فاعلية بعض المطهرات الكيماوية فى بعض التدوى الفيروسية للداوجن

٢ ـ فيروس السيلو

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الملخص العربى

فى هذه الدراسة أمكن اختبار فاعلية كل من ٢٪ فورمالين ، ٢٪ ليزوفيت ، ٢٪ صوديوم هيبوكلوريت كمطهر لفيروس السيلو الواسع الانتشار وذلك باستخدام الطريقة القياسية المقنعة من الجمعية البيطرية الألمانية فى عام ١٩٧٤.

وأمكن تلخيص النتائج فى الآتى :

١ ـ أبدى فيروس السيلو مقاومة كبيرة لتاثير المطهرات ٠

٢ ـ فى التجارب السائلة (Suspension experiments) وفى وجود مواد حامية (مصل الأبقار الخالى من الأجسام المناعية) كانت المدة اللازمة للقضاء على الفيروس باستعمال الفورمالين واللزوفيت هى ٦٠ دقيقة ٠

٣ ـ لم يثبت الصوديوم هيبوكلوريت أى فاعلية فى القضاء على الفيروس مع وجود المادة الحامية حتى ولو بعد ١٢٠ دقيقة ٠

٤ ـ فى تجارب تطهير الأسطح الملوثة (Snrface carrier cxper.) بالفيروس أمكن تطهير الفيروس على قشر البيض والشلش الملوث بعد التعرض للفورمالين لمدة ١٢٠ دقيقة ، بينما صعب تطهير الخشب الملوث وكانت نسبة التطهير بالنسبة له أقل من ٩٠٪ حتى ولو بعد ١٢٠ دقيقة من المعاملة ٠

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ANTIVIRAL ACTIVITY OF SOME CHEMICAL DISINFECTANTS AGAINST SOME POULTRY PATHOGENIC VIRUSES

II. CELO-VIRUSS.

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(With 5 tables one figure)

By

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SUMMARY

In the course of this investigation, the antiviral activity of 2% formalin, 2% Lysovet[R]J-forte and 2% sodium hypochlorite was studied on the wide host range CELO-virus. Experimental procedure used, was that discribed by GVS (1974).

Results obtained could be summerised in the following :

1. CELO-virus showed comparativly greater resistance to disinfection.

2. In suspension experiments 2% formalin and 2% Lysovet[R]J-forte needed at least 60 min. exposure to inactivate CELO-virus in the presence of protecting substance.

3. 2% Sodium hypochlorite failed to show any disinfecting activity against CELO-virus in suspension in presence of protecting substance ever after 120 min exposure.

4. In CELO-carrier surface experiments with 2% formalin an effective disinfection could be obtained in case of gauze and egg shell carriers only after 120 min. exposure. Wood-carrier showed difficulty to disinfection. < 90% disinfection success was obtained even after 120 min. exposure.

INTRODUCTION

Chicken Embryo Lethal Orphan (CELO-Viruses), which were identified as avian adeno viruses have a wide host range. They were reported in quail (Du BosE et al 1958, Du Bose and GRUMBLES 1959), in chickens (WOERNLE and BRUNNER 1963, ERDOS 1964, KRAUSS 1965, ISMAIL

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

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1966, AHMED et al 1968 a.b and 1969), in ducks (GRENEL 1966, AHMED *et al* 1969 AHMED 1971 b), in geese (KALETA 1969 and CSONTOS 1967), in turkeys (AHMED 1971 a.b 1973). CELO-viruses might be present in other spieces of birds not yet investigated GREUEL (1966).

Numerous investigations had revealed that there were variations in pathogenicity among CELO-Viruses for poultry. They caused motsly inapperant or latent infection in chicken (YATES and FRY 1957, YATES *et al* 1960, CHOMIAK et al 1961, BURKE et al 1965 and COOK 1968). Due to this latency the vius was at one time thought to be the cause of lymphomatosis in chicken (FONTES et al 1958, SHARPLESS et al 1958 and BURMESTER *et al* 1960)., then it was identified as avian adenovirus (BURMESTER et al 1960, SHARPLESS and JUNGHERR 1961 and SHARPLESS 1962).

In chickens CELO-infections were sometimes associated with mild respiratory manifestation, conjunctivitis, diarrhea, low egg production and nervous manifestation (WOERNI E and BRUNNER 1963,KAWAMURA et al 1963, 1964, ISMAIL 1966 and AHMED and EL SISI 1969). Strains of CELO-viruses were also isolated from appearently healthy chickens (CLEMMER 1964, 1965, KHANNA 1964). In some experimental trials CELO infections passed symptomless (BURKE *et al* 1959, KOHN 1962, - and MONREAL 1968).

On the other hand certain CELO-virus strains were responsible for a specific disease in quail "Quail Bronchitis" (DU BOSE et al 1958 and DU BOSE and GRUMBLES 1959).

CELO-infections could not be neglected as a complicating factor in the course of some diseases of poultry i.e. mycoplasmosis and infectious bronchitis (IB) (MONREAL and AHMED 1963), ISMAIL 1966, GESSLER 1966, MONREAL 1966, 1968 and AWAD et al 1973). GESSLER (1966) is of the openion that a corrilation existed between hygenic condition and the appearance of CELO and infectious bronchitis antibodies in chicken sera.

The aim of this study was to test the antiviral activity of 2% formalin, 2% Lysovet[R]-J-forte and 2% sodium hypochlorite on CELO-virus following the German Veterinary Sociaty (GVS) "Guidelines for testing chemical disinfectants (1974).

MATERIALS and METHODS

1.1: Virus-strain.

CELO-PHELIPS Strain in 13 th egg passage was used as model for non enveloped, lipid-free viruses.

1.2: Disinfectant:

Formalin 2%, lysovert[R]-J forte 2% and sodium hypochlorite 2% in distelled water.

1.3: Test system:

9 to 11 days living embryos were used in this study. Embryonated eggs were obtained, from poultry diseases Institut's SPF-Farm (Giessen, W. Germany). Numebr of embryos used in these experiments were included in the tables.

1.4: Cattle serum:

40% inactivated antibodies free sterile cattel serum with 6% protein was used as protecting substance in this study.

2. Methods:

The following procedures were described in details ISMAIL et al (1975).

A. prelminary testing:

2.1: pH-value : PH- of disinfectant in applicable concentration as well as disinfectant virus serum mixture were determined by electrical PH-meter.

2.2 Determination of disinfectant toxicity on test system.

2.3 Viricidal activity of disinfectant in suspension.

2.4 Viricidal activity of disinfectant in suspension in presence of protecting substance (cattle serum).

B. Main testing:

2.5 CELO-carrier surfaces: gauze, egg shell and wood.

2.6 Titer reduction.

2.7 Virus assays:

Demonstration of viable (multiplication cabaple) virus was Judged by the pathological manifestation induced in incoulated chicken embryos. These included death, petechial hemmorrhage especially on extremites, dwarfing, liver necrosis and sometimes defective feathering were observed. Titer calculation was done after SPERMAN & KARBER (1973).

RESULTS

1. Virus assay:

phelips strain-CELO- virus was propagated in chicken embryos. Two successive passages were needed to reach $10^{7.5}$ ELD_{50} 0.1ml. (concentration required for test virus GVS-1974).

2. Disinfectant toxicity to test system and PH value of disinfectant:

Results of disinfectant toxcicity to test system and pH value are summerised in table (1).

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

Disinfectant	pH-value	observation period in days						
		1	2	3	4	5	6	Control
Formalin . . . . . (0.2%)[a]2%	3.7	0/5*	0/5	0/5	0/5	0/5	0/5	0/5
Lysovet . . . . . (0.2%)[a]2%	2.6	0.5	0/5	0/5	0/5	0/5	0/5	0/5
Sodium hypochlorite (0.2%)[a]2%	8.7	0/5	0/5	0/5	0/5	0/5	0/5	0/5

* embfyos showing lesions / embroys inoculated
a) end concentration for inoculation (GVS — 1974).

From the results in (table 1) it was proved that 2%, formalin, 2% lysovet[R] and 2% sodium hypochlorite has no toxic effect on test system.

3. Suspension experiments:

Disinfecting activity of 2% formalin, 2% lysovert[R]-J forte and 2% sodium hypochlorite against CELO-vorus in presence and absence of Organic matter protecting substance) were determined in suspension trials. Results obtained are summerised in tables (2, 3 & 4) respectively.

TABLE 2. **Effect of 2% formalin on CELO virus in suspension**

in presence of serum exposure/min						in absence of serum exposure/min				
dil	pH	15	30	60	virus cont .	pH	15	30	60	virus cont
10^4		0/5*	0/4	0/5	5/5		0/5	0/5	0/5	5/5
10^3	7.05	3/5	2/5	0/5	5/5	6.7	1/5	0/3	0/4	5/5
10^2		5/5	3/5	0/5	5/5		3/5	1/5	0/5	5/5
tier reduction		$10^{4.4}$	$10^{5}>$	$10^{6.5}$			$10^{5.2}$	$10^{6.2}$	$>10^{6.5}$	
disinurscess .		<99.9%		99.9%			<99.9%		>99.9%	

From table 2 it is appearent that 2% formalin inactivated CELO-virus only after 60 min. exposure even in absence of protecting substance.

* embroys with CLO lesions embryos inoculated.

TABLE 3. Effect of 2% Lysovet J-forte on CELO-virus in suspension.

With serum exposure time/min.						Without serum exposure time/min.				
dil.	pH	15	30	60	virus cont.	pH.	15	30	60	virus cont.
10^4		4/5*	2/5	0/4	5/5		2/5	1/5	0/5	5/5
10^3	5.75	5/5	4/5	0/5	5/5	4/2	3/5	1/4	0/5	5/5
10^2		5/5	5/5	0/4	5/5		4/5	3/5	0/5	5/5
titer reduction .		0	0	$>10^{4.5}$			0	0	$>10^{4.5}$	
disinfsucess . .		0	0	>99.9%			0	0	>99.9%	

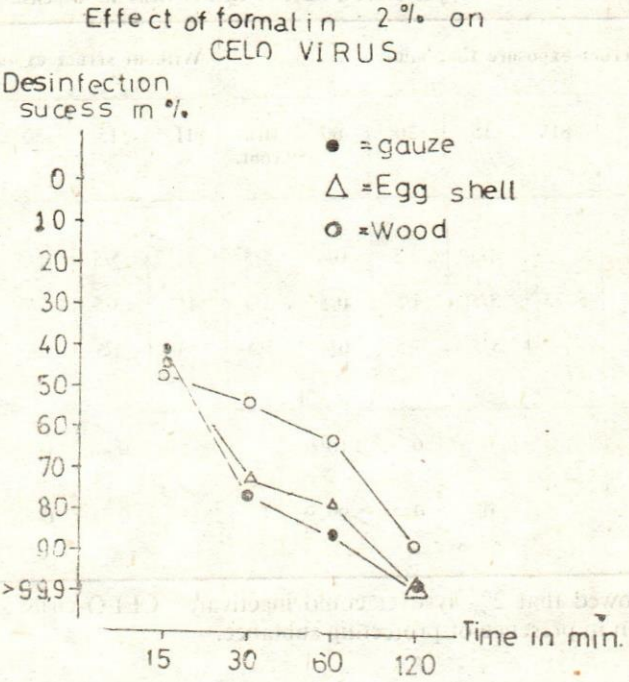
Table (3) showed that 2% lysovet could inactivate CELO-virus after 60 min exposure even in presence of protceting subtance.

TABLE 4. Effect of 2% sodium hypochlorite on CELO-Virus in suspension

With serum exposure time/min.							Without serum exposure time/mix					
dil.	pH.	15	30	60	120	virus cont.	pH.	15	30	60	120	virus cont.
10^4		4/4*	5/5	2/5	2/5	5/5		5/5	5/5	0/4	0/5	5/5
10^3	10.5	5/5	5/5	3/4	2/5	5/5	9/7	4/4	4/5	0/5	0/5	5/5
10^2		5/5	5/5	4/5	4/5	5/5		5/5	4/4	0/5	0/5	5/5
titer reduction		0	0	0	0	0		0	0	$>10^{4.5}$	$<10^{4.5}$	
disinfsucess		0	0	0	0	0		0	0	>99.9%		

2% Sodium hypochlorite failed to show any activity against CELO- virus in presence of cattle serum even after 120 min. exposure. In absence of protecting subtance, 60 min. were sufficient to inactivate the same virus.

Fig.1



4. CELO-carrier surfaces experiments:

From economic point of view formalin was chosen for such experiments. Results of two separate trials (GVS 1974) are summerised in tabel (5

TABLE 5. Effect of 2% formalin on CELO-carrier surfaces

Carrier surface	1st trial exposure/min. 15	30	60	120	virus cont.	2nd trial exposure/min. 15	30	60	120	virus cont.
Gauze . . .	+ 15/37	9/40	7/38	0/38	$10^{7.1}$	17/39	9/39	4/40	0/40	$10^{7.1}$
Disinfsucess	59.5%	77.5%	81.5%	>99.9%	—	56.5%	76.4%	90.1%	>99.9%	—
Egg shell .	18/38	11/38	8/38	0.37	$10^{7.3}$	16/40	9/40	8/40	0/40	$10^{7.3}$
Disinfsucess	47.3%	71.1%	79%	>99.9%	—	60%	77.5	80%	>99.9%	—
Wood . . .	23/40/	17/40	14/40	6/40	$10^{6.9}$	12/38	19/38	16/40	4/38	$10^{6.9}$
Disinfsucess	42.5%	57.5%	65%	85%	—	44.7%	50%	60%	89%	—

+ Embroys with CELO - lesions / Embroys inoculated

In two trials CELO- carrier surfaces were subjected to disinfection with 2% formalin. Gauze and egg shell carriers could be disinfected sucessfully after 120 min. exposure. On the other hand wood showed difficulty to disinfect. It remained in both trials under 90% A better look could be seen in figure (1).

DISCUSSION

Intensive poultry breeding to obtain optimal production is now the interest of veterinarians. In this breeding system high productive birds are easily desturbed by specific and nonspecific noxiousness. Therefor the problem of infections control appears in fore-ground. From this stand point of view, desinfection gained more interest in comparison with older times (SCHLIESSER 1974 a. b) as tool in infection control.

The importance of avian adenoviruses was not only dut to their preliminary pathogenicity which is seldom, but also due to the complicating role of such viruses in other infections (ISMAIL 1966, GESSLER 1966, MONREAL 1966, 1968 and AHMED 1971) Beside this Gessler (1966) assumed that a relationsip was present between hygenic condition of a flock and the appearance of CELO and infectiuos bronchitis antibodies in poultry.

Results obtained in this study reveald that 2% formalin and 2% lysovet J forte could inactivate CELO- virus after 60 min. exposure in the presence of protedting substances. 2% Sodium chypoc hlorite inactivated -CELO virus after 60 min : only in absence of protecting substances. It failed inactivate CELO- virus even after 120 minexposure in presence of inactivated antibodies free steriile cattle serum at 20- 20°c. Similar viricidal activites against CELO- VIRUS were obtained by MAHNEL (1974). He could obtain disinfection effect with jodophore and aldehydes while cholorinated compounds were of limited values in suspension experiments. OXFORD & POTTER (1969) obtained complete inactivation of avian adeno type 1 with formalin 0.004% after 24 hours exposure at 35-36 C° but not at 4C°E even after 14 days exposure using the same concentration. PETEK et al (1963) a, b) could inactivate CELO- Virus in 30 min., when a mixture from 7 parts ethyl alcohol 95% and 1 part tencture iodine was used. They failed to obtain inactivation effect of the same virus with phenol 2%, ethyle alchol 50 % after 24 hours exposure.

From this investigation and other results obtained with Newcastle disease virus ISMAIL et al (1975) it appeared that CELO- virus was more defficult to disinfect. This conclusion agreed with (MAHNEL 1974& GEISSLER 1974),where lipid free viruses showed great resistance to desinfection.

Desinfection of CELO- carrier surfaces were carried out in two trials (GVS 1974). 2% Formalin proved effective for ganze and egg-shell- carrier, deinfection occured only after 120 min. exposure. On the other hand wood carrier showed grate defficulty to desinfection as the disinfection sucess

was under 90% even after 120 min. exposure. This was better demonstrated in (Figure 1 and Table 5).

Results of CELO- virus carrier experiments showed no deviation from that obtained in Newcastle - carrier experiments, in that wood was always more dificult to desinfect than gauze and egg shell carrier surfaces ISMAIL et al (1976) .

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