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LABORATORY EVALUATION OF SELECTED DISINFECTANTS ON GUMBORO DISEASE VIRUS

(With 2 Tables)

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التقييم المعملى لبعض المطهرات المختارة ضد فيروس الجامبورو

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تم اجراء هذه الدراسة لتقييم فاعلية مركبات اليود والجلوتر الدهيد معمليا ضد فيروسات مرض الجامبورو. تم عزل فيروس الجامبورو من طيور بلدى ساسو عمر ٣٥ يوم. تم تقييم فاعلية المطهرات عن طريق حساب عيارية الفيروس قبل وبعد المعاملة بالمطهر سواء فى وجود مواد عضوية أو فى غير ذلك وقد اختبرنا ثلاثة تخفيفات للمطهرين محل الدراسة. وتم قياس تركيز أيون الهيدروجين فى تركيزات المطهرات المستخدمة. من خلال هذه الدراسة لاحظنا قصور فى طريقة الحساب المتبعة. لحساب الانخفاض الحادث فى عيارية الفيروس بعد المعاملة بالمطهرات فقد وجدنا فرضاً أنه لو كانت عيارية الفيروس 10^6 EID_{50}/0.1 ml أن هذه العيارية قد انخفضت بعد المعاملة بالمطهرات إلى 10^2 EID_{50} /0.1 ml. فإن الجزء الذى انخفض فى عيار الفيروس يتم حسابه على أنه 10^4 EID_{50}/0.1 ml ولكن اذا ما حسبنا المقابل الحسابى للوغاريتم للعيارات السابقة لوجدنا ١٠٠٠٠٠٠ وحدة فيروس للعيار 10^6 EID_{50}/0.1ml ، ١٠٠ وحدة فيروس للعيار 10^2EID_{50}/0.1 ml ، ١٠٠٠٠ وحدة فيروس للعيار 10^4EID_{50}/0.1 ml وعند طرح ١٠٠ من ١٠٠٠٠٠٠ يكون الناتج ٩٩٩٩٠٠ وحدة فيروسية وهذا الناتج لا يعبر عنه العيار 10^4EID_{50}/0.1 ml و أن هذه النتيجة سوف تؤدى الى خطأ فى الحساب ولذلك لجأنا الى حساب انخفاض عياريه الفيروس عن طريق حساب المقابل للوغاريتم وطرح قيمة الانخفاض فى عدد الوحدات الفيروسية لحساب كفاءة التطهير. من خلال هذه الدراسة وجدنا أن تركيز أيون الهيدروجين فى محلول اليود ١ : ٥٠ كان ٦,٢ وأن هذا التخفيف قد أدى إلى انخفاض عياريه الفيروس بنسبة ٩٩,٩٣% بعد ١٥ دقيقة وبنسبة ٩٩,٩٧ بعد ٦٠ دقيقة وعند التخفيف بنسبة ١: ١٠٠ كان تركيز أيون الهيدروجين ٦,٥. وقد أحدثت هذا التركيز انخفاض فى عياريه الفيروس بنسبة ٩٩,٧% بعد ١٥ دقيقة و ٩٩,٩٩% بعد ٦٠ دقيقة. أما التخفيف ١ : ٢٠٠ فقد كان تركيز أيون الهيدروجين له ٦,٧ وقد أحدث انخفاض فى عيارية الفيروس بنسبة ٩٩,٦% ، ٩٩,٨، ٩٩,٩% بعد ١٥ ، ٣٠ و٦٠ دقيقة على التوالي. وقد حصلنا على

نتائج مقاربة لهذه عند استخدام مواد عضوية بنسبة ٤٠% وبالرغم من اختلاف هذه النتائج عن نتائج الآخرين الذن أشاروا إلى تأثر مركبات اليود بالمواد العضوية. فقد وجدنا أن تركيز أيون الهيدروجين فى المحلول بعد اضافة المواد العضوية قد تحولت إلى القلوية وهو السبب وراء هذا التأثير على فيروس الجامبورو لتأثرالأخير بالوسط القاعدى وهذه النتيجة لن تكون موجودة فى عائلات فيروسية أخرى. ويمكن أيضا أن نضع فى الاعتبار إجراء التجربة تحت الظروف المعملية وهو ما يختلف كثيرا عن الظروف الحقلية. أما عند استعمال المطهرات التى تحتوى على مركب الجلوترالدهيد فقد لاحظنا فاعلية المركب عند تخفيفه بنسبة ١:١٠٠ ، ١:٢٠٠ الا أن التركيز الاخير كان أكثر أماناً على أجنة البيض المستعملة كنموذج بيولوجى للتقييم وأن قدرته على خفض عيارية الفيروس محل الدراسة كانت مماثلة للتخفيف ١:١٠٠بنسبة ٩٩,٩% وأحدث المطهر نتائج مماثلة عند استعماله فى وجود المواد العضوية ويمكن أن نعزى السبب فى ذلك إلى زيادة تركيز أيون الهيدروجين تجاه القلوية وهذا الأخير له قدرة على التاثير على فيروس الجامبورو.

SUMMARY

The efficacy of iodine and glutraldehyde containing compounds against infectious bursal disease virus (IBDV) was assayed by comparing the virus titer before and after exposure to each disinfectant. The test was conducted at room temperature in presence or absence of organic matter "40% foetal bovine serum". The two tested disinfectants were effective against IBDV under all test conditions and at the dilutions used 1:50, 1:100 and 1:200. In this paper a new method for calculation of viral regression after disinfection, was devised as it gives a more reasonable calculation.

Key words: *Chicken, gumboro disease, disinfectant*

INTRODUCTION

Infectious bursal disease is one of the widely spreaded acute, highly contagious diseases of young chickens that had lymphoid tissue target with special predilection for the bursa of fabricius (Lukert and Saif, 2003).

Disease prevention depends on proper disinfection of poultry premises, proper management and specific active immunization (Bayoumie, 1997).

Faragher (1972) stated that contaminated poultry premises is the main source for IBDV infection, this is helped by the very stable physicochemical properties of IBDV (Benton *et al.*, 1967).

The short life span of broilers (30-35 day) is insufficient to generate active immunity. For this, disease prevention strategies should run parallel to ensure successful poultry operation (Bayoumie, 1997).

Few scientific researches about chemical disinfection of IBDV are available. The present work evaluates commercially available iodine, and glutraldehyde containing disinfectant on IBDV in presence or absence of organic materials after different reaction times.

MATERIALS and METHODS

A-Materials:

1- Samples for isolation of IBDV:

Samples for IBDV isolation were collected from a native saso chicken flock 35 day old exhibiting 80% morbidity and 50% mortality. The affected flock was showing signs and post mortem lesion specific for IBD as described by Lukert and Saif (2003). Severely hemorrhagic and inflammed bursae were collected for virus isolation (Rosenberger *et al.*, 1998).

2- IBDV live vaccine:

An intermediate (D-78) live vaccine strain kindly obtained form Prof. Dr. S.Assily, Poultry Vaccine Dept., Serum & Vaccine Res. Inst. El-Abbassia.

3- IBDV reference antigen and antisera:

IBDV "serotype 1" reference antigen and antisera were obtained from the international marketing center, Cairo, Egypt.

4- Test system:

Embryonated chicken eggs (ECE) from a small native breeder flock that received influenza vaccination only were used for virus isolation, viral propagation, viral identification and testing disinfection potency.

5- Foetal bovine serum:

Foetal bovine serum produced by "life technologies" obtained from Bardisi medical. This serum was virus and mycoplasma tested, and it was heat inactivated at 56°C for 30 min before use.

6- Tested disinfectants:

a- Glutraldehyde containing compound, (62.5 gm/liter) was tested as antiviral agent against IBDV, at dilutions 1:50, 1:100 and 1:200.

b- Iodine containing compound 2.5% active iodine w/v. was similarity tested.

7- Water diluents:

Under ground water from a commercial poultry farm was used after being autoclaved.

8- Source of organic matter (OM):

40% solution of foetal bovine serum in autoclaved distilled water was prepared, and was used as a source for organic matters as adapted by Ismail *et al.* (1976).

B- Methods:

1- Sample preparation for viral assay:

Collected bursae were grounded. AGPT against reference IBDV antigen and antisera was performed (Beard 1980), positively reacting bursae, were further processed for virus isolation (Rosenberger *et al.* 1998, Senne, 1998). The CAM route was chosen for virus isolation, specific mortality and PM lesion were recorded as previously described by Hitchner (1970). Examination for heat resistance (56°C for 5 hrs) was performed as described by Benton *et al.* (1967). Virus neutralization was performed according to Thayer and Beard (1998).

2- pH measurement:

pH of tested concentration for both disinfectants in presence or absence of (OM) was determined using an electrical pH meter (Jenway 3510).

3- Safety of disinfectant for the test system:

The tested dilutions of each disinfectant was inoculated in 5 ECE, daily mortality was recorded after neglecting the non specific mortality.

4- Testing Virucidal activity of the selected disinfectants in presence or absence of organic matters:

Isolated IBDV was 10 fold serially diluted once using sterile saline and another in 40% foetal bovine serum. Selected test disinfectant were diluted 1:50, 1:100 and 1:200 using the autoclaved under ground water as diluent. A 0.5 ml of the viral saline suspension or viral serum suspension was added to 0.5 ml of the tested disinfectant dilutions. A reaction time 15, 30 and 60 min. was given for this mixture. Each

dilution at every reaction time was inoculated in 5 ECE via allantioc sac route (AS) to ensure precise reaction time limits.

5- Calculation of disinfectant potency:

Mortality of inoculated chicken eggs was daily recorded and was specifically confirmed through the examination of inoculated CAM in AGPT. Titer calculation was preformed according to Spearman and Karber Cunningham (1973). Antilog 10 of the obtained titer was calculated. Reduced virus titer (c) was calculated by subtracting the antilog of post disinfection titer (b) from the antilog of virus control (a). The percent of disinfection success (d) is obtained by dividing c/a.

6- Preparation of IBDV hyperimmune sera:

Some of the fertile chicken eggs were used to hatch a day old chicks. The latter were eye inestillated repeatedly every four days with the live intermediate IBDV vaccine until birds became 60 day old, collected sera were examined with AGPT for IBDV precipitating antibodies.

RESULTS

1- Clinical picture and necropsy findings in the field case:

The examined native saso flock was 35 day old, showed 80% morbidity and 50% mortality. The PM lesion observed was, dehydrated darkened skeletal muscles with subcutaneous hemorrhage on thigh muscles; urate deposition in the urters; enteritis, the liver is enlarged with peripheral areas of infarction, hemorrhages at the juncture of proventriculus and gizzard, spleen is mildly enlarged with necrotic foci on its surface, and the bursae were enlarged and surrounded by gelatinous hemorrhagic fluid. Their plicae were also hemorrhagic.

2- Results of viral assay:

a- Virus isolation:

Embryo mortality due to inoculated bursal homogenate is shown in Table (1). Precipitins specific for IBDV was observed after the 2nd passage. The 3rd passage was performed for further adaptation on test system. The PM lesion observed in inoculated eggs via CAM route was congestion of embryo, paleness of liver with mild thickening of CAM. Positive reaction of infected CAM in AGPT against reference IBDV antigen and antisera is a preliminary indication for successful isolation of IBDV.

b- Results of viral titration, resistance to heat inactivation and virus neutralization:

Results are shown in Table (1)

Table 1: Virological assay.

Items	Tested dilutions						AGPT	Virus titer	Antilog 10 for virus titer	Neutralized viruses
	-1	-2	-3	-4	-5	-6				
Viral propagation P1	0/5						–			
P2	2/5						+			
P3	3/5						+			
Viral titration	5/5	5/5	5/5	5/5	3/5	2/5		$10^{5.5}$ EID_{50}/ 0.1 ml	316228	
Heat inactivation 56°C for 5 hrs.	5/5						+			
V. neutralization										
V.+ positive serum	5/5	3/5	1/5	0/5	0/5	0/5		$10^{2.3}$ EID_{50}/ 0.1 ml	199	125693 (99.8%)
V.+ negative serum	5/5	5/5	5/5	4/5	3/5	1/5		$10^{5.1}$ EID_{50}/ 0.1 ml	125892	

3-Results of pH measurement and disinfection potency with iodine:

The pH of diluted iodine 1:50 was 6.2 this dilution reduced virus titer 99.93% after 15 min. reaction and the reduction was 99.97% after 60 min. the pH of diluted iodine 1:100 was 6.5, disinfection success was 99.7% after 15 min. and 99.9% after 60 min., while the pH of diluted iodine 1:200 was 6.7 and the disinfection success was 99.7, 99.84 and 99.9% after 15, 30 and 60 min. respectively.

The pH of diluted iodine 1:50 in 40% foetal bovine serum was 7.26 this dilution reduced virus titer 99.6, 99.8 and 99.9% at 15, 30 and 60 min. reaction times respectively, comparable results were obtained at dilutions 1:100 and 1:200 at the same test conditions (Table 2).

4- Results of pH measurement and disinfection potency with glutraldehyde containing compound:

The disinfection potency of dilution 1:50 of the glutraldehyde containing compound couldn't be tested because it killed all inoculated ECE in the safety trial. The pH measurement of dilutions 1:100 and 1:200 was 7.5 and 7.6 respectively and the disinfection success was 99.9% similar results of disinfection success was obtained in presence of organic matter at pH measurement 8.6 and 8.78 for dilutions 1:100 and 1: 200 respectively.

Table 2: Virucidal activity of iodine, glutraldehyde containing disinfectant in presence or absence of organic matters, with different dilutions and reaction times.

				In absence of organic matter										In presence of organic matter										
Disinfectant	DC	Safety	pH	Reaction duration	Tested dilutions -2	-3	-4	Post. disinf. titer (PD)	a Antilog 10 for PD titer	Virus control titer (VC)	b Antilog 10 for VC titer	c reduced titer after disinfec. c=b-a	% of disinfection success c/b	pH	Reaction time	Tested dilutions -2	-3	-4	PD titer	a* antilog 10 for PD titer	Virus control titer	b* Antilog 10 for VC titer	c* reduced titer after disinfec. C*=b*-a*	% of disinfect. Success c*/b*
Iodine	1:50	2/5	6.2	15	2/5	2/5	0/5	2.3	199 VP	5.5 10 EID_{50} /0.1ml	316228	316029 VP	99.93		15	4/5	35	1/5	3.1	1259 VP	5.5 10 EID_{50} /0.1ml	316228	314969 VP	99.6
				30	3/5	0/5	0/5	2.1	126 VP			316102 VP	99.96	7.26	30	3/5	2/5	1/5	2.7	501 VP			315727 VP	99.8
				60	2/5	0/5	0/5	1.9	80 VP			316148 VP	99.87		60	3/5	1/5	1/5	2/5	316 VP			315912 VP	99.9
	1:100	1/5	6.5	15	3/5	3/5	1/5	2.9	794 VP			315434 VP	99.7		15	4/5	3/5	2/5	3.3	1995 VP			314233 VP	99.3
				30	3/5	2/5	0/5	2.5	316 VP			315912 VP	99.9	7.74	30	4/5	2/5	1/5	2.9	794 VP			315434 VP	99.7
				60	2/5	1/5	0/5	2.1	126 VP			316102 VP	99.96		60	3/5	1/5	1/5	2.5	315 VP			315912 VP	99.9
	1:200	0/5	6.7	15	1/5	1/5	0/5	2.9	794 VP			315434 VP	99.97		15	4/5	1/5	1/5	3.3	1995 VP			314233 VP	99.3
				30	3/5	2/5	1/5	2.7	501 VP			315727 VP	99.84	8.8	30	4/5	3/5	1/5	3.1	1259 VP			315727 VP	99.8
				60	3/5	2/5	0/5	2.5	316 VP			315912 VP	99.9		60	4/5	2/5	1/5	2.9	794 VP			315434 VP	99.7
Glutraldehyde containing disinfectant	1:50	5/5	7.4	15	ND							ND		8.4	ND									
				30																				
				60																				
	1:100	2/5	7.6	15	2/4	1/4	0/4	2.25	177 VP			316051 VP	99.94		15	1/4	0/4	0/4	1.5	31 VP			316197 VP	99.99
				30	¼	0/4	0/4	1.5	31 VP			316197 VP	99.99	8.6	30	1/4	0/4	0/4	1.5					
				60	¼	0/4	0/4	1.5	31 VP						60	1/4	0/4	0/4	1.5					
	1:200	0/5	7.7	15	2/4	1/4	0/4	2.25	31 VP						15	1/4	1/4	0/4	1.5					
				30	1/4	0/4	0/4	1.5	31 VP					8.78	30	1/4	0/4	0/4	1.5					
				60	1/4	0/4	0/4	1.5	31 VP						60	1/4	0/4	0/4	1.5					

PD = Post disinfection titer EID_{50}/0.1 ml
VP = Virus particle
DC = disinfection concentration

ND = Not done

DISCUSSION

Disinfection is one of the measures taken to break the cycle of infectious diseases but it is not adequate alone Linton *et al.* (1987). For implementing a disinfection plan, there are several important areas to be addressed, this include assessment of cleaning, washing, disinfection and evaluation (Dvorak, 2005).

Before selecting a disinfectant to use, several factors must be considered. Some disinfectants are effective for routine disinfection protocols while others are necessary for outbreak situation.

For effective disinfection protocol, consideration should be given to the targeted microorganism, this involves the characteristics of a specific disinfectant and environmental issues, additionally the health and safety of personals (Ewart, 2001; Quinn, 2001; Sawicki, 2002; Shulaw and Bowman, 2001; Grooms, 2003).

Test methods for evaluating virucide are more complex than those adapted for evaluating bactericides, because the living host required for the recovery of virus is susceptible for the toxic effect of disinfectant (Linton *et al.,* 1987). This was limiting factor prevented us from testing the potency of 1:50 dilution of the glutraldehyde containing disinfectant because this dilution killed the inoculated ECE, but the mortality was 40% in ECE at dilution 1:100 of glutraldehyde containing compound (Table 2). As for iodine dilution 1:50 killed 40% of ECE while dilution 1:100 killed 20% ECE in the safety trials. Dialysis has been proposed as a mean of removing or reducing the concentration of disinfectant in a mixture to a level that wouldn't be toxic for the host system. (Blackwell and Chen, 1970). Boudouma *et al.* (1984) had overcome this limitation by ultra filtration, while Linton *et al.* (1987) pointed to the value of density gradient ultracentrifugation to solve this problem. In our study dilution 1:200 proved safe for ECE and the percent of disinfection success was comparable to the concentrated dilutions 1:100, 1:200 for both tested disinfectants. So we didn't had to go through this troubling procedures.

Glutraldehyde is saturated 5-carbon dialdehyde ($C5H_8O_2$) (CHO-CH_2-CH_2-CH_2-CHO) (Linton, 1987). It is characterized by high efficiency and broad spectrum. It achieves its effect through denaturation of protein and disrupting nucleic acid (Ewart 2001). It is non corrosive to metal, rubber or plastics (Morley, 2003). Thus it avoids the limitations

met with formaldehyde (Lenton, 1987), but they are highly irritating to humans by contact or inhalation and they are potentially carcinogenic (Green, 1998; Quinn, 2001; Morley, 2002). Thus protective equipments should be worn during its usage (Dvorak, 2005). The antimicrobial efficacy of glutraldehyde depends mainly on pH and it is more active in alkaline pH, and not affected by the presence of organic matter in disinfection of IBDV (Linton, 1987). This may be due to the increased alkalinity of solution which in turn kills IBDV.

In the present study the glutraldehyde containing compound couldn't be tested at dilution 1:50. At dilution 1:100 40% mortality in ECE eggs was obtained; dilution 1:200 was safe for ECE and produced a disinfection success 99.9% in presence or absence of organic matter these results were similar to those obtained at 1:100 dilution so from economic point of view 1:200 dilution can be used without risk for disinfection of IBDV. Meulemans and Halen (1982) found that aldehyde and complex disinfectant containing aldehyde reduced 4 log 10 or more in the titer of IBDV; the virucidal activity was maximum after 60 min. In the present study results of disinfection success were nearly equal at 15, 30 and 60 min. respectively.

Iodine compounds are broad spectrum compounds of low toxicity, low cost, easy to use, they do lose potency overtime, and not active at high temperature (Jeffrey, 1995). Since these compounds lose activity quickly in the presence of organic matter; they must be applied to a thoroughly cleaned surfaces (Green, 1998; Kennedy *et al.,* 2000; Shulaw and Bowman 2001; Grooms, 2003).

Iodine function by denaturating proteins, thus interfere with enzymatic system of microorganisms (Jeffrey, 1995); concentrated iodines irritates the skin, stains clothes, damages rubber and metals (Shulaw and Bowman, 2001), they are also inactivated by QACS and organic matter. Benton (1967) treated IBDV with various concentrations of iodine complex, phenolic derivatives and QACS for a period of 2 min. at 23°C and found that iodine is the only disinfectant having deleterious effect on IBDV. On the other hand, Meulemans and Halen (1982) found that iodines were not effective as disinfectant for IBDV so they didn't test its efficacy in presence of organic matter.

In the present study iodines were proved effective for disinfection against IBDV at the tested dilutions and the different reaction times, their activity in presence of organic matter was 99% .This

effectiveness may be due to the alkaline pH recorded in presence of organic matter, the later had a deleterious effect on IBDV as mentioned by (Benton, *et al.*, 1967) and this may not be the situation with other viruses resisting alkalinity.

In the present study, disinfection success was evaluated by comparing log virus titer before and after exposure to each dsinfectant. Suppose!! Virus control is $\log 10^6$ EID_{50}/0.1 ml and the titer after disinfection was reduced to log 10^2 EID_{50}/0.1 ml. The difference is 10^4 as adapted by Thayer and Beard (1998). The antilog of 10^6=1000000 VP(a)., the anti log of 10^2= 100 VP (b)., and the antilog of 10^4=10000 VP(c) calculation of titer reduction c/a % = 1% in one hand ,and the 10^4 reduction titer doesn't signify the actual drop in viral titer in another hand .For this reason we adapted another method for calculation as follow:

Antilog of 10^6=1000000 as virus control; antilog 10^2=100 after disinfection for calculation of disinfection success =1000000 VP – 100 VP = 999900 so the percent of disinfection success is 99.99% and this is more logic calculation. This method can be adapted in calculation of neutralization and viral regression in calculation of relatedness.

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