Original Article

Culex tritaeniorhynchus is Unlikely to be a Vector for the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

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Abstract

At least, 4 mosquito species, *Culex tritaeniorhynchus, Culex gelidus, Anopheles vagus* and *Mansonia uniformis* were identified on a pig farm in Nakorn Pathom, Thailand between March 2004 to February 2005. *C. tritaeniorhynchus* was found to be the predominant species (88.38 7.63%). To determine whether *C. tritaeniorhynchus* could serve as a mechanical vector for PRRSV transmission, 2 experiments were conducted on: the duration of PRRSV within the mosquitoes and PRRSV transmission from the PRRSV-infected pigs to naive pigs by the infected mosquitoes. PRRSV nucleic acid could be detected in the whole pooled mosquito samples for up to 48 hours post feeding in the PRRSV-infected pig (PFP) using RT-PCR, whereas the PRRSV could be isolated from the whole pooled mosquito samples for up to 2 hours PFP. However, no PRRSV was detected in the pooled mosquito legs. The results of the PRRSV transmission showed that all the naive pigs used in the direct mosquito feeding were negative, whereas, the swine bioassay using pooled mosquito injection at 30 minutes PFP was positive for PRRSV detection in both RT-PCR and ELISA tests. The results of this study demonstrate that *C. tritaeniorhychus,* a predominant mosquito species found on a pig farm in Thailand is unlikely to serve as a potential vector for PRRSV transmission on pig farms.

Keywords : Mechanical, mosquitoes, porcine reproductive and respiratory syndrome virus (PRRSV), pigs, transmission, vector.

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บทคัดย่อ

ยุงรำคาญ *Culex tritaeniorhynchus* ไม่น่าจะเป็นตัวนำโรคพีอาร์อาร์เอส

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จากการสำรวจชนิดของยุงในฟาร์มสุกร จ. นครปฐม ระหว่างเดือนมีนาคม พ.ศ. 2547 ถึง เดือน กุมภาพันธุ์ พ.ศ. 2548 พบยุงทั้งหมด 4 ชนิด คือ *Culex tritaeniorhynchus, Culex gelidus, Anopheles vagus* และ *Mansonia uniformis* โดยพบยุงรำคาญ *Culex tritaeniorhynchus* มากที่สุด (ร้อยละ 88.38 ±7.63) การทดสอบความสามารถการนำไวรัสพีอาร์อาร์เอส แบบเชิงกล ของยุงรำคาญ *(Culex tritaeniorhynchus)* ได้วางแผน 2 การทดลอง คือ การทดสอบระยะเวลาการมีชีวิตอยู่ภายในตัวยุง ของไวรัสพีอาร์อาร์เอส และความสามารถในการนำโรคแบบเชิงกลของไวรัสพีอาร์อาร์เอสโดยยุง จากสุกรติดเชื้อไปยังสุกร ปลอดโรค พบว่าสามารถตรวจพบสารพันธุกรรมของไวรัสพีอาร์อาร์เอส โดยวีธี อาร์ทีพีซีอาร์ ภายในตัวอย่างรวมของยุง ที่ดูดเลือดจากสุกรที่มีไวรัสได้นานถึง 48 ชั่วโมง ในขณะที่สามารถเพาะแยกไวรัสที่มีชีวิตจากตัวอย่างรวมดังกล่าวได้เพียง 2 ชั่วโมง ทั้งนี้ไม่พบสารพันธุกรรมของไวรัสพีอาร์อาร์เอส จากขาของยุงที่ดูดเลือดจากสุกรติดเชื้อเลย ส่วนการทดลองการนำโรค แบบเชิงกลของยุงนั้น ไม่พบการติดโรคพีอาร์อาร์เอส ในสุกรที่ถูกยุงกัด แต่พบการติดโรคในสุกรที่ได้รับการฉีดตัวอย่าง ยุงบดที่ดูดเลือดสุกรติดเชื้อมาก่อน 30 นาที โดยวิธีอาร์ทีพีซีอาร์และวิธีอิไลซ่า ผลการศึกษาครั้งนี้แสดงให้เห็นว่า ยุงรำคาญ *Culex tritaeniorhynchus* ที่พบมากที่สุดในฟาร์มสุกรในประเทศไทยนั้น มีศักยภาพต่ำในการนำโรคพีอาร์อาร์เอสในธรรมชาติ

คำสำคัญ: เชิงกล ยุง ไวรัสพีอาร์อาร์เอส สุกร การติดต่อ ตัวนำโรค

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) caused by the PRRS virus (PRRSV), is one of the most economically devastating diseases of the pig industry today. PRRSV affects pigs of all ages causing a poor conception rate, late - term abortions, stillborn and weak live-born pigs, post-weaning pneumonia and an increase in the mortality rate in nursery pigs. PRRSV emerged in the late 1980s resulting in reproductive failures and respiratory diseases in infected pigs in North America and Europe (Albina, 1997) and later in Asia (Saito et al., 1996). The first retrospective report of PRRSV infection in Thailand revealed that Thai pigs had had seroconversion to PRRSV since 1989 and the genomic organization of the first Thai isolate was similar to the North American (US) genotype (Damrongwatanapokin et al., 1996). Later, both the US and the European (EU) genotypes were reported in Thailand (Thanawongnuwech et al., 2004).

PRRSV is a small, enveloped RNA virus and is classified as a member of the genus Arterivirus, family Arteriviridae in the order Nidovirales. Other viruses in the genus Arterivirus are the lactate

dehydrogenase-elevating virus (LDV) in mice, equine arteritis virus (EAV) and the simian hemorrhagic fever virus (SHFV) (Yoon, 2002). The Arteriviruses have common biological properties, including primary replication in host macrophages and the establishment of asymptomatic persistent infection in the host (Yoon, 2002). Currently, the known routes of PRRSV transmission are direct contact, contaminated semen, contaminated needles, fomites, insect vectors and mammary secretion and transplacental infection as well as airborne transmission (Rossow, 1998; Wagstrom et al., 2001). In addition, previous reports have found that some insects such as houseflies *(Musca domestica Linnaeus)* and mosquitoes *(Aedes vexans)* can serve as mechanical vectors for PRRSV transmission (Otake et al., 2003[a,b]). These reports also indicate that the infectious PRRSV can survive in the intestinal tract of mosquitoes for up to 6 hours following its feeding on an infected pig. These findings also suggest that PRRSV does not replicate within the mosquitoes to establish a sufficient titer during the 14-days incubation period and the mosquitoes, therefore, cannot serve as a biological vector for the PRRSV transmission (Otake et al., 2003[a]).

Since Thailand differs in terms of geographical region and its variety of the mosquito species from North America, the objectives of this study were to identify the mosquito species seen on a pig farm in Nakorn Pathom province, the region with the highest pig population in Thailand and to determine whether *Culex tritaeniorhynchus,* a predominant species found on pig farms, could serve as a mechanical vector for PRRSV transmission.

Materials and Methods

The protocol used in this study was approved by the committee on laboratory animal care, Faculty of Veterinary Science, Chulalongkorn University #18/2547.

Mosquito survey and mosquito colonization

Between March 2004 and February 2005, mosquitoes were captured once a month by 6 people from a PRRSV-positive pig farm in Nakorn Prathom province, Thailand, using mouth aspirators (Fig. 1). The mosquitoes were then brought to the Insectary room of the Veterinary Parasitology Unit at Chulalongkorn University for species identification (Rattanarithikul and Panthusiri, 1994) and the number of the mosquitoes in each species was counted. Based on the preliminary study, *C. tritaeniorhynchus* was the predominant mosquito species found on the pig farm. This particular species was then used in this study. The colony of *C. tritaeniorhynchus* was established and the adults (female) were maintained with mice or a 10% sucrose solution until used.

Figure 1 Mosquito collection using a mouth aspirator.

Experiment 1*: Assessment of the presence of PRRSV within the mosquitoes after feeding on a PRRSV- infected pig*

Source of mosquitoes*:* The mosquitoes used in this study were from the established colony of *C. tritaeniorhynchus.* Three to five days old of adults were starved for 8 hours prior to the study. To ensure their PRRSV-negative status, 50 mosquitoes were randomly selected and tested for the presence of PRRSV using a reverse transcriptase-polymerase chain reaction (RT-PCR) (Thanawongnuwech et al., 2004).

Experimental pigs and virus inoculation*:* Four 3-week-old piglets were purchased from a commercial, PRRSV free herd. The RT-PCR and a commercial ELISA (HerdChek PRRS, IDEXX, Westbrook, Maine, USA) were also performed to verify PRRSV-negative status. Two pigs from each group were housed in a separate room in the isolation facility of the Faculty of Veterinary Science, Chulalongkorn University to prevent the cross contamination of the pathogens between groups. After acclimatization for 3 days, the pigs in the infected group were inoculated intranasally with 4 ml of the Thai PRRSV (01NP1, a US genotype) at a concentration of 10^4 $TCID_{50}$/ml as previously described (Talummug et al., 2004). The other group was served as a negative control group. In order to prevent cross contamination of PRRSV between groups, a standard biosecurity protocol was implemented.

Experimental design*:* At 7 days post infection (DPI), PRRSV transmission by *C. tritaeniorhynchus* was performed. The experimental time was selected based on previous published data indicating the sufficient titers of PRRSV viremia in infected pigs (Talummug et al., 2004). To allow the mosquitoes to feed on the pigs, the pigs in both groups were anaesthetised using Pentobarbital sodium (Ceva animal health , Switzerland). The anaesthetised pigs were placed upon the mosquito cages, and approximately 500 mosquitoes were allowed to feed on each pig through the mesh roof of the cage for at least 30 minutes (Fig. 2). Three hundred engorged mosquitoes in each group were collected and placed in new mosquito cages. The fully-fed mosquitoes were kept in the insectary room and were given a 10% sucrose solution until used. Blood samples from those pigs were also tested for virus titration at the same time.

PRRSV detection from the fed mosquitoes

Figure 2 A model for mosquitoes feeding on the PRRSV-infected pig.

was performed using 30 pooled mosquitoes for each test at 0, 2, 4, 6, 12, 24, 48, 72 hour(s), 7 and 14 days post feeding on the PRRSV-infected pigs (PFP). At each time point, the mosquitoes were exposed to a low temperature (-20 C) and pooled in the sterile tubes. In order to prevent cross contamination, an exterior surface wash of mosquitoes from the pooled samples was done with 1 ml of minimum essential medium (MEM, Hyclone , Logan, Utah) by centrifuging it at 5000 rpm for 1 min before collecting the media and testing it. The legs of those mosquitoes were removed and placed in a new microcentrifuge tube containing 1 ml of MEM. The mosquito legs were labeled according to each sampling time and tested for the presence of PRRSV nucleic acid by RT-PCR in order to determine replication within the mosquitoes in a way similar to the West Nile virus study (Sardelis et al., 2001). The remaining mosquitoes were crushed against the tube wall with a sterile swab containing MEM and centrifuged at 4500 rpm for 5 min. The supernatants derived from the pooled samples were also tested for the presence of PRRSV by both RT-PCR and virus isolation (Thanawongnuwech et al., 2004).

***Experiment 2**: PRRSV transmission by C. tritaeniorhynchus*

***Source of mosquitoes**:* The mosquitoes used in this study were from an established colony of *C. tritaeniorhynchus*. The mosquitoes were sampled and tested for their PRRSV negative status as mentioned above. Approximately 8 hours prior to the study, the mosquitoes were starved to ensure feeding success.

***Experimental pigs and virus inoculation**:* Nine 3-week-old piglets were purchased from a commercial, PRRSV-free herd. One pig served as the donor pig (group A), while the others were divided into 4 groups (2 pigs each) as group B, group C, group D and group E. PRRSV inoculation was performed in the donor pig as mentioned before using the same PRRSV batch of the inoculum as in the experiment 1.

***Experimental design**:* The PRRSV-infected pig (group A) was anaesthetised with Pentobarbital sodium at 7 DPI. Blood sampling was done for the PRRSV titration and the mosquitoes were allowed to feed on the infected pig for 30 minutes. During feeding, the mosquitoes were interrupted to prevent the full feeding and 150 mosquitoes were collected and placed in a new small plastic cage. Four small plastic cages containing 150 mosquitoes each were placed in the humidified incubation room. An attempt at PRRSV transmission by the infected mosquitoes was performed at the appropriate time as mentioned below.

Approximately 30 minutes PFP, 100 mosquitoes were allowed to feed on one pig (group B) in the same manner as the donor pigs. A pooled sample of the 50 remaining mosquitoes was tested for the presence of infectious PRRSV using the swine bioassay as described previously by intramuscular injection of the grounded mosquitoes into the other pig (Stewart et al., 1975). Similarly, the other 3 small plastic cages containing partially fed mosquitoes were allowed to feed on the recipient pigs in group C, group D and group E at 6 hours, 24 hours and 7 days PFP, respectively.

Blood sampling was done on all the pigs at 3, 5, 7, 9 and 11 days after contact with the infected mosquitoes and tested for the presence of PRRSV by RT-PCR and ELISA. After contact with the mosquitoes for 14 days, the pigs were euthanized and necropsied. Samples from the lungs, lymph nodes and bronchial alveolar larvage fluid were collected and tested for the presence of PRRSV by RT-PCR and virus isolation (Thanawongnuwech et al., 2004). The lungs and lymph nodes of all the pigs were also tested with immunohistochemistry (Laohasittikul et al., 2004) for the presence of PRRSV antigen using SDOW-17 (Kindly provided by Dr. E. Thacker, Iowa State University, Ames, Iowa).

Results

Mosquito survey

The results of the mosquito survey from the pig farm in Nakorn Pathom Province, Thailand between March 2004 and February 2005 are shown in Table 1. The numbers of mosquitoes collected each month are demonstrated and the mosquitoes are identified. *Culex tritaeniorhynchus* (88.38 7.63%), *C. gelidus* (9.17 8.50%), *Anopheles vagus* (2.26 1.42%). and *Mansonia uniformis* (0.19 0.15%) were found in this study. The mosquito population density was found to be its highest point in October (Table 1) corresponding with the rainy season in Thailand (June-November).

Table 1 Numbers and percentages of mosquito species identified from a pig farm in Nakorn Pathom Province, Thailand.

Month	Number	*Culex tritaeniorhynchus*	*Culex gelidus*	*Anopheles vagus*	*Mansonia uniformis*
March, 04	4,200	78.92 %	19.73 %	1.28 %	0.07 %
April, 04	5,400	93.67 %	0.78 %	5.50 %	0.05 %
May, 04	5,400	92.68 %	4.72 %	2.59 %	0.01 %
June, 04	8,000	69.44 %	29.76 %	0.69 %	0.11 %
July, 04	11,600	88.53 %	10.00 %	1.06 %	0.41 %
August, 04	7,600	90.83 %	7.80 %	1.24 %	0.13 %
September, 04	8,200	91.76 %	6.17 %	1.83 %	0.24 %
October, 04	13,400	83.48 %	15. 00 %	1.15 %	0.37 %
November, 04	8,800	89.04 %	7.50 %	3.41 %	0.05 %
December, 04	4,400	95.75 %	1.73 %	2.07 %	0.45 %
January, 05	5,400	91.95 %	3.81 %	3.96 %	0.28 %
February, 05	9,400	94.48 %	3.06 %	2.34 %	0.12 %
Total	**91,800**	**88.38 %**	**9.17 %**	**2.26 %**	**0.19 %**

Experiment 1*: Assessment of the presence of PRRSV within the mosquitoes post feeding on the PRRSV-infected pig (PFP)*

At 7 DPI with RRRSV, viremia with a titer of $10^{2.70}$ $TCID_{50}$/ml was detected in the infected pigs. The PRRSV detection from mosquito samples is summarized in Table 2. Pooled mosquito legs and pooled washing fluid from the exterior surface of the mosquito samples tested negative for PRRSV by RT-PCR. The pooled mosquitoes from the samples collected between 0 to 48 hours PFP tested positive for PRRSV by the RT-PCR, whereas virus isolation was able to detect the infectious PRRSV from the mosquito samples at only 0 and 2 hour(s) PFP.

Experiment 2*: PRRSV transmission by C. tritaeniorhynchus*

The PRRSV titer in the serum of the donor pig at 7 DPI was $10^{2.23}$ $TCID_{50}$/ml. On day 14 after contact with the infected mosquitoes or after received the homogenized mosquitoes, all the recipient pigs were euthanized and necropsied. Only the recipient

Table 2 PRRSV detection in mosquitoes post feeding on the PRRSV-infected pig (PFP).

Time (PFP)	RT-PCR			VI
	Whole body	Legs	Washing fluid	
0 hr	+*	-	-	+
2 hr	+	-	-	+
4 hr	+	-	-	-
6 hr	+	-	-	-
12 hr	+	-	-	-
24 hr	+	-	-	-
48 hr	+	-	-	-
72 hr	-	-	-	-
7 days	-	-	-	-
14 days	-	-	-	-

*+ = Positive, - = Negative (All tests were done once with a pool of 30 mosquitoes)

RT-PCR = Reverse transcriptase polymerase chain reaction, Legs = Mosquito legs (pooled samples), VI = Virus isolation.

pig from group B (30 minutes PFP) treated as the swine bioassay pig tested positive for the PRRSV. The only swine bioassay pig had a seroconversion to PRRSV as early as 10 DPI by a commercial ELISA. Likewise, PRRSV was also isolated from the serum of this pig and the RT-PCR assay demonstrated PRRSV positive results in all tissues tested when necropsied. Immunohistochemistry could not demonstrate any PRRSV antigen in the tissues of any of the pigs. It is suggested that immunohistochemistry is less sensitive than the RT-PCR or virus isolation.

Discussion

The results of this study suggest that *C. tritaeniorhynchus,* a predominant mosquito species seen on a pig farm in Thailand, is possible but unlikely to serve as a mechanical vector for PRRSV transmission. However, the role of *C. gelidus, A. vagus* and *M. uniformis* in PRRSV transmission is needed since those species were also regularly seen on the pig farm. It should be noted that the results of this study are similar to the previous report in Malaysia that *C. tritaeniorhynchus* was the predominant mosquito specie found on pig farms (Vythilingam et al., 1994). Since it has been reported that *C. tritaeniorhynchus* has a preference for pigs rather than humans (Macdonald et al., 1967), this mosquito species may play a major role in the mosquito borne diseases in pigs.

In experiment 1, we confirmed our preliminary report that PRRSV nucleic acid could be detected from the pooled mosquito samples *(C. tritaeniorhynchus)* for up to 48 hours PFP by the RT-PCR. No evidence of virus multiplication in the mosquitoes was detected over 14 days PFP. In general, the positive results of RT-PCR indicated the presence of the virus genetic materials. However, this does not necessarily indicate the presence of the infectious virus (Benson et al., 2002; Yoon and

Stevenson, 2002). Therefore, we confirmed the presence of infectious PRRSV by virus isolation and the infectious virus was able to survive in the mosquitoes *(C. tritaeniorhynchus)* for up to 2 hours PFP. None of the pooled mosquito samples at 4, 6, 12, 24, 48, or 72 hours, 7 or 14 days PFP tested positive for the PRRSV. Since the extrinsic incubation periods of mosquito-borne viruses were approximately 5 to 14 days post feeding on the infected animals (Stewart et al., 1975; Beerntsen et al., 2000), it should be noted that PRRSV replication did not occur within the mosquitoes *(C. tritaeniorhynchus)* used in this study.

However, a previous report found that PRRSV persisted in the gut of the mosquitoes *(Aedes vaxans)* for up to 6 hours PFP (Otake et al, 2003[a]). One possible explanation is that the difference in mosquito species was used in the study was different and may have had different capabilities in carrying the pathogens (Beerntsen et al., 2000). Moreover, it should be noted that, differences in the amount of PRRSV in the blood of the infected pigs may have led to differences in the viral load and the survival time of the virus within the mosquitoes. In addition, several reports have indicated that PRRSV titers vary depending either on the pig age (Thanawongnuwech et al., 1998) or on the strains of the virus (Johnson et al., 2004).

The presence of the infectious virus for a longer time inside the mosquitoes may represent the possible status of its being a biological vector, since the character of the biological vector of mosquito borne diseases is determined by the extrinsic incubation period, which is variable depending on the family of virus, the initial dose, the mosquito species and the environmental temperature (Meller, 2000). Moreover, the replication of the virus within the mosquitoes before reaching a sufficient virus titer and the presence of the virus in the hemocoel are major characteristics of the biological vector (Beerntsen et al., 2000). However, our study demonstrated that there was no evidence of the PRRSV nucleic acid in the legs of the mosquitoes tested by RT-PCR and an inability to detect PRRSV several days PFP. Therefore, *C. tritaeniorhynchus* is unlikely to serve as a biological vector for PRRSV transmission.

During the experiment, it was essential to minimize the risk of cross contamination of PRRSV by the plastic containers or contaminated mosquitoes, since it has been reported that PRRSV can be detected in contaminated containers in warm and moist conditions (Dee et al., 2003). In this study, the contamination was minimized by using new plastic containers at each stage, and the contaminated mosquitoes were tested using the washing fluid from the exterior surface of the mosquitoes. No PRRSV contamination was observed in this study. In conclusion, our study suggests that the infectious PRRSV is not able to survive within *C. tritaeniorhynchus* for longer than 2 hours and again this mosquito was not able to serve as a biological vector for the PRRSV transmission.

In experiment 2, we demonstrated whether the mosquitoes were able to transmit the infectious PRRSV from donor pigs to naive pigs at different time points. Concurrently, the swine bioassay was done along with the mosquito feeding protocol. The results of this study show that only the pig in group B (30 minutes PFP) tested positive for PRRSV by swine bioassay, which is considered to be the most sensitive test for PRRSV detection (Benson et al., 2002). As has been known previously, a mosquito may take a volume of 0.001-0.002 ml blood meal (Lensen et al., 1997), which may contain approximately $10^{1.23}$ $TCID_{50}$/50 mosquitoes of PRRSV in this study. The amount of the virus titer in the homogenized mosquitoes was sufficient to cause infection when injected into the swine bioassay pig. Only 10 or fewer infectious PRRSV particles either by intranasal or by intramuscular route is sufficient to cause infection

(Yoon et al., 1999). The PRRSV positive swine bioassay in this study indicated that the virus could survive within the mosquitoes for up to 30 minutes PFP. In contrast to the swine bioassay, the pigs used in the mosquito feeding protocol did not reveal the positive results when tested by any test. It should be noted that *C. tritaeniorhynchus* used in this study were less likely to transmit PRRSV mechanically in this experiment or even in field conditions. The possible explanation is that the PRRSV titers of the infected donor pigs did not exceed the thresholds of the infection or the number of mosquitoes used in this study did not reach the threshold for the mechanical transmission. Moreover, the success of mechanical transmission depends on the virus concentration in the mouthpart of the mosquitoes (Webb et al., 1989) and the greater the number of mosquitoes used in the experiment, the more susceptible to infect the recipient pigs are expected to be.

According to the results of experiment 1, the viability of PRRSV was demonstrated within the mosquitoes for up to 2 hours PFP when tested by virus isolation. However, the results of experiment 2 showed that PRRSV remained infective only 30 minutes PFP, while the previous study showed that the virus was still infective in the mosquitoes for up to 6 hours when using the swine bioassay (Otake et al., 2003[a]). Between 30 minutes and 6 hours PFP in this study, the PRRSV might not survive in *C. tritaeniorhynchus* due to many factors such as a lower titer in the viremic donor pig, the differences of the mosquito species used or, especially, the warmer environmental temperatures in Thailand.

PRRSV could be isolated only from the serum of the swine bioassay pig receiving homogenized mosquitoes after 30 minutes PFP, whereas the results of virus isolation from bronchioalveolar larvage fluid, lungs and lymph nodes remained negative at necropsy. In young pigs, PRRS viremia persists for a longer period and the virus is more stable in the serum than in the tissues (Yoon and Stevenson, 2002). The PRRSV isolation from the serum is, therefore, more sensitive than that from the tissue samples in this study. In addition, PRRSV persistence may depend on the pig's age and the stage of infection. Although previous study has shown that the PRRSV antigen was detected frequently in the lungs, lymph nodes and tonsils, PRRSV antigen distribution depends on the stage of infection and the viral strain (Halbur et al., 1995).

Our study indicated that *C. tritaeniorhynchus*, a predominant mosquito species found on a pig farm in Nakorn Prathom Province, Thailand was unable to transmit PRRSV biologically. Although, the inability of *C. tritaeniorhynchus* to transmit PRRSV mechanically from the infected donor pig to the susceptible pigs indicated that this mosquito species was less likely to transmit PRRSV mechanically in field conditions, the PRRSV positive result from the swine bioassay indicated that mechanical transmission could occur and *C. tritaeniorhynchus* could serve as a vector for the PRRSV transmission in pig farms in the same way as the houseflies (Otake et al., 2003[b]). Further studies of other mosquito species are needed. The results are useful to the PRRSV epidemiology on PRRSV transmission by mosquitoes especially in PRRSV-negative herds located in PRRSV endemic areas.

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