.2% (2%) . . .	3.8	+ 0/5	0/5	0/5	0/5	0/5	0/5	0/5
lysovet 0.2% (2%) . . .	2.7	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Sod. hyp. 0.2% (2%) . .	10.6	0/5	0/5	0/5	0/5	0/5	0/5	0/5

cont = Contrl

+ = embryos showing lesions / embryos inoculated

It was proved that D C inoculated non toxic, as Lesions of any kind could not be detected.

TABLE 2. **Effect of formalin 2% on NDV in suspension**

in presence of cattle serum exposure time (min.)						in absence of cattle serum exposure time (min.)					
dil.	pH.	15	30	60	Virus cont.	dil.	pH.	15	30	60	Virus cont.
		+									
10^{-4}		0/5	0/5	0/5	5/5	10^{-4}		0/5	0/5	0/5	5/5
10^{-3}	6.9	0/5	0/4	0/5	5/5	10^{-3}	6.2	0/5	0/5	0/5	5/5
10^{-2}		0/5	0/5	0/4	5/5	10^{-2}		0/5	0/5	0/5	5/5
titer reduction			$> 10^{6.1}$					$> 10^{6.1}$			
Disinf succ. %			$> 99.9\%$					$> 99.9\%$			

\+ embryos showing HA activity embryos inoculated

From table (2) is apparent that 2% formalin inactivated NDV completely in 15 min, even in the presence ofprotectign substance.

TABLE 3. **Effect of lysovet[R] J-forte 2% on NDV in suspension**

in presence of cattle serum exposure/min.						in absence of cattle serum exposure/min.					
dil.	pH.	1	30	60	virus cont.	dil.	pH.	15	30	60	virus cont.
10^{-4}		0/3	0/5	0/4	5/5	10^{-4}		0/3	0/5	0/4	5/5
10^{-3}	6.9	0/5	0/5	0/4	5/5	10^{-3}	6.2	0.5	0/5	0/4	5/5
10^{-2}		0/5	0/5	0/5	5/5	10^{-2}		0/5	0/5	0/5	5/5
titer reduction			$> 10^{6.1}$					$> 10^{6.1}$			
Disinfection success %			$> 99.9\%$					$> 99.9\%$			

2% lysovet[R] J-forte could easily inactivate NDV in suspension irrespective of the presence of a protecting substance.

TABLE 4. Effect of 2% sodium hypochlorite on NDV in suspension

in presence of cattle serum exposure/min						in absence of cattle serum exposure/min					
dil.	pH.	15	30	60	virus cont.	dil.	pH.	15	30	60	virus cont.
10^{-1}		5/5	2/5	1/5	5/5	10^{-1}		0/5	0/4	0/5	5/5
10^{-2}	8.8	5/5	4/5	2/5	5/5	10^{-2}	8.5	0/5	0/4	0/4	5/5
10^{-3}		5/5	4/4	3/5	5/5	10^{-3}		0/4	0/3	0/5	5/5
titer reduction			0					> $10^{6.1}$			
Disinfection succ. %			0%					> 99.9%			

TABLE 5. Effect of formalin 2% on NDV carrier surfaces

	1st Trial				2nd Trial			
org.-carrier	exposure time/min 15	30	60	virus cont.	exposure time/min 15	30	60	virus cont.
Gauze . . .	+ 0/38	0/40	0/40	$10^{6.9}$	0/40	0/38	0/37	$10^{6.9}$
Disinf succ.%	>99.9%	>99.9%	>99.9%			>99.9%		
egg shell . .	4/40	0/37	0/38		7/40	0/36	0/38	$10^{6.9}$
Disinf. succ. %	90%	>99.9%	>99.9%	$10^{6.9}$	82%	>99.9%	>99.9%	
wood	32/40	14/40	0/40	$10^{6.7}$	38/40	16/40	3/40	$110^{6.7}$
Disinf succ. %	20%	65%	>99.9%		5%	40%	92.5%	

Embryos showing HA. activity
Emprvos inoculated.

It was proved that DC inoculated in embryonated chiken eggs is non toxic, as lesions of any type could not be detected. (Table 1)

2. *NDV-partial purification and titration*:

Virus assays on original and partially pruifed with Frigen 113 revealed virus titers of $10^{8.1}$ and $10^{7.1}$ EID_{50} 0. lml. respectively.

3. *Suspension experiments:*

Disinfecting activities of each of 2% solution of formalin lysovet and sodium hypochlorite against NDV in the presence and abscence of cattle serum as protecting substance are shown in Tables (2, 3 and 4)

Table (4) shows that 2% sodium hypochlorite could only inactivate NDV in the absenc of protecting substance.

4. *NDV carrier surface experiments:*

Since it was shown in suspension experiments that formalin and lysovet in 2% concentraion hav the same inactivation capacity against NDV under favourable or undfavourable conditions, formalin was chosen for disinfection of carrier surfaces from economic point of view (REBER 1973 b).

The results of NDV carrier surface experiments treated with 2% formalin are summerized in Table (5) and diagramaticall illustrated in fig. (1).

In two trials NDV-carrier experiments were done under conditions simulating practical field disinfection (under unfavourably influencing factors). They revealed that gauze carrier surface could be easily disinfected with formalin 2% even within 15 min. exposure. Egg shell carrier treated in the same manner needed at least 30 min. On the conterary the wood carrier chowed dificulty to disinfect even after 60 min. exposure.

5. *Effect of homoginziation on the NDV on wood carrier:*

In 3 trials homogenization of the carrier surface for 45 & 90 seconds was carried out. NDV titer and temperature of suspension were recorded and given in Table (6).

TABLE 6. **Effect of homoginization on NDV.**

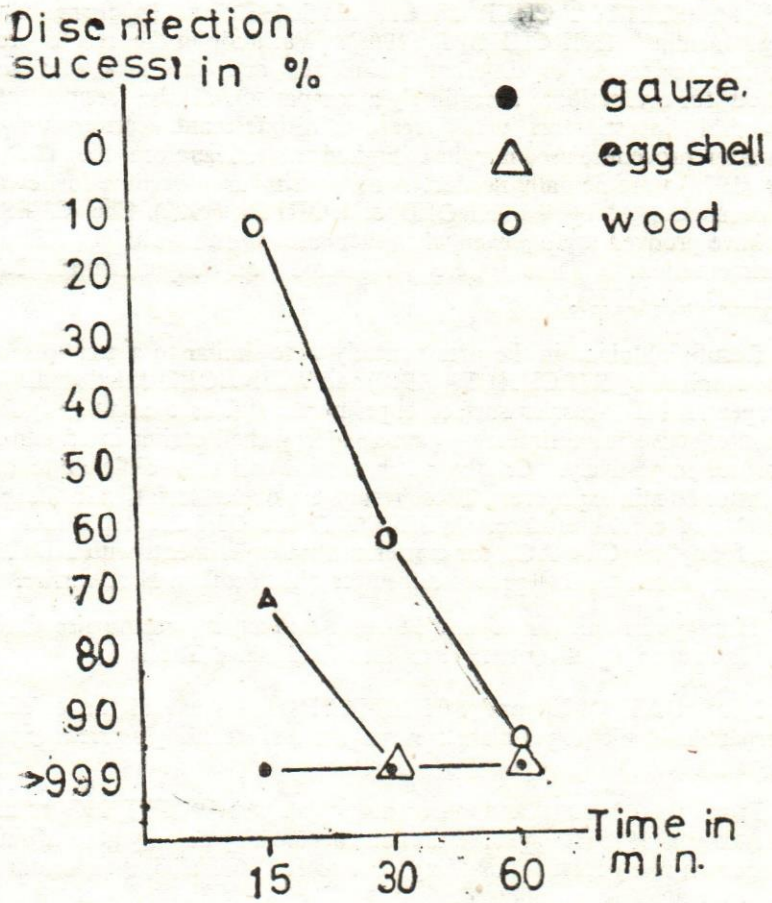
	45 second homoginization			90 second homoginization		
	Trials			Trials		
Virus titer	1	2	3	1	2	3
EID50	$10^{6.9}$	$10^{6.5}$	$10^{6.9}$	$10^{6.9}$	$10^{5.3}$	$10^{6.5}$
Temperature . . .	35.6	34.7	35 C°	51	49.1	49.4C

The titer fall was + 0.43 (log 10) at = 14.7 C°

DISCUSSION

Disinfection is a process and not a state where its serves a specific purpose and is intended to prevent transmission of infection or microbial contamination.

Fig. I
Effect of Formalin 2 %
on: ND-WIRUS



The NDV was subjected to disinfection studies by several investigators using suspension experiments (TILLEY & ANDERSON 1947, CUNNIGHAN 1948, BEAMER & PRIER 1950 and WRIGHT 1974). The results obtained in our in suspension experiment revieled that 2% sodium hypochlorite was unable to have any disinfecting activity on NDV in the presence of protecting susbtances. Formalin and lysovet in 2% concentrations have an effieicient disinfectant effect on NDV. The addition of serum as protecting substance made it uneasy to compare with the results obtianed by the fore mentioned investigators.

Oragnism carrier surface was introduced by HOLZ (1958), REUSS (1962 1963), WASIELEWSKI & KOBERG(1961), KIRCHHOFF (1968) and NEUMANN 1964, & 1971. The results obtained in those investigations were contradiciting but in some cases were considerable; fore example the formalin concentration needed for efficient disinfection varied from 3% to 7.5% (HOLZ, 1958) 5% (STELLMACHER & SCHWEBS, 1970) and 1% formalin containing "Incidin" (KIRCHHOFF 1968). Variation in the results reported were due to the use of different techniques and the lack of a standard method for evaluation, accordingly a comparison of the results obtained by several investigators with regard to disinfestnant, carrier surface and evaluation procedure used may be of limited value. Therefore the GVS-guidelines (1974) were actually needed. A nearly similar procedure for evaluation has been adopted by WEINHOLD & KOHLER (1972). The GVES-Guide lines have prodved useful, even when ther are some technical difficulties in experimentation. These were dissucsed on experimental basis by WOELFING (1975).

Results obtained in the present study were similar to a great extent with those obtained by KIRCHHOFF (1968) and WEINHOLD & KOHLER (1972). In repeated NDV-carrier surface experiments (tables 5 and fig 1) formalin proved effective for disinfecting gauze and egg shell carrier in 15 and 30 min exposure, respestively. On the other hand wood was difficiult to disinfect even after 60 min exposure. These results are due to variation in the physical condition of carrier surface. WEINHOLD & KOHLER (1972) used lorasol -V a Jodophor (Ciba AG.) for carrier surface experiments with NDV. After 45 min exposure egg shell proved difficult to disinfect than wood carrier.

Homoginization for 45 and 90 second and temperature rise during the process had no significant effect on results obtained Table (6).

MUSSGAY (1972) and SCHLIESSER (1974) are of the opinion that an organism carrier experiment can substitute successfully a praxis experiment (field-trial).

From these results and other investigations (WOELFING et al 1975, ISMAIL et al 1975) it appeared that the disinfesting activity of a disinfectant depends on many factors including virus to be disinfested, disinfectant chosen and exposure time allowed.

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