Assessment of *Stomoxys calcitrans* (Diptera: Muscidae) as a Vector of Porcine Reproductive and Respiratory Syndrome Virus

K. ROCHON,1 R. B. BAKER,2 G. W. ALMOND,3 AND D. W. WATSON4


**ABSTRACT** Porcine Reproductive and respiratory syndrome (PRRS) is a globally significant swine disease caused by an arterivirus. The virus replicates in alveolar macrophages of infected pigs, resulting in pneumonia in growing pigs and late-term abortions in sows. Outbreaks occur on disparate farms within an area despite biosecurity measures, suggesting mechanical transport by arthropods. We investigated the vector potential of stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), in the transmission of porcine reproductive and respiratory syndrome virus (family *Arteriviridae*, genus *Arterivirus*, PRRSV) under laboratory conditions. Stable flies were collected around PRRS-negative boar stud barns in North Carolina and tested for presence of the virus. Stable flies were collected on alsynite traps placed near the exhaust fan of the close-sided tunnel-ventilated buildings, suggesting blood seeking flies are attracted by olfactory cues. No flies were positive for PRRSV. We assessed transmission of the virus through an infective bite by feeding laboratory reared stable flies on blood containing virus and transferring them to naïve pigs for subsequent bloodmeals. Transmission of the virus to naïve pigs by infective bites failed in all attempts. The volume of blood contained within the closed mouthparts of the stable fly seems to be insufficient to deliver an infective dose of the virus. Stable flies are unlikely to transmit PRRSV from one pig to another while blood feeding. The fate of the virus after a bloodmeal remains to be determined.

**KEY WORDS** stable fly, virus transmission, infective dose, porcine reproductive and respiratory syndrome

Porcine reproductive and respiratory syndrome (PRRS) is considered one of the most economically significant swine diseases in the United States, causing annual losses estimated at US$560 million in breeding herds and in growing pig populations (Neumann et al. 2005). The disease was first described in North Carolina in 1987 (Keffer and Baker 1989), and European cases were first reported in Germany in 1990 (Lindhaus and Lindhaus 1991). Porcine reproductive and respiratory syndrome virus (family *Artericirdae*, genus *Arterivirus*, PRRSV) was added to the Office International des Epizooties, World Organisation for Animal Health (OIE) list of reportable diseases in 1992. The disease has now spread to most swine-producing countries and is globally enzootic, with the exception of New Zealand and Australia (OIE 2009). The disease is caused by PRRSV, a positive-sense, single-strand enveloped RNA virus. Clinical signs vary with host age and may include fever, lethargy, increased mortality among nursing pigs, failure to thrive, and pneumonia in growing pigs (Horter et al. 2000). Sows and gilts suffer from late-term abortions, increased number of mummies (embryonic death between day 35 and 70 of gestation), stillborn piglets (death a few days before or during farrowing), and poor conception rates (Zimmerman et al. 2006). Boars have reduced semen quality (Prieto and Castro 2005) and shed virus in semen, which can result in venereal transmission to sows. Viremia is highest 7–14 d postinfection, with titers of $10^6$–$10^7$ tissue culture infectious dose50/ml serum, and then decreases over a period of a month (Zimmerman et al. 2006). Acute phases of disease can span 2–3 mo, followed by an apparent return to normal reproduction. Persistent infections lead to decreased farrowing rate and irregular estrus cycle (Albina 1997).

The virus is highly transmissible from pig to pig within confinement buildings by direct contact and contact with contaminated secretions such as semen, blood, and saliva (Cho and Dee 2006). Indirect transmission also occurs through contaminated objects such as boots, clothing (Otaké et al. 2002c), and needles (Otaké et al. 2002b). Transport vehicles serve as a source of virus transmission, during cold and damp weather (Dee et al. 2002, 2003). Implementation of biosecurity measures does not prevent spread of the virus, suggesting the virus is spread by airborne routes (Mortensen et al. 2002). In the laboratory, PRRSV was
transmitted from infected to naïve pigs over short distances (Brockmeier and Lager 2002, Lager et al. 2002, Kristensen et al. 2004, Trincado et al. 2004) and by aerosolized virus particles over longer distances (Dee et al. 2005). Transmission in the field has been successful over a distance of 120 m (Pitkin et al. 2009), and low amounts of infective virus have been detected in air samples 4.7 km from a source population (Dee et al. 2009). PRRSV continues to spread from farm to farm despite the presence of rigorous biosecurity protocols, suggesting insects may be involved in the dissemination of this disease.

Under laboratory conditions, house flies (Musca domestica L.) and mosquitoes [Aedes vexans (Meigen)] can mechanically transmit the virus from pig to pig (Otake et al. 2002a, Otake et al. 2004). Clearly, fly transmission potential is evident as the house fly may acquire PRRSV (Schurrer et al. 2005) and disperse from the site of origin (Schurrer et al. 2004). In a recent investigation, field captured stable flies, Stomoxys calcitrans (L.) (Diptera: Muscidae), near a large production system were positive for PRRSV by using polymerase chain reaction (PCR) (Baker 2006).

Stable flies are blood-feeding flies associated with livestock. These persistent biters take one to two bloodmeals each day, usually from different animals. Animals react vigorously to the painful bite, dislodge the flies, resulting in movement to adjacent hosts. As a result, flies rarely complete a bloodmeal without interruption (Schofield and Torr 2002) contributing to potential disease transmission from animal to animal. Because of their association with swine (Moon et al. 1987), the frequent interrupted blood feeding, and the high virus titers in the blood of PRRSV-infected pigs, we investigated the potential role of stable flies in the transmission of PRRSV among swine. The objectives of this study were to demonstrate the association of stable flies with swine by examining the prevalence of stable flies around boar barns and to evaluate the potential of stable flies to transmit PRRSV to naïve pigs from an infective bite.

Materials and Methods

Association With Swine. The prevalence of stable flies was determined at 12 boar stud barns from two swine production companies in southeastern North Carolina. Boar stud barns are close-sided and fenced in, with strict biosecurity protocols. The boars were tested for PRRSV weekly as part of the swine production company’s routine screening procedures, and remained negative throughout the study. Routine PRRSV detection procedure included RNA extraction from blood samples by using QIAmp Viral RNA Mini kit (QIAGEN, Valencia, CA), following the manufacturer’s vacuum procedure. PCR amplification was performed with the QIAGEN One Step Reverse Transcription (RT)-PCR kit (QIAGEN). We monitored the presence of stable flies by using alsynite cylinder traps (Broce 1988) placed ≈45 cm above the ground. Traps were placed around the selected boar stud barns (Fig. 1). The adhesive sleeve was changed weekly, and the number of stable flies caught was recorded. This was performed in July and August 2005, for 2–4 wk depending on the location. In addition, stable flies surrounding PRRS-negative boar barns were collected with a modified leaf blower fitted with a collection container (Stewart and Wright 1995) to determine whether they carried PRRSV. Although stable flies are known to enter buildings (Moon et al. 1987, Kaufman et al. 2005), we were interested in flies potentially bringing the virus from an outside source, and concentrated the sampling effort to flies located outside the barns. Each collection container was chilled on ice to immobilize the insects and allow the transfer to a labeled (date, time, and location) plastic bag, which was then frozen at −70°C. The contents of each bag were examined, and the stable flies removed for processing. Flies from each collecting event were transferred to labeled 1.5-ml microcentrifuge tubes and sent for PCR analysis by the swine production company diagnostic services laboratory. There were 53 collection events in total where one or more stable flies were captured, and all stable flies from one collection event were pooled. Flies were kept frozen (−50°C) to prevent degradation until PCR analysis was completed. Statistical analysis was performed with SAS for Windows, version 8.2 (SAS Institute 2001). Differences in the number of stable flies caught between companies, barns, or trap locations were determined with the GLM procedure, and means were compared using a Student–Newman–Keuls multiple range test.

PRRSV. Transmission studies were conducted using reconstituted modified live vaccine (Vetmedica RespPRRS, Boehringer Ingelheim USA, St. Joseph, MO) and a North Carolina isolate of the virus known as the NC Powell strain (McCaw et al. 2003). Modified live vaccine virus was used because it can easily be recovered using the MARK-145 cell culture line (Kim et al. 1993), whereas wild-type viruses are less adapted to culture in laboratory, resulting in inconsistent growth. The vaccine was prepared according to the manufacturer’s instructions and mixed with pig blood to a final concentration of ≈10^5.7 virions/ml whole blood. The NC Powell strain virus solution was prepared from a frozen aliquot propagated on an alveolar macrophage cell culture. The virus was harvested from the infected cells, titrated, and used as inoculum. The final con-
centration of virus fed to flies was ≈10^7 virions/ml whole blood, to mimic peak viremia in naturally infected pigs (Zimmerman et al. 2006). For experiment 1, the NC Powell strain was used in parallel with the modified live vaccine strain because the vaccine strain produced very low viremia in the positive control animals. For experiment 2, only the NC Powell strain was used because greater fly mortality was observed in the vaccine group compared with the NC Powell strain group at the end of experiment 1.

**Insect Rearing.** The stable flies used in transmission studies came from a stock colony which originated from flies reared at USDA–ARS in Gainesville, FL, and was maintained at North Carolina State University since 2005. Adults were kept in cages held at 25°C with a photoperiod of 16:8 (L:D) h. Flies were fed bovine blood, treated with sodium citrate (50 ml of Na_3C_6H_5O_7, 150 ml of H_2O, and 3 liters of fresh blood) and a 10% sucrose solution in small cups fitted with a loose paper towel top. Ovipositional substrate consisted of used house fly larval media wrapped in three layers of laboratory grade tissue (KimWipes, Kimberly-Clark Corporation, Irving, TX) placed in a small medicinal measuring cup and kept moist. Eggs were placed on a mixture of 5% Milk Plus pellets (Cargill, Minneapolis, MN), 37% wheat bran, 20% vermiculite, and 38% water by removing the outer paper layer from the ovipositional medium and placing it face down on the larval medium. Tops of rearing containers were removed, and the containers were placed in clean cages for adult emergence.

**Pigs.** PRRSV-naïve 8-wk-old piglets were obtained from North Carolina State University’s Teaching Animal Unit. The animals were screened for PRRSV by the Rollins Laboratory (North Carolina Department of Agriculture & Consumer Services, Raleigh, NC) to confirm negative status. Pigs were housed in pairs in individual isolation air spaces at Laboratory Animal Resource unit of the College of Veterinary Medicine. Animal euthanasia was performed at the end of the study (Institutional Animal Care and Use Committee protocol 05-069-A).

**Fly Transfer.** Measures were taken to prevent the transmission of PRRSV by fomites and to ensure stable flies, not the containers, were the point of transmission. The general procedure was to transfer flies from contaminated cups to sterile cups by suction to avoid direct contact between the containers. Transfers were made using custom-made containers with double lids to allow easy and sterile fly transfer (Fig. 2). This procedure allowed us to avoid direct or indirect contact between cups.

**Experiment 1: Full Bloodmeal.** Transmission of PRRSV by adult stable flies after a complete bloodmeal of viremic blood was evaluated in this experiment. Groups of flies were removed from the colony with a modified hand-held vacuum and held at room temperature in a screened container and starved for 18 h. Flies were anesthetized with CO_2 and blood solutions (≈10^7 virions/ml, vaccine strain or 10^5 virions/ml, NC Powell strain) placed into the containers. Control flies were given blood without added virus. The flies were allowed to feed for 30 min, after which they were anesthetized by cold for 5 min in a −20°C freezer. The flies were transferred into sterile experimental specimen cups with screened bottoms to recover and were provided water from a cotton wick.

Twelve PRRSV-naïve piglets were randomly assigned to pairs and separated into treatment groups. Positive control pigs (n = 2) were inoculated with 2 ml intramuscular plus 1 ml in each nostril of the RespPRRS modified live vaccine virus. Two pigs served as negative controls and eight as treatment pigs (two pigs per virus strain – vaccine and NC Powell – and time interval – 12 and 24 h after the initial bloodmeal). At 12 and 24 h after the initial artificial bloodmeal, four treatment pigs were tranquilized with acepromazine (0.55 mg/kg) and suspended in a Paneponito sling (Britz & Company, Inc., Wheatland, WY). Two small areas (≈7 cm in diameter) on the back of the animals were shaved, two cups containing 10 flies each were placed on each naïve pig (20 flies per pig), and the flies were allowed to feed to repletion. One pair of pigs was treated with vaccine-fed flies, the other pair was treated with NC Powell-fed flies. For untreated controls, sterile specimen cups containing stable flies not exposed to PRRSV were placed on the negative control pigs and allowed to feed to repletion. After feeding, the flies were transferred by suction to a sterile cup, placed in a cooler kept at 18°C, and provided water on a cotton wick. Flies were frozen immediately after the last feeding (24 h) and transferred to sterile microfuge tubes until analysis. Presence of virus was detected by real-time PCR and virus isolation. Pigs were bled weekly to monitor for viremia by real-time PCR. The experiment was performed once, each pig from a pair considered an experimental unit.

**Virus Assays.** Thawed flies were crushed with a sterile pestle in 500 µl of Dulbecco minimal essential medium and centrifuged at 14,000 rpm for 20 min to separate the debris from the supernatant used for virus detection. Active virus was detected by adding 100 µl of supernatant to duplicate microplate wells containing monolayers of MARC-145 cells (Kim et al. 1993). Cells were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum and an antibiotic solution (100 IU/ml penicillin, 100 µg/ml streptomycin, 50 µg/ml gentamicin, and 25 µg/ml Fungizone). Inoculated plates were incubated at 37°C with 5% CO_2 for 4 d. Cytopathic effect indicated the presence of PRRSV. Results were recorded after two passages on cell culture.

We also assessed the presence of PRRSV nucleic acid by real-time PCR. Total RNA was extracted from the supernatant with QIAamp Viral RNA Mini kit (QIAGEN) according to manufacturer’s spin column protocol. Transcription and amplification were performed in a single tube with the Quantitect Probe RT-PCR kit (QIAGEN). Final concentrations were 0.4 µM each reverse and forward primers and 0.2 µM probe plus 15.5 µl of water and 5 µl of template RNA (50-µl reaction volume). The probe and primer sequences were probe, 5'-HEX/TGTGGTGAATG-GCActGATTGACA/BHQ3/-3'; forward primer 1, 5'-ATGATGCGCTGGCATTCT-3'; forward primer
2, 5'-ATATGRGC TGGCATTCC-3'; and reverse primer, 5'-ACACGGTCGCCCTAATTG-3' (Kleiboeker et al. 2005). The tubes were loaded into an iCycler (Bio-Rad Laboratories, Hercules, CA) thermocycler. The program was 50°C for 30 min, 95°C for 15 min, 40 cycles of 94°C for 15 s and 60°C for 1 min. A serial dilution (10^1–10^8 copies/µl) of purified viral RNA was used to create standards used for template quantification. These standards were loaded on each plate and assayed with the samples, providing a standard curve specific to each plate reading. Real-time PCR efficiency was 108%.

**Experiment 2: Interrupted Bloodmeal.** Transmission of PRRSV by adult stable flies after a partial bloodmeal of viremic blood was evaluated in this experiment. Because stable flies are often dislodged while blood feeding, interrupted bloodmeals are more representative of what occurs in the field. Flies were prepared as described for the previous experiment, except they were interrupted while feeding on the viremic blood solution (≈10^7 virions/ml, NC Powell strain) by a small paintbrush inserted on the side of the feeding container. The flies were in contact with the blood for 5 min to allow all flies an opportunity to obtain a partial bloodmeal. The flies were then anesthetized by cold for 5 min in a −20°C freezer, transferred to sterile experimental specimen cups with screened bottoms and provided with water from a cotton wick. Sixty flies were randomly sampled and frozen immediately after feeding to serve as reference and determine the amount of virus found in the flies after feeding.

Eight PRRSV-naïve piglets were randomly assigned to pairs and separated into treatment groups. Two pigs served as negative controls and six as treatment pigs (two pigs per time interval.) At 3, 6, and 12 h after the partial bloodmeal, a pair of treatment pigs were tranquillized and prepared as outlined above. Two cups containing 15 flies each were placed on each naïve pig (30 flies per pig), and the flies were allowed to feed to repletion. Stable flies not exposed to PRRSV were placed on the negative control pigs and allowed to feed to ensure they were not previously contaminated. The flies were frozen after completing the bloodmeal, and pigs were bled weekly to detect viremia. Presence of virus was detected by real-time PCR and virus isolation in the heads and body of the flies separately.

Fig. 2. Modified vacuum used to transfer stable flies from one container to another without direct contact between cups. (A) Double lid shows almost aligned openings. (B) Modified hand-held vacuum. (C) From left to right: cup containing flies, double lid, sleeve attached to double lid, recipient cup, and vacuum opening. (D) All pieces inserted into vacuum opening. (E) Lid rotated to align openings and allow fly movement. (F) Completed transfer. Cups shown have screened bottom to allow fly feeding when placed on the pigs.
The experiment was performed once, each pig from a pair considered an experimental unit.

Results and Discussion

Association With Swine. Stable flies were captured around pig barns in southeastern North Carolina. Stable fly populations varied around the different barns (F = 4.69; df = 11, 166; P < 0.0001), with barns A, B, and C having most stable flies (Table 1). Flies were more abundant near the exhaust fans compared with the air intake of the evaporative cooling system (Table 1). Even when low numbers were captured, significantly more stable flies were collected from the exhaust fan side of the barns (P < 0.05; Student–Newman–Keuls test). PRRSV was not detected on the stable flies captured in the vicinity of known PRRSV-negative boar barns.

The goal of this experiment was to determine whether stable flies were present around boar barns in North Carolina. The collection of high numbers of stable flies around boar stud barns clearly indicates that stable flies use olfactory cues to locate potential hosts because the animals were not visible through closed wall buildings. These undetermined stimuli present at pig barns function as attractants for host seeking stable flies. The greatest numbers of flies were collected near the exhaust fans. Flies and other blood-feeding insects typically fly upwind toward a stimulus. Presumably if the velocity of the air is sufficient to prevent building ingress there will be little risk associated with the ventilation. However, if air velocity is variable, some insects may be able to enter the buildings, as was observed in the dairy industry (Lysyk 1993, Kaufman et al. 2005). In the present case, the boars were housed inside solid walled ventilated structures. Although the pigs were not within sight of the flies, a large number of stable flies were caught in a short period of time at some barns and pose a concern for swine. Interestingly, the source of the stable flies around the pig barns was unknown. Many factors may influence the abundance of stable flies in an area. Presumably, the presence of other animal production facilities, especially cattle feeding, will increase local stable fly populations (Broce et al. 2005). An examination of satellite imagery of the areas surrounding the barns revealed poultry was the most common alternative animal production in the area. Although poultry litter may support the growth and development of stable flies under rare circumstances, we suspect poultry facilities are an unlikely source of stable flies. No dairy, beef feedlots, or clearly identifiable pastures were identified from aerial photographs. Barn A had the highest number of stable flies and was located <2 km from a large lake. It is possible that fermenting overgrown algae may have served as larval breeding sites (Newson 1977). Larger facilities with many buildings, open-sided buildings, more animals, and more waste may attract and support more flies. If the flies migrate from remote breeding sites (Hogsette et al. 1989, Jones et al. 1991), then barns surrounded by dense windbreaks may have lower stable fly densities (Fried et al. 2005).

Experiment 1: Full Bloodmeal. Stable flies fed on viremic blood were transferred to naïve pigs for subsequent bloodmeals. All groups of virus-fed stable flies were positive for infective virus by using virus isolation after feeding on a naïve pig at 24 h. Control flies remained PRRSV negative. None of the treatment pigs developed viremia. Positive control pigs inoculated with live modified vaccine virus developed low viremia 7 d postinoculation, and blind passages on cell culture were necessary to detect the virus.

Virus isolated from all groups of flies was successfully grown on cell culture, indicating the virus remained infectious for ≈24 h after the initial infective bloodmeal. Because the flies were only feeding on naïve pigs, they were not reinoculated with virus.

Experiment 2: Interrupted Bloodmeal. Gut tissues of stable flies partially fed from the infective bloodmeal were positive for PRRSV by using real-time PCR. Fly heads were processed separately, and virus was
Table 2. PCR detection of PRRS viral RNA (copies per microliter) from fly tissues (body and head) and from cell culture for virus isolation for flies interrupted during their first bloodmeal

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Time (h)</th>
<th>PCR on fly</th>
<th>PCR on cell culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Body</td>
<td>Head</td>
</tr>
<tr>
<td>Group 1</td>
<td>15</td>
<td>0</td>
<td>2.44 × 10^3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3</td>
<td>4.55 × 10^4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>6</td>
<td>7.18 × 10^4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 2</td>
<td>30</td>
<td>3</td>
<td>1.16 × 10^5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>6</td>
<td>1.08 × 10^5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>12</td>
<td>2.28 × 10^4</td>
<td>0</td>
</tr>
</tbody>
</table>

Only detected at time zero, immediately after feeding (Table 2). Bacterial contamination of the cell culture made it impossible to discern whether the observed cytopathic effect was caused by PRRSV. We performed PCR on the cell culture to determine whether there was an increase in the amount of virus, which would indicate virus activity. The increase in the amount of virus present after two passages on cell culture indicated the virus multiplied. Therefore, there was active virus in fly bodies 6–12 h after partial feeding in one of each group. There was also active virus in the mouthparts of the flies immediately after feeding.

Despite the presence of active virus in the flies, none of the pigs became viremic. This brings forward two important parameters of mechanical transmission: the quantity of pathogens transferred and the stability of the pathogen. In an investigation of transmission of enzootic bovine leukosis by stable flies, Weber et al. (1988) used electron microscopy to estimate insect mouthparts volumetrically and concluded the approximate maximal volume of the closed labellum was 29.4 × 10^-6 μl (0.0294 nl). They estimated that under their experimental conditions, 3,950 mouthpart volumes were required to transfer an infective dose and concluded that the capacity of the stable fly mouthparts makes it an unlikely vector of bovine leukemia virus. More recent work based on empirical observations estimates the mouthparts volume to be 10 times greater at 4 × 10^-7 ml (0.4 nl) (Kloft 1992, Scoles et al. 2005). Using this larger volume estimate and the virus concentrations we used, the mouthparts of stable flies would contain less than one virion per fly. The volume of blood contained in the mouthparts may not all be transferred to a second host. For example, mosquitoes acting as mechanical vectors of myxoma virus leave ≈12% of the residues in the second host (Day et al. 1956). To determine a possible range of deposited virus particles, we assumed 20–100% of the bloodmeal residue was transferred (Foil et al. 1987). Even if we assume 100% deposition of mouthpart residues into a second host, no more than four virions would be transferred by 20 flies in experiment 1 and two virions by 30 flies in experiment 2. PRRSV is highly infectious, and pigs seem to be most susceptible via the parenteral route of infection (through a break in the skin) (Hermann et al. 2005). Doses as low as 20 virions, in a 2-ml inoculum, cause infection when administered intramuscularly (Yoon et al. 1999). However, the modified live vaccine virus is a low pathogenicity strain and a larger inoculum is required to cause infection. This was demonstrated by the low viremia produced in our inoculated pigs. In addition, the volume of liquid injected by a fly is <2 ml, and the inoculation is intracutaneous rather than intramuscular. Because active virus was only detected in the mouthparts (head) of the flies immediately after feeding, it is likely that the time elapsed between the infectious bloodmeal and the contact with the naïve animals was sufficient to inactivate the virus. In a study of bloodmeal residues in tabanid mouthparts, it was revealed that the level of blood residue after feeding decreased over time when the flies were kept alive, probably due to grooming (Foil et al. 1987). PRRSV is also a fragile virus and it does not persist in the environment (Zimmerman et al. 2006, Hermann et al. 2007). These two factors, combined with the minute volume of bloodmeal residue in stable fly mouthparts, and the time elapsed postfeeding of the infective bloodmeal, contributed to the failure of PRRSV transmission in our experiments. Regurgitation of previously ingested contaminated blood by stable flies is considered an unlikely event, because blood is a valuable protein source to the fly and is usually sent directly to the gut of the insect rather than stored in the crop. This aspect of feeding is different from house flies and may explain why the latter successfully transmitted PRRSV under laboratory conditions (Otake et al. 2004).

The viremia in our positive control animals was so low and slow to develop that if transmission of the virus had occurred, it would not have been detected under our experimental design. However, these results indicate that stable flies are not likely to play a role in area spread of PRRSV by transmitting an infective dose of the virus directly to animals through a bite because of the low volume contained within their mouthparts. Although each experiment was performed only once, these data also reveal the virus remains active in stable fly gut for a period of at least 24 h. The part stable flies may play in dissemination of the virus remains to be more thoroughly investigated. We suspect the virus is protected from the environment and desiccation while in the fly gut and that it could be deposited onto feed or fomites that could indirectly infect the animals, particularly if a highly infectious strain is present in sufficient amounts.
Acknowledgments

We thank Jinsheng Xu and Patty Routh (College of Veterinary Medicine) and Steve Denning (College of Agriculture and Life Sciences) for technical assistance. We are grateful to Isabel Gimeno for expert advice and to Tim Lysyk for helpful comments on the manuscript. This work was funded by Boehringer Ingelheim Vetmedica through the Advancement in PRRS Research Award 2005 and by North Carolina State University Colleges of Veterinary Medicine and Agriculture and Life Sciences.

References Cited


Received 21 January 2010; accepted 27 March 2011.