Short Communication

Field Efficacy of Potassium Peroxymonosulfate (PMS) Oxidizing Disinfectant (Virusnip™) against Porcine Circovirus Type 2 (PCV2) in Gilt Acclimatization Unit

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Abstract

Virusnip™ is one of the commercial potassium peroxymonosulfate disinfectants widely used in swine farms. This disinfectant is well studied and proves to be excellent in antimicrobial activity previously. However, there is no information about its efficacy under field condition. Thus, the objective of this study was to determine the virucidal activity of Virusnip® against PCV2 in farm condition. Consequently, 1:200 concentration of Virusnip™ was sprayed for 15 minutes once or twice daily depending on the acclimatization period in gilts isolation units compared to the untreated group in the same building. Environmental swabs and oral fluid samples were collected and pooled to determine virucidal effectiveness of PCV2 in the environment as well as virus shedding in the oral secretion of the studied animals. Viral load in each group was quantified by a modified real-time PCR specific for ORF1 of PCV2. Result showed significant reduction in the viral load from the environment contamination and also from the oral fluid samples of the treatment group compared to the control group. This indicates that potassium peroxymonosulfate disinfectant (Virusnip™) can be a disinfectant of choice used for reducing the viral contamination and viral transmission among animals under field situation.

Keywords: acclimatization, disinfectant, porcine circovirus type 2, potassium peroxymonosulfate

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บทคัดย่อ

ประสิทธิภาพของ Potassium Peroxymonosulfate (PMS) Oxidizing Disinfectant (Virusnip™) ต่อเชอร์โคไวรัส ชนิดที่ 2 ในคอกสุกรสาวปรับสภาพ

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สารเคมีในกลุ่มโพแทสเซียม เปอร์ออกซีโมโนซัลเฟตเป็นสารเคมีที่ได้รับการทดลองในห้องปฏิบัติการมาแล้วว่ามีฤทธิ์ยับยั้งการเจริญของไวรัสและแบคทีเรียได้เป็นวงกว้าง อย่างไรก็ตามในปัจจุบันยังไม่มีการทดสอบประสิทธิภาพของสารฆ่าเชื้อชนิดนี้ภายใต้สภาพแวดล้อมจริงในภาคสนาม การศึกษาในครั้งนี้จึงเป็นการทดสอบประสิทธิภาพในภาคสนาม ในการลดปริมาณเชอร์โคไวรัสของสารฆ่าเชื้อไวรูสนิป ซึ่งเป็นตัวแทนของสารเคมีในกลุ่มโพแทสเซียม เปอร์ออกซีโมโนซัลเฟต โดยใช้ไวรูสนิปความเข้มข้น 1 : 200 พ่นในพื้นที่เลี้ยงสุกรที่แยกจากกัน วันละหนึ่งหรือสองครั้ง ทำการเก็บตัวอย่างไวรัสจากสิ่งแวดล้อมและน้ำลายสุกร เพื่อตรวจปริมาณเชอร์โคไวรัส โดยวิธีเรียลไทม์ พีซีอาร์ และเปรียบเทียบปริมาณไวรัสที่พบ ระหว่างกลุ่มทดลองที่มีการพ่นไวรูสนิป และกลุ่มควบคุมที่ไม่มีการพ่นไวรูสนิป จากการศึกษาพบว่าปริมาณไวรัสที่พบจากสิ่งแวดล้อมและน้ำลายสุกรในกลุ่มทดลอง มีปริมาณลดลง แตกต่างจากกลุ่มควบคุมที่มีปริมาณไวรัสเพิ่มขึ้น แสดงให้เห็นว่า การใช้สารฆ่าเชื้อไวรูสนิป ซึ่งเป็นสารเคมีในกลุ่มโพแทสเซียม เปอร์ออกซีโมโนซัลเฟต สามารถลดปริมาณไวรัสที่ปนเปื้อนในสิ่งแวดล้อม และยังลดการแพร่กระจายไวรัสระหว่างสุกรโดยตรวจวัดจากตัวอย่างน้ำลายภายใต้สภาพแวดล้อมในภาคสนามได้อย่างมีนัยสำคัญ

คำสำคัญ: การปรับสภาพ สารฆ่าเชื้อโรค เชอร์โคไวรัสชนิดที่สอง โพแทสเซียม เปอร์ออกซีโมโนซัลเฟต

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Introduction

Porcine circovirus type 2 (PCV2) is one of the most important infectious virus in swine industry. Routes of PCV2 transmission compose of many means, mainly by direct contact of virus particles in mucus secretion, feces and urine (Rose et al., 2012). Moreover, PCV2 is a durable virus and is tolerant to high temperature, up to 75^{0}C for 15 minutes and also in dry-heat condition of 120^{0}C for 30 minutes (O'Dea et al., 2008). Therefore, cleaning and disinfecting the environment is an important practice to minimize the spread of the disease among animals. According to previous studies, many *in vitro* researches determining virucidal activity of a number of disinfectant solutions have been accomplished and demonstrated the efficacy under the *in vitro* conditions including oxidizing agent class, containing potassium peroxymonosulfate (PMS; potassium monopersulfate, PMP) as major active ingredient (Royer et al., 2001; Kim et al., 2009). However, there have been no field experiments, especially PMS compound, except for the study of Patterson and colleagues (2011) determining the effectiveness of various disinfectants to PCV2 reduction on transport vehicle model. Various factors in field conditions including organic materials, environmental surface and water quality more or less impact on the disinfectant activity (Amess, 2004). Thus, knowledge about antiviral activity of PMS compound disinfectant in field condition is needed especially in gilt acclimatization unit because horizontal transmission of PCV2 commonly occurs. Reduction in viral load in the environment will be very helpful in maintaining good health of a herd.

Virusnip™ is one of the commercial potassium peroxymonosulfate disinfectants widely used in swine farms. This advanced soluble powder disinfectant contains two active ingredients, PMS and sodium dichloroisocyanurate (SDIC). This agent is well-studied and proves to be excellent antimicrobial activity. One per thousand concentration of Virusnip™ solution significantly reduces *E. coli* viability within 2 minutes. Similarly, within 2 minutes the reagent could destroy *Salmonella spp*. in various concentrations, 1 : 400 for completely deactivate S. Infantis and S. Virchow and 1 : 200 for destroying S. Enteritidis, respectively (Kramomtong et al., 2010). Similarly, recent *in vitro* study of Virusnip™ efficacy on various important pig viruses including classical swine fever (CSFV), pseudorabies virus (PRV), swine influenza (SIV) and also porcine reproductive and respiratory syndrome virus (PRRSV) were accomplished. The study proved that CSFV, PRRSV and SIV were completely destroyed within only 30 seconds using 1 : 100 concentration of Virusnip™ solution while PRV was completely destroyed within 5 minutes. This indicates that Virusnip™ is one of the most interesting disinfectants used in the swine farms. Therefore, the objective of this study was to determine the virucidal activity of Virusnip™, a representative of disinfectants containing PMP as an active ingredient, against PCV2 under field condition.

In the present study, 1:200 concentration of Virusnip™ was sprayed for 15 minutes once or twice daily in gilts isolation units compared to the untreated group in the same building. Environmental swabs and oral fluid samples were collected and pooled to determine the virucidal effectiveness of PCV2 in the environment and virus shedding in the oral secretion of the studied animals. The viral load in each group was determined by a modified real-time PCR specific for ORF1 of PCV2.

Materials and Methods

Experimental design: According to the objective of the study, a PCV2 infected pig farm was selected. Based on the farm owner's interest in being an endemic PCV2-affected farm, a gilt acclimatization unit was selected as the place of this study in order to measure PCV2 viral reduction in the environment. Briefly, the gilt barn was divided into 2 rows with 12 pens (4.5 x 6 meters) on each side specified as A1 to A12 and B1 to B12, respectively. Twenty-week-old gilts were randomly allocated into each pen, 8-10 animals per pen. For an orderly grouping and acclimatization, all gilts were kept together for 4 weeks. Five environmental swabs and 2 oral fluid samples were collected in pens 3, 4, 5, 8, 9 and 10 of each side (Fig 1A). Samples collected in the period before the treatment were designated as "pre-treated" samples and were tested by PCV2 ORF1 qPCR to determine the presence and quantity of PCV2 nucleic acid before the Virusnip™ treatment. Consequently, 1:200 concentration of Virusnip™ solution was prepared and sprayed (Amess, 2004). Pairs of sprayers were located in the Virusnip™ treated pens # A3, A4, A5, B3, B4 and B5, respectively. To spray thoroughly, the sprayers were installed 2 meters above each pen, with approximately 3-meter spraying diameter (Fig 1B). In the treatment groups (A3, A4, A5, B3, B4 and B5), the solution was sprayed twice a day when the gilts were around 24-28 weeks old and only once a day when they were 29-32 weeks old. In the last week of the acclimatization, oral fluid and environmental swab samples of all pens were collected as previously described. The collected samples after the Virusnip™ treatment were designated as "post-treated" samples. Those samples were tested to determine the quantity of PCV2 specific nucleotide and to evaluate the efficacy of the Virusnip™ solution against PCV2 under field condition.

Sample collection: Environmental swabs were collected by using 6 pieces of 10x10 cm^2 cloths, soaked with normal saline solution. The cloths were used to wipe 5 floor areas of 1 square meter and on the feed track of each pen. The cloths collected from each pen were pooled together as one sample, squeezed to collect fluid and stored at -20^{0}C until tested. As described in a previous study, cotton ropes were used for oral fluid collection (Fig 2) (Prickett et al., 2008). The ropes were hung in each pen for 15 minutes, giving an opportunity to each pig to chew on the rope. Similar to the cloths, the ropes collected from each pen were squeezed to collect oral fluid samples and stored at -20^{0}C until used.

Quantitative virus detection: Viral DNA of all samples was extracted and tested for a quantitative examination. One-step SYBR green real-time PCR was conducted based on the routine procedure performed at the Chulalongkorn University-Veterinary Diagnostic Laboratory (CU-VDL). The primers were designed specifically conserve ORF1 region of the PCV2 virus (PCV2-ORF1Forward primer: 5′ ATGCCCAGCAAGAAGAGTGGAAGAAG 3′ and PCV2-ORF1 Reverse primer: 5′ AGGTCACTCCGTTGTCCTTGAGATC 3′) and product size was 356bp. The modified real-time PCR amplification was carried out with a Real-time Themocycler (The Corbett Research RG 3000, Australia). The 25 µl reaction contained 12.5 µl QuantiTect® SYBR® Green PCR Kit, 1 µl forward and reverse primers (stock concentration 10 mM) 3 µl, DNA template and 7.5 µl RNase-free water. The PCR conditions were composed of 15 min at 95^{0}C, followed by 35 cycles of denaturation for 30 sec at 94^{0}C, annealing for 30 sec at 50^{0}C and extension for 45 sec at

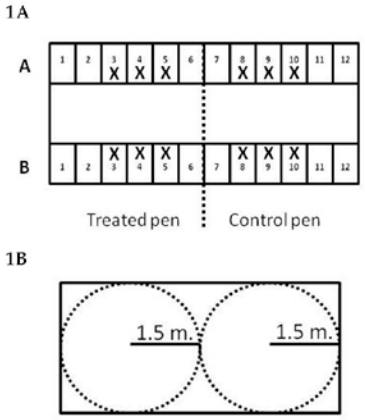

Figure 1 (A) Lay out of the gilt barn. Samples were collected in 3 pens in the middle of each group (X). **(B)** Lay out of the sprayers in each pen. A pair of sprayers were located in the Virusnip™ treated pens with approximately 3-meter spraying diameter.

Figure 2 Oral fluid collection using cotton rope

72^{0}C. Fluorescence was detected at the end of the extension step. Internal PCR-product plasmids were cloned by pGEM easy Vector System I (Promega, USA), transferred to JM109 (Promega, USA), amplified and used as standard DNA.

Data analysis: To evaluate the efficacy of Virusnip™ against PCV2 in the oral fluid and in the environmental samples, quantity of nucleotide between pre-treated and post-treated samples were

compared. Data were analyzed by a pair *t*-test (*p*-value < 0.05).

Results and Discussion

The efficacy of 1:200 concentration of Virusnip™ solution is summarized in Table 1. The results of PCV2 the modified real-time PCR demonstrated that all pre-treated samples including the environmental swabs and oral fluid samples obtained from both treatment and control groups were positive as expected. Interestingly, average quantities of PCV2 specific nucleotide (copies/ml) collected from the treatment group from environmental swabs and oral fluid samples (272.98 copies/ml and 1417.23 copies/ml) were higher than those of the untreated control (5.60 copies/ml and 2.88 copies/ml). Possibly, there were higher numbers of PCV2-infected gilts in the treatment group continuingly shedding the virus into the environment before the treatment was implemented. However, after the treatment with Virusnip™, the average quantities of PCV2 specific nucleotide from the treatment group was significantly reduced while significantly increased average quantities of PCV2 specific nucleotide was found in the untreated group (Table 1). The result demonstrated that 1 : 200 concentration Virusnip™ solutions had the virucidal capability against PCV2 under field condition since the viral load in the environment clearly diminished and re-infection of susceptible animals was minimized. The infected gilts could clear up the virus from the body significantly, therefore, shortening the virus shedding in oral fluid samples and reducing the period of horizontal transmission among infected and susceptible pigs.

It should be noted that PCV2 vaccination was implemented twice when the gilts were around 25 and 29 weeks old in this selected herd due to high viral load when the owner noticed initial results. This inactivated vaccine might more or less activate the pigs' immunity, having a direct effect on the decrease in and shortening of PCV2 viremia and shedding of all experimental gilts. Even though the PCV2 vaccine was used, at the end of the present study, the gilts in the control group still shed the virus in higher amount during the acclimatization compared to the results before the study. Unlike the treatment group, significant reduction in PCV2 shedding in oral fluid samples was shown. This raises up a major point of concern when a proper disinfection practice in combination with a PCV2 vaccination might play a significant role in the endemic PCV2 infected herds. According to the results shown in the control group, it can be indicated that basic cleaning practice is inadequate to clear up PCV2 contamination in the field condition. Consequently, proper disinfection performing virucidal effects in the environment together with specific immunity activated by a good vaccination program could have a positive impact on decreasing PCV2 viremia and minimizing viral shedding in gilts during acclimatization period. However, further research works are needed to verify these combination effects in the future.

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Table 1 Quantity of porcine circovirus type 2 (PCV2) specific nucleotide detected (copies/ml) from pooled environmental swabs and oral fluid samples

		Quantity of PCV2 specific nucleotide (copies/ml)		Virus Reduction
		Pre-treated	Post-treated	
Treatment group	Environmental contamination	272.98	8.51	Yes
	(n = 6)	(± 364.94)	(± 19.54)	
	Oral fluid shedding	1417.23	13.12	Yes
	(n = 6)	(±3035.04)	(±20.22)	
Control group	Environmental contamination	5.60	10.56	No
	(n = 6)	(±5.29)	(±19.55)	
	Oral fluid shedding	2.88	14.08	No
	(n = 6)	(±2.32)	(±18.60)	