Original Article

Inactivation of Infectious Bronchitis Virus with Various Kinds of Disinfectants

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Abstract

This study aimed to perform an *in vitro* testing of the efficacy of various kinds of disinfectant on infectious bronchitis virus (IBV). Four kinds of disinfectants including Virusnip, Omnicide, CID2000 and Virkon S were used for the virus inactivation test. After challenge with each strain of IBV for each contact time (30 seconds, 1, 5 and 30 minutes), we recorded number of dead embryo/6 inoculated eggs after incubation. Results showed that there was no significant difference among the contact times except 1:800 Virusnip tested for Tha07 ($p < 0.05$). For Tha03, there was a significant difference among the disinfectants at 30 seconds and 1 minute of contact times ($p < 0.05$). For Tha08, there was a significant difference among the disinfectants at 1 minute of contact time ($p < 0.05$). For Tha09, there was a significant difference among the disinfectants at 5 and 30 minutes of contact times ($p < 0.05$). For Tha10, there was a significant difference among the disinfectants at 5 minutes of contact time ($p < 0.05$). Virusnip revealed the ability to inactivate the activity of 9 IBV strains in all exposure times, especially at dilutions of 1 : 100 and 1 : 200.

Keywords: disinfectants, inactivation, infectious bronchitis virus

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บทคัดย่อ

การทำลายฤทธิ์ของไวรัสหลอดลมอักเสบติดต่อด้วยสารฆ่าเชื้อชนิดต่างๆ

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การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบประสิทธิภาพของสารฆ่าเชื้อหลายชนิดต่อเชื้อไวรัสหลอดลมอักเสบติดต่อในไก่ในหลอดทดลอง สารฆ่าเชื้อไว รูสนิป ออมนิไซด์ ซีไอดี 2000 และเวอร์คอน เอส ถูกนำมาใช้ในการทดสอบการทำลายฤทธิ์ไวรัส โดยสังเกตจำนวนเอ็มบริโอที่ตาย หลังจากที่ฉีดไข่ไก่ฟักด้วยเชื้อไวรัสที่ได้สัมผัสกับสารฆ่าเชื้อที่เวลาแตกต่างกัน ได้แก่ 30 วินาที, 1, 5 และ 30 นาที พบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญ ยกเว้นในกลุ่มที่ใช้ไวรูสนิป ความเข้มข้น 1 : 800 สัมผัสต่อเชื้อ Tha07 ($p < 0.05$) เชื้อ Tha03 พบความแตกต่างที่เวลา 30 วินาที และ 1 นาที ($p < 0.05$) เชื้อ Tha09 พบความแตกต่างที่เวลา 5 นาทีและ 30 นาที ($p < 0.05$) เชื้อ Tha10 พบความแตกต่างที่เวลา 5 นาที ($p < 0.05$) จากการศึกษาครั้งนี้ พบว่าไวรูสนิปมีความสามารถในการทำลายฤทธิ์ไวรัสหลอดลมอักเสบติดต่อในไก่ ทั้ง 9 สายพันธุ์ โดยเฉพาะที่ความเข้มข้น 1 : 100 และ 1 : 200

คำสำคัญ: สารฆ่าเชื้อ การทำลายฤทธิ์ ไวรัสหลอดลมอักเสบติดต่อในไก่

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Introduction

Infectious bronchitis virus (IBV) is an enveloped coronavirus which is characterized by respiratory signs and may result in renal damage, decreased egg production and decreased quality of eggs (Dolz et al., 2012). IBV is acquired via horizontal transmission; for example, inhalation or direct contact with contaminated poultry, litter, equipment or other fomites (de Wit et al., 1998). Although vertical transmission of the virus within the embryo has never been reported, the virus may be present on the shell surface of hatching eggs via shedding from the oviduct or the alimentary tract (Gallardo et al., 2011).

Development of a disease's prevention system is the most important part of poultry disease control. Biosecurity, good management and efficacy of disinfectant application are key points to the success of poultry disease prevention. The principle of biosecurity is based on reducing the risk of infection (Fasina et al., 2012). Although IBV is considered to be sensitive to common disinfectants, *in vitro* testing of disinfectants is an important guideline to apply to the disinfectants in field conditions (McDonnell and Russell, 1999). The disinfectants used in this study were composed of 4 kinds. Firstly, Virusnip contains two active ingredients, which are potassium peroxymonopersulphate (PMP) as an oxidizing agent and sodium dichloroisocyanurate (SDIC) as an organic releasing chlorine. Secondly, Virkon S contains two active ingredients, which are sodium dodecyl benzene sulphonate as an anionic surfactant and potassium monopersulphate as an oxidizing agent. Thirdly, CID 2000 contains two active ingredients, which are hydrogen peroxide and peroxyacetic acid as oxidizing agents. Lastly, Omnicide contains two active ingredients, which are glutaraldehyde and alkyl benzyl dimethyl ammonium chloride as a subclass of quarternary ammonium compounds (QACs). These disinfectants are commonly used in Thailand's poultry farm. Therefore, the aim of this study was to perform the *in vitro* testing of the efficacy of various kinds of disinfectants on IBV.

Materials and Methods

Embryonated eggs: Seven-day-old embryonic eggs were bought from Kasetsart University and immediately transported for incubation in a hatchery of the Avian Health Research Unit, Chulalongkorn University. Nine- to eleven-day embryonic eggs were used in our experiments. Six embryonic eggs were taken to test virucidal activity of disinfectant in each contact time (Table 1).

Infectious bronchitis virus: Nine isolates of Thai IBV were used for the testing of virus inactivation by disinfectants. A dosage of virus was prepared in a solution containing approximately 1.0×10^6 ELD_{50}. A half ml of virus was also mixed with a half ml of disinfectant.

Disinfectants: All tested disinfectants were registered by the Department of Livestock Development,

Ministry of Agriculture and Cooperation, Thailand. The disinfectants used in this study were composed of 4 kinds including Virusnip (Novartis Animal Health, Switzerland), Omnicide (Metrex, USA), Virkon S (Antec International Ltd, UK) and CID 2000 (CID LINES, Belgium). Test protocol was modified from Suarez et al. (2003). Briefly, all the disinfectants were diluted with distilled water following the manufacturers' recommendation for each product. Omnicide, Virkon S and CID 2000 were diluted and tested at concentrations of 1 : 150, 1 : 200 and 2%, respectively, except that Virusnip was diluted and tested at concentrations of 1 : 100, 1 : 200 1 : 400 and 1 : 800. All diluted disinfectants were aliquoted and kept at 25^0C. A half ml of IBV containing approximately 1.0 x 10^6 ELD_{50} was mixed with 0.5 ml of diluted disinfectants and incubated for 30 sec, 1, 5 and 30 min at room temperature. In addition, 0.5 ml of IBVs was mixed with 0.5 ml of phosphate buffered saline (PBS), and 0.5 ml of disinfectant was mixed with 0.5 ml of distilled water, serving as positive and negative controls, respectively (Table 1). The positive control and the negative control was inoculated into nine- to eleven-day old chicken embryonic eggs in six replications and candled twice a day for 7 days. Inoculated embryonic eggs dying prior to 24 hours were discarded. Allantoic fluid was harvested from each egg on day 7 post inoculation or upon death. Secondary egg inoculations were performed to determine the presence of the virus.

Statistical analysis: The Kruskal-Wallis and Mann-Whitney U test were used to define difference between strains of IBV in each contact time of a disinfectant and among all disinfectants of each IBV strain.

Results

Results of IBV inactivation with various kinds of disinfectants and exposure times are shown in Table 2. In each strain of IBV, we compared the results at each contact time of different disinfectants and at each contact time we compared the results from different strains of IBV. From all groups of these tests, there was no significant difference among their durations (30 sec, 1 min, 5 min and 30 min) except 1 : 800 Virusnip tested for Tha07 which had a significant difference among their durations ($p < 0.05$).

For the Tha03 strain of IBV, at 30 seconds of contact time, the number of embryonic deaths of 1 : 200 Virusnip, CID 2000 and Virkon S was significantly less than 1 : 800 Virusnip ($p < 0.05$) and at 1 minute of contact time, the number of embryonic deaths of 1 : 100 Virusnip, CID 2000, Omnicide and Virkon S was significantly less than 1 : 800 Virusnip ($p < 0.05$).

For the Tha08 strain of IBV, at 1 minute of contact time, the number of embryonic deaths of 1 : 100, 1 : 200, 1 : 400 and 1 : 800 Virusnip and Omnicide was significantly less than Virkon S ($p < 0.05$).

For the Tha09 strain of IBV, at 5 minutes of contact time, the number of embryonic deaths of 1 : 100, Virusnip and Virkon S was significantly less than 1 : 400 and 1 : 800 Virusnip ($p < 0.05$) and at 30 minutes of contact time, the number of embryonic deaths of 1 : 100, 1 : 200 and 1 : 400 Virusnip, Omnicide and Virkon S was significantly less than 1:800 Virusnip ($p < 0.05$).

For the Tha10 strain of IBV, at 5 minutes of contact time, the number of embryonic deaths of 1 : 100, 1 : 200, 1 : 400 and 1 : 800 Virusnip and Virkon S was significantly less than Omnicide ($p < 0.05$).

Discussion

In this study, we investigated the *in vitro* testing of the efficacy of various kinds of disinfectants including Virusnip, Omnicide, CID 2000 and Virkon S on nine strains of IBV. In this study, we used a concentration of disinfectants according to the recommendation of the manufacturers except for Virusnip whose concentration we varied at 1 : 100, 1 : 200, 1 : 400 and 1 : 800. The results indicated that the proper concentration and contact time to inactivate nine strains of IBV was Virusnip 1 : 200 for 1 minute. The virucidal activity of Virusnip was derived from both the oxidizing agent (PMP) and the organic releasing chlorine (SDIC). The oxidizing agent (PMP) probably denatures proteins and enzymes and increases cell wall permeability by disrupting sulfhydryl (-SH) and sulfur (S-S) bonds and organic releasing chlorine (SDIC) which provides a higher concentration of available chlorine is less susceptible to be inactivated by organic matter than sodium hypochlorite (McDonnell and Russell, 1999). In water, SDIC generates hypochlorites that are ready to disinfect instantly, destroying the cellular activity of proteins and inhibiting DNA synthesis of microorganisms (McDonnell and Russell, 1999) and

Table 1 Disinfectants and contact time of each group

Group	Contact time			
	30 seconds	1 minute	5 minutes	30 minutes
1	IBV + PBS	IBV + PBS	IBV + PBS	IBV + PBS
2	Disinfectant + PBS	Disinfectant + PBS	Disinfectant + PBS	Disinfectant + PBS
3	IBV+ Virusnip (1 : 100)	IBV+ Virusnip (1 : 100)	IBV+ Virusnip (1 : 100)	IBV+ Virusnip (1 : 100)
4	IBV+ Virusnip (1 : 200)	IBV+ Virusnip (1 : 200)	IBV+ Virusnip (1 : 200)	IBV+ Virusnip (1 : 200)
5	IBV+ Virusnip (1 : 400)	IBV+ Virusnip (1 : 400)	IBV+ Virusnip (1 : 400)	IBV+ Virusnip (1 : 400)
6	IBV+ Virusnip (1 : 800)	IBV+ Virusnip (1 : 800)	IBV+ Virusnip (1 : 800)	IBV+ Virusnip (1 : 800)
7	IBV+ Omnicide (1 : 150)	IBV+ Omnicide (1 : 150)	IBV+ Omnicide (1 : 150)	IBV+ Omnicide (1 : 150)
8	IBV+ CID 2000 (2%)	IBV+ CID 2000 (2%)	IBV+ CID 2000 (2%)	IBV+ CID 2000 (2%)
9	IBV+ Virkon S (1 : 200)	IBV+ Virkon S (1 : 200)	IBV+ Virkon S (1 : 200)	IBV+ Virkon S (1 : 200)

Table 2 Number of dead embryo/6 inoculated eggs after incubation with each IBV strain.

Disinfectants	Dilution	Exposure times	Number of dead embryo/6 inoculated eggs of each IBV strains									
			Tha03	Tha04	Tha05	Tha07	Tha08	Tha09	Tha10	Tha28	Tha29	Total
Virusnip	1 : 100	30 sec	1	0	0	0	0	0	0	0	0	1
		1 min	0[I]	0	0	0	0[a]	1	0	0	0	1
		5 min	0	0	0	0	0	0[a]	0[a]	0	0	0
		30 min	0	0	0	0	0	0[I]	0	1	0	1
Virusnip	1 : 200	30 sec	0[a]	0	0	0	1	2	0	0	0	3
		1 min	1	0	0	0	0[a]	0	0	0	0	1
		5 min	0	0	0	0	0	2	0[a]	0	1	3
		30 min	0	0	0	1	0	0[I]	0	0	1	2
Virusnip	1 : 400	30 sec	2	0	0	0	2	4	2	1	0	11
		1 min	2	0	0	0	0[a]	3	2	1	0	8
		5 min	1	0	0	0	0	4[b]	0[a]	0	0	5
		30 min	0	0	0	0	0	0[I]	0	0	0	0
Virusnip	1 : 800	30 sec	4[b]	3	0	3[a]	2	4	0	2	0	18
		1 min	4[II]	2	0	0[b]	0[a]	4	0	1	0	11
		5 min	3	0	0	0[b]	0	5[b]	0[a]	0	1	9
		30 min	1	0	1	0[b]	0	6[II]	0	0	0	8
Omnicide	1 : 150	30 sec	1	2	1	1	3	3	2	2	2	17
		1 min	0[I]	3	2	1	0[a]	1	2	1	2	12
		5 min	1	0	0	1	1	3	4[b]	2	1	13
		30 min	0	1	2	1	0	2[I]	1	2	1	10
CID 2000	2%	30 sec	0[a]	2	0	2	2	2	1	2	0	11
		1 min	0[I]	1	2	1	2	1	1	3	3	14
		5 min	1	1	2	1	1	2	1	3	1	13
		30 min	0	0	0	1	1	3	0	1	1	7
Virkon S	1 : 200	30 sec	0[a]	1	0	1	2	1	0	2	0	7
		1 min	0[I]	0	0	0	4[b]	1	1	1	0	7
		5 min	0	1	0	0	1	0[a]	0[a]	2	0	4
		30 min	0	0	0	0	2	0[I]	0	2	0	4
Positive	NA	NA	6	6	6	5	6	6	5	6	6	52
Negative	NA	NA	0	0	0	0	0	0	0	0	0	0

NA: not applicable

are immediately regenerated back into new active SDIC by PMP, providing continuous action until all the monopersulphate is used up (manufacturer's information) which causes a higher performance than with only oxidizing agents (Virkon S, CID 2000). The Virusnip dilution of 1 : 200 also completely killed classical swine fever virus, porcine reproductive and respiratory syndrome virus and pseudorabies virus in 30 seconds to 5 minutes (Bunpapong et al., 2010). This is in contrast to a study in Japan which indicated that 0.1% benzalkonium chloride (BC) was an effective virucidal agent against avian bronchitis virus in the allantoic fluid of chicken eggs. Nonetheless, disinfectant effectiveness depends on many factors, for example, contact time, temperature, activity in organic matter or protein-containing material, type of chemical, and concentration and quantity of the chemical (Kennedy et al., 2000).

In conclusion, Virusnip revealed the best inactivating activity in all exposure times, especially at dilutions of 1 : 100 and 1 : 200. At the dilution of 1 : 100, all 9 IBV strains were inactivated within 30 min. except for Tha28. At the dilution of 1 : 200, most 9 IBV strains were inactivated within 30 min. except for Tha07 and Tha29. Virkon S seemed to be in the second rank of the inactivated efficacy to IBV. At the dilution of 1:200 of Virkon S, most of the IBV strains were killed within 30 min. except for Tha08 and Tha28. CID2000 and Omnicide were the third and fourth ranks of inactivated efficacy to IBV. In conclusion, Virusnip revealed the best inactivated efficacy to 9 strains of Thai IBV isolates.

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