https://doi.org/10.52973/rcfcv-e35732 Revista Científica, FCV-LUZ / Vol. XXXV

Detection of PRRS virus in housefly (Musca domestica) in a pig farm in southeastern Mexico

Detección del virus de PRRS en la mosca doméstica (Musca domestica) en una granja porcina en el sureste de México

Gladys Noh-Cuxim[1], Alejandro Alzina-López[1], Guadalupe Ayora-Talavera[2], Jesús Ek-Mex[3], José Segura-Correa[1]

[1] Universidad Autónoma de Yucatán, Facultad de Medicina Veterinaria y Zootecnia, Campus de Ciencias Biológicas y Agropecuarias. Carretera Mérida-Xmatkuil, Km 15.5, Apartado postal 4-116, CP. 97100, Mérida Yucatán, México.

[2] Universidad Autónoma de Yucatán, Centro de Investigaciones Regionales "Dr. Hideyo Noguchi". C.43 s/n entre 96 Paseo de las Fuentes y 40 Col. Inalámbrica. Apartado postal 97225, Mérida Yucatán, México.

[3] Universidad Autónoma de Baja California, Instituto de Investigaciones en Ciencias Veterinarias.Carretera a San Felipe Km.3.5 Fraccionamiento Laguna Campestre, CP. 21386, Mexicali, Baja California, México.

**Corresponding autor: jose.segura@correo.uady.mx*

ABSTRACT

The goal of this study was to detect the presence of the porcine respiratory and reproductive syndrome virus in housefly (*Musca domestica*) in a pig farm. To know the porcine respiratory and reproductive syndrome virus circulation in the farm, 1277 blood samples, and 26 samples of oral fluids from pigs housed in pens were taken to detect the nucleic acid of the porcine respiratory and reproductive syndrome virus using real time RT-PCR test. Three-hundred flies were collected, using an entomological net, and 50 samples of 6 flies each were formed. Data was analyzed using descriptive statistics. From the sera tested, 95.74% (1224/1277) were positive in the ELISA test. The nucleic acid of the porcine respiratory and reproductive syndrome virus was detected in 34.6% (9/26) of the oral fluids and in 4% (2/50) of the fly samples analyzed. The real time-PCR test allowed detecting of the porcine respiratory and reproductive syndrome virus in houseflies. Therefore, further virus sequencing studies are needed to better understand the role of flies in the porcine respiratory and reproductive syndrome transmission.

Key words: Houseflies; PRRSV; real-time-PCR; swine

RESUMEN

El objetivo de este estudio fue detectar la presencia del virus del síndrome respiratorio y reproductivo porcino en moscas domésticas (*Musca domestica*) en una granja comercial de cerdos. Para conocer la circulación del virus del síndrome respiratorio y reproductivo porcino en la granja, se tomaron muestras de sangre de 1277 y 26 muestras de fluidos orales de cerdos alojados en corrales para detectar el ácido nucleico del virus del síndrome respiratorio y reproductivo porcino utilizando la prueba de RT-PCR en tiempo real. Se recolectaron 300 moscas, utilizando una red entomológica, y se formaron 50 grupos de 6 moscas cada una. Los datos se analizaron mediante estadísticas descriptivas. De los sueros analizados el 95,74% (1224/1277) fueron positivos en la prueba ELISA. El ácido nucleico del virus del síndrome respiratorio y reproductivo porcino se detectó en el 34,6% (9/26) de los fluidos orales y en el 4% (2/50) de las muestras de moscas analizadas. La prueba de PCR en tiempo real permitió detectar el virus del síndrome respiratorio y reproductivo porcino en moscas domésticas. En conclusión, más estudios de secuenciación del virus son necesarios para comprender mejor la función de las moscas en la transmisión del síndrome respiratorio y reproductivo porcino.

Palabras clave: Mosca domestica; PRRSV; PCR tiempo real, cerdos

Recibido: 30/06/2025 Aceptado: 11/09/2025 Publicado: 17/10/2025

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) virus is a pathogen of wide world distribution, with negative effects for the swine industry [1]. The cost caused on pigs by the PRRS virus in the USA is around $664 million per year [2, 3]. The PRRS virus, a single-stranded, positive-sense, enveloped RNA belongs to the family Arteriviridae, genus *Arterivirus* and order Nidovirales [4].

PRRS virus classified into two major genotypes, European or type 1 (PRRSV-1), Lelystad strain, and the North American or type 2 (PRRSV-2), isolated ATCC-VR-2332. Both genotypes share 63.4% identity at genomic level [5, 6, 7]. There are different control strategies for PRRS virus, such as vaccination, use of inoculates, natural infection and the use of gilts grown in the own farm [8, 9, 10]. Transmission commonly occurs by direct contact between pigs because of high population density or by exposition to contaminated body fluids contaminated blood with PRRS virus, semen, secretions and saliva [4], contaminated needles, fomites (coveralls and boots) and vectors such as Diptera [11].

Houseflies (*Musca domestica*) are insects that belong to the Diptera order, which are considered potential agents in the transmission of PRRS disease [12]. Flies are common insects in pig farms, where they reproduce and feed animal wastes, transmitting bacteria, viruses or parasites when they defecate or regurgitate the food, infecting other animals and humans with acquired pathogens [13]. Previous studies have demonstrated that houseflies could serve as vectors for PRRS virus; however, experimental studies indicate that this virus seems not to replicate in flies, which means that they could not act as a biological vector [11, 14, 15, 16]. However, experimental studies have limitations, such as laboratory conditions and the size of fly populations. The goal of the present study was to detect, through RT-PCR test, the presence of PRRS virus in houseflies in a commercial pig farm.

MATERIALS AND METHODS

Study design, site and animals

A transversal descriptive study was carried out in a population of houseflies, in a replacement gilts barn, where gilts were distributed in 72 pens with approximately 11 animals per pen.

The study was conducted in a pig (*Sus scrofa domesticus*) farm located in Yucatán, Mexico. The climate of the region is tropical subhumid with average temperature, rainfall, and relative humidity of 26 °C, 1200 mm, and 78 %, respectively [17]. The farm was a continuous flow and complete cycle farm with approximately 5500 sows, PRRS positive stable, according to the classification of Holtkamp *et al*. [18]. To control PRRS effects on animals, the farm produced its own gilt replacement, which are taken to an acclimatization area. The farm did not applied vaccines against PRRS. Serum samples were taken from gilts before they were introduced to the breeding herd, at approximately 30 weeks of age. Only gilts positive to the ELISA test were kept.

Biological samples from pigs and houseflies

To detect PRRS virus antibodies in pigs in a commercial farm, 1227 gilt serum samples were taken. The samples were analyzed with a commercial kit (IDEXX HerdCheck PRRS X3, USA), and they were considered seropositive when the S/P ratio was ≥ 0.4, according to the manufacturer instructions. The ELISA test was carried out at the clinical laboratory of the Faculty of Veterinary Medicine and Animal Science of the *Universidad Autónoma de Yucatán*.

Oral fluid samples from 26 pens, out of a total of 72 pens, were collected by allowing pigs to interact with 20 cm cotton ropes for 20 min, using the protocol described by Prickett *et al.* [19, 20]. Thereafter, the oral fluids were deposited in collection tubes, stored in coolers, and then sent to the laboratory for analysis.

Housefly samplings were carried out using an entomological net [21, 22] throughout the length of the barn. Three-hundred houseflies were collected, placed in a mesh cage, and stored at −80 °C in an ultra-low temperature freezer (VWR model 5703, USA). Then pools of six flies were deposited in sterile Eppendorf tubes for a total of 50 samples [11, 23]. Pools were pressed in the wall of the tube, added 500 µL of phosphate buffered saline (PBS) and centrifuge at 450 G per 5 min, to obtain the supernatant [11].

To detect PRRS virus nucleic acid, all oral fluid samples and housefly homogenates were tested by real-time RT-PCR (VetMAX™ PRRSVNA & EU Controls of Therma Fisher Scientific, USA) following the manufacturer instructions and using the sequence of the ORF7 gene. A PCR reaction was considered positive if the cycle threshold (Ct) level was obtained at <37 cycles. The quality control of the real-time RT-PCR process included negative (nuclease-free water) and positive (PRRSV RNA) controls, provided by the kit (VetMAX™ PRRSV NA & EU Controls, Thermo Fisher Scientific, USA)

Statistical analysis

Data were analyzed using descriptive statistics through the Excel software, and the kappa test to compare the diagnostic assays (oral fluid vs serology).

RESULTS AND DISCUSSION

A total of 1,277 serum samples from gilts distributed across six groups were analyzed. The ELISA test indicated that 1239 serum (97,02%) were seropositive. In the six groups, percentages of seropositive cases were 98,90% (179/181), 99,45% (181/182), 98,74% (236/239), 99,30% (283/285), 100% (194/194) and 84,69% (166/196), respectively.

This is the first study that detects the PRRS virus in housefly under field conditions in Mexico. In addition, farm PRRS virus seropositive was confirmed by the presence of specific antibodies in replacement gilts. The high prevalence (>95%) of antibodies indicates the titers circulation of the virus and the development of antibodies in an endemically infected farms [24, 25]. The highest percentage of positive animals could be because the gilts were sampled 19 weeks after staying in the same barn with pigs (lots) of different ages, ranging from

6 to 20 weeks of age. However, that does not mean that pigs were shedding the PRRS virus. The short duration of passive immunity in piglets could make them susceptible to infection or natural reinfection. Geldhof *et al*. [26] found antibodies against PRRS virus in piglets, transmitted through colostrum and still detectable in serum up to 5 weeks of age. The gilts, in this study, were sampled at 200 days (d) of age, which agree with Flores-Mendoza and Hernández [27] results, who mention that the antibodies produced by the infection with PRRS virus can remain for long periods but with low titers.

The high percentage of seropositive gilts here found shows the presence of infection or natural reinfection. IgG antibodies could be found between 7- and 15-d post infection, remaining constant for months and declining between 135- and 300-d post infection [9 , 28 , 29].

PRRS virus nucleic acid was detected by RT-PCR in 9 of the 26 oral fluid samples (34,6%). In fly homogenates, 2 of 50 samples were detected positive (4%). Diagnostic results from fly homogenates and oral fluid are shown in FIG. 1. The positive control amplified in cycle 29.

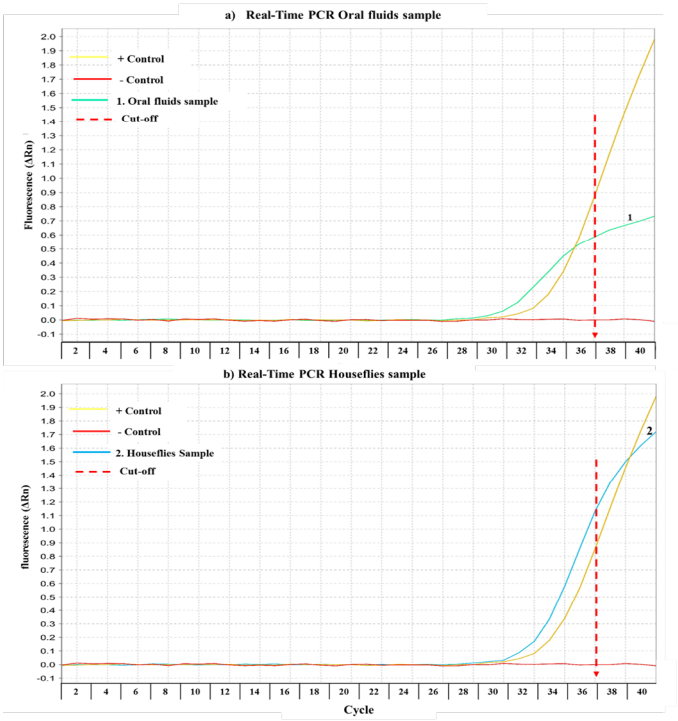

FIGURE 1. Amplification of the ORF 7 gene by real-time PCR of oral fluids (a) from pigs and housefly homogenates (b). The cut point is represented by a dotted red line. The positive and negative controls of the amplified ones are represented in each figure (yellow and red lines respectively). Number 1 corresponds to a sample of oral fluid from pigs, and number 2 corresponds to a homogenate sample of houseflies. The "X" axis corresponds to the amplification cycles, and the "Y" axis is the fluorescence production

The 34,6% (9/26) positive samples detected in oral fluid by real-time PCR test, agree with De Regge and Cay [30] who found that oral fluid samples would be PCR positive when more than 30% of individual pigs are serum positive. On other hand, Olsen *et al.* [31] reported 90% positive animals to PRRS virus using the PCR test in oral fluid, when 36% of the pigs were vaccinated. Prickett *et al*. [19] tested samples of oral fluid and serum for the detection of PRRS virus with pigs of 3, 5, 8, 12 and 16 weeks of age, that were infected with the PRRS virus and obtained a 77% concordance between oral fluid and serum samples.

An explanation of the low detection of the virus in oral fluid, is that probably few animals were shedding the virus in the saliva, as the transmission of PRRS virus in the animals here studied occurs naturally, causing the moments of contact to be different. This generates a dilution with negative saliva, whereby oral fluid samples could be below the detection limit of the test. Seddon *et al*. [32] reported that 80% of the pigs in a corral chew the rope within 30 min of its placement. The real-time PCR test in oral fluid has a high sensitivity when 30% of the pigs are individually shedding the virus [30].

Several authors mention that when the viral load is very low, the samples deteriorate during the conservation period, which could result in a false negative case, which was not very likely in this study, since the cold chain was taken care of [33 , 34].

Houseflies are insects that belong to the order Diptera, considered potential agents in the transmission of diseases [12]. They are common in pig farms, where they develop and feed on animal waste and this is where these insects can acquire bacteria, viruses or parasites, and disseminate by flies hairs, legs, body and proboscis (by ingesting liquids from animal tissues, including blood, serum, saliva, mucus and tear secretions). These insects, by defecating and regurgitating [16 , 35] can infect animals and humans with the acquired agents [13]. Previous studies have shown that flies act as vectors for pathogen transmission [36] as well as PRRS virus [11].

Previous studies reported that the houseflies are a mechanical vector of PRRS virus [37], which can transport the pathogen through its morphological structure [38].

PRRS virus do not require many viral particles to cause an infection, according to Yoon [39] who found that transmission only requires ≤20 virions.

However, here, the nucleic acid of PRRS virus was detected in only 4% (2/50) of the samples, which could be due to the handling of the samples, because the frozen of the extracted RNA could have suffered some type of degradation [40]. However, based on the sample size, the amount of viruses here found could be important in the dissemination, as few viral particles are required to establish an infection [41]. A possible explanation for the absence of PRRS virus in fly homogenates could be due to a low number of viremic pigs or low loads of the virus shedding by infected pigs [42]; and to the amount of virus in the flies that could be detected [37]. Schurrer *et al.* [16] propose that virus retention in flies appears to be related to the initial viral load after ingestion and ambient temperature.

Otake *et al.* [11] using real-time PCR observed that the PRRS virus could remain viable in the intestinal tract of the houseflies up to 12 h after feeding fluids from an infected pig, but only for a short period on outer surface of the flies. On the other hand, Schurrer *et al.* [16] found that the PRRS virus could remain 48 h in the fly after its exposure to the virus; however, the prevalence of positive flies and the viral load decreased with time. The ability of the PRRS virus to remain within the body

of a housefly suggests that it could protect the virus against certain environmental factors that affect the survival of this microorganism outside the host, such as ultraviolet light and drying factors [43]. However, Grübel *et al.* [44] mention that the midgut of the housefly has a pH of 3,1, which could be another explanation for the low number of positive samples, since the PRRS virus remains viable at a pH between 6,0 and 7,65 [45], thus decreasing the possibility of finding the virus in the macerates. There are studies that mention that the flight capacity of housefly could increase the risk of the transmission of disease. Schurrer *et al.* [15] reported that the housefly flight exceeds 5 km, with a distance dispersion of up to 33 km. It is very relevant to mention that the farm here studied was in a high pig density area with a large population of pigs within 10 km. In the present study, the season effect was not evaluated; however, it could be a risk factor, since there are reports of increments in the number of outbreaks, with low incidence during spring and summer, and high during the fall and winter seasons [46].

CONCLUSION

The porcine reproductive and respiratory syndrome virus was detected in domestic flies using the real-time PCR test; however, more epidemiological and molecular studies are needed before being able to reach a conclusion regarding the importance of domestic flies in the transmission of the porcine reproductive and respiratory syndrome virus among and between pig farms.

Conflict of interest statement

The authors proclaim that there is no conflict of interest with regard the publication of this manuscript.

Ethics approval

Animal ethic committee approval was not necessary because oral fluid collection was not invasive, not painful and pigs were not forced to interact. Blood samples included in the study were obtained from routine veterinary work in the farm.

BIBLIOGRAPHIC REFERENCES

[1] Neumann EJ, Kliebenstein JB, Johnson CD, Mabry JW, Bush EJ, Seitzinger AH, Green Al, Zimmerman JJ. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. J. Am. Vet. Med. Assoc. [Internet]. 2005; 227(3):385-392. doi: https://doi.org/fjmgq3

[2] Holtkamp DJ, Kliebenstein JB, Neumann EJ, Zimmerman JJ, Rotto HF, Yoder TK, Wang C, Yeske PE, Mowrer CL, Haley CA. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. J. Swine Health Prod. [Internet]. 2013 [cited 22 May 2025]; 21(2):72-84. Available in: https://goo.su/UCJXvn

[3] Taira O, Kato A, Tsutsumi N, Sugiura K. Epidemiological review of porcine reproductive and respiratory syndrome virus (PRRSV) in Japan: From Discovery and Spread to Economic Losses and Future Prospects. Vet. Sci. [Internet]. 2025; 12(6):554. doi: https://doi.org/g9vrqq

[4] Cho JG, Dee SA. Porcine reproductive and respiratory syndrome virus. Theriogenology. [Internet]. 2006; 66(3):655-662. doi: https://doi.org/ctkxs6

[5] Wensvoort G, de Kluyver EP, Luijtze EA, den Besten A, Harris L, Collins JE, Christianson WT, Chladek D. Antigenic comparison of Lelystad virus and swine infertility and respiratory syndrome (SIRS) virus. J. Vet. Diagn. Investig. [Internet]. 1992; 4(2):134-138. doi: https://doi.org/fw8k5v

[6] Collins JE, Benfield DA, Christianson WT, Harris L, Hennings JC, Shaw DP, Goyal SM, McCullough S, Morrison RB, Joo HS, Gorcyca D, Chladek D. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs. J. Vet. Diagn. Investig. [Internet]. 1992; 4(2):117-126. doi: https://doi.org/cwjw9z

[7] Allende R, Lewis TL, Lu Z, Rock DL, Kutish GF, Ali A, Doster AR, Osorio FA. North American and European porcine reproductive and respiratory syndrome viruses differ in non-structural protein coding regions. J. Gen. Virol. [Internet]. 1999; 80(2):307-315. doi: https://doi.org/p8f2

[8] Batista L, Pijoan C, Torremorell M. Experimental injection of gilts with porcine reproductive and respiratory syndrome virus (PRRSV) during acclimatization. J. Swine Health Prod. [Internet]. 2002; 10(4):147-150. doi: https://doi.org/p8f3

[9] Batista L, Pijoan C, Dee S, Olin M, Molitor T, Joo HS, Xiao Z, Murtaugh M. Virological and immunological responses to porcine reproductive and respiratory syndrome virus in a large population of gilts. Can. J. Vet. Res. [Internet]. 2004 [cited 12 May 2025]; 68(4):267-273. Available in: https://goo.su/PLo0qI

[10] Fano E., Olea L, Pijoan C. Eradication of porcine reproductive and respiratory syndrome virus by serum inoculation of naïve gilts. Can. J. Vet. Res. [Internet]. 2005 [cited 12 Feb 2025]; 69(1):71–74. Available in: https://goo.su/x7JkS

[11] Otake S, Dee SA, Moon RD, Rossow KD, Trincado C, Farnham M, Pijoan C. Survival of porcine reproductive and respiratory syndrome virus in houseflies. Can. J. Vet. Res. [Internet]. 2003 [cited 30 May 2025]; 67(3):198-203. Available in: https://goo.su/oyZGY

[12] Fasanella A., Scasciamacchia S, Garofolo G, Giangaspero A, Tarsitano E, Adone R. Evaluation of the house fly *Musca domestica* as a mechanical vector for an anthrax. PLoS One. [Internet]. 2010; 5(8):e12219. doi: https://doi.org/c3xwbf

[13] Hung KY, Michailides TJ, Millar JG, Wayadande A, Gerry AC. House fly (*Musca domestica L.*) attraction to insect honeydew. PLoS One. [Internet]. 2015; 10(5):e0124746. doi: https://doi.org/f7kx7j

[14] Dee SA, Schurrer JA, Moon RD, Fano E, Trincado C, Pijoan C. Transmission of porcine reproductive and respiratory syndrome virus under field conditions during a putative increase in the fly population. J. Swine Health Prod. [Internet]. 2004; 12(5):242-245. doi: https://doi.org/p8g2

[15] Schurrer JA, Dee SA, Moon RD, Rossow KD, Mahlum C, Mondaca E, Otake S, Fano E, Collins JE, Pijoan C.

Spatial dispersal of porcine reproductive and respiratory syndrome virus-contaminated flies after contact with experimentally infected pigs. Am. J. Vet. Res. [Internet]. 2004; 65(9):1284-1292. doi: https://doi.org/cnz6fr

[16] Schurrer JA, Dee SA, Moon RD, Murtaugh MP, Finnegan CP, Deen J, Kleiboeker SB, Pijoan CBJ. Retention of ingested porcine reproductive and respiratory syndrome virus in houseflies. Am. J. Vet. Res. [Internet]. 2005; 66(9):1517-1525. doi: https://doi.org/c3g82f

[17] Instituto Nacional de Estadística y Geografía (INEGI). Prontuario de información geográfica municipal de los Estados Unidos Mexicanos. INEGI. [Internet]. 2009 [cited 10 Jun 2025]; 2:1-9 Available in: https://goo.su/GxP2QU

[18] Holtkamp DJ, Polson DD, Torremorell M, Morrison B, Classen DM, Becton L, Henry S, Rodibaugh MT, Rowland RR, Snelson H, Straw B, Yeske P, Zimmerman J. Terminology for classifying siwne herd by porcine reproductive and respiratory syndrome virus status. J. Swine Health Prod. [Internet]. 2011; 19(1):44-56. doi: https://doi.org/p8g6

[19] Prickett J, Simer R, Christopher-Hennings J, Yoon K, Evans RB, Zimmerman JJ. Detection of porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: a longitudinal study under experimental conditions. J. Vet. Diagn. Investig. [Internet]. 2008; 20(2):156-163. doi: https://doi.org/d3xqnm

[20] Prickett J, Kim W, Simer R, Yoon K, Zimmerman J. Oral-fluid samples for surveillance of commercial growing pigs for porcine reproductive and respiratory syndrome virus and porcine circovirus type 2 infections. J. Swine Health Prod. [Internet]. 2008; 16(2):86–91. doi: https://doi.org/p8g7

[21] Roulston TH, Smith SA, Brewster AL. A comparison of pan trap and intensive net sampling techniques for documenting a bee (Hymenoptera: Apiformes) fauna. J. Kans. Entomol. Soc. [Internet]. 2007; 80(2):179-181. doi: https://doi.org/csft2w

[22] Ramírez-Freire L, Alanís-Flores G, Ayala-Barajas R, Velazco-Macias C, Favela-Lara S. El uso de platos trampa y red entomológica en la captura de abejas nativas en el estado de Nuevo León, México. Acta Zool. Mex. [Internet]. 2014 [cited 15 May 2025]; 30(3):508-538. Available in: https://goo.su/7Q7v0sn

[23] Otake S, Dee SA, Moon RD, Rossow KD, Trincado C, Pijoan C. Studies on the carriage and transmission of porcine reproductive and respiratory syndrome virus by individual houseflies (*Musca domestica*). Vet. Rec. [Internet]. 2004; 154(3):80-85. doi: https://doi.org/cjdd8k

[24] Albina E. Epidemiology of porcine reproductive and respiratory syndrome (PRRS): An overview. Vet. Microbiol. [Internet]. 1997; 55(1-4):309-316. doi: https://doi.org/fnxcr9

[25] Nodelijk G, van Leengoed LAMG, Schoevers EJ, Kroese AH, De Jong MCM, Wensvoort G, Verheijden JHM. Seroprevalence of porcine reproductive and respiratory syndrome virus in Dutch weaning pigs. Vet. Microbiol. [Internet]. 1997; 56(1-2):21-32. doi: https://doi.org/bftz75

[26] Geldhof MF, Van Breedam W, De Jong E, Lopez-Rodriguez A, Karniychuk UU, Vanhee M, Van Doorsselaere J, Maes D, Nauwynk HJ. Antibody response and maternal immunity upon boosting PRRSV-immune sows with experimental farm-specific and commercial PRRSV vaccines. Vet Microbiol. [Internet]. 2013; 167(3-4):260-271. doi: https://doi.org/f5qrv7

[27] Flores-Mendoza L, Hernández J. Vacunas contra el virus del síndrome reproductivo y respiratorio porcino (PRRSV): escribiendo una historia. Vet. Mex. [Internet]. 2010 [cited 10 Jun 2025]; 41(2):139-159. Available in: https://goo.su/RaJUlR1

[28] Nelson EA, Christopher-Hennings J, Benfield DA. Serum immune responses to the proteins of porcine reproductive and respiratory syndrome (PRRS) virus. J. Vet. Diagn. Investig. [Internet]. 1994; 6(4):410–415. doi: https://doi.org/dtxqhg

[29] Li X, Galliher-Beckley A, Pappan L, Trible B, Kerrigan M, Beck A, Hesse R, Blecha F, Nietfeld JC, Rowland RR, Shi J. Comparison of host immune responses to homologous and heterologous type II porcine reproductive and respiratory syndrome virus (PRRSV) challenge in vaccinated and unvaccinated pigs. Biomed. Res. Int. [Internet]. 2014; 2014:416727. doi: https://doi.org/gb83qv

[30] De Regge N, Cay B. Comparison of PRRSV nucleic acid and antibody detection in pen-based oral fluid and individual serum samples in three different age categories of post-weaning pigs from endemically infected farms. PLoS One. [Internet]. 2016; 11(11):e0166300. doi: https://doi.org/f9rj2r

[31] Olsen C, Karriker L, Wang C, Binjawadagi B, Renukaradhya G, Kittawornrat A, Lizano S, Coetzee J, Main R, Meiszberg A, Panyasing Y, Zimmerman J. Effect of collection material and sample processing on pig oral fluid testing results. Vet. J. [Internet]. 2013; 198(1):158-163. doi: https://doi.org/f5j6qg

[32] Seddon YM, Guy JH, Edwards SA. Optimising oral fluid collection from groups of pigs: effect of housing system and provision of ropes. Vet. J. [Internet]. 2012; 193(1):180-184. doi: https://doi.org/b6gx89

[33] Prickett JR, Cutler S, Kinyon JM, Naberhaus N, Stensland WR, Yoo K, Zimmerman JJ. Stability of porcine reproductive and respiratory syndrome virus and antibody in swine oral fluid. J. Swine Health Prod. [Internet]. 2010 [cited 10 Jun 2025]; 18(4):187–195. Available in: https://goo.su/tZ1D5N

[34] Decorte I, Van der Stede Y, Nauwynck H, De Regge N, Cay AB. Effect of saliva stabilisers on detection of porcine reproductive and respiratory syndrome virus in oral fluid by quantitative reverse transcriptase real-time PCR. Vet. J. [Internet]. 2013; 197(2):224-228. doi: https://doi.org/f5chnp

[35] Wang YC, Chang YC, Chuang HL, Chiu CC, Yeh KS, Chang CC, Hsuan SL, Lin WH, Chen TH. Transmission of Salmonella between swine farms by the housefly (*Musca domestica*). J. Food Prot. [Internet]. 2011; 74(6):1012-1016. doi: https://doi.org/d79tmb

[36] Murvosh CM, Thaggard CW. Ecological studies of the house fly. Ann. Entomol. Soc. Am. [Internet]. 1996; 59(3):533-547. doi: https://doi.org/p8g8

[37] Otake S, Dee SA, Rossow KD, Moon RD, Pijoan C. Mechanical transmission of porcine reproductive and respiratory syndrome virus by mosquitoes, *Aedes vexans* (Meigen). Can. J. Vet. Res. [Internet]. 2002 [cited 25

Jun 2025]; 66(3):191-195. Available in: https://goo.su/zx6HDn

[38] Förster M, Klimpel S, Mehlhorn H, Sievert K, Messler S, Pfeffer K. Pilot study on synanthropic flies (e.g. *Musca, Sarcophaga, Calliphora, Fannia, Lucilia, Stomoxys*) as vectors of pathogenic microorganisms. Parasitol. Res. [Internet]. 2007; 101(1):243-246. doi: https://doi.org/fkrkm8

[39] Yoon KJ, Zimmerman JJ, Chang CC, Cancel-Tirado S, Harmon KM, McGinley MJ. Effect of challenge dose and route on porcine reproductive and respiratory syndrome virus (PRRSV) infection in young swine. Vet. Res. [Internet]. 1999 [cited 15 Jun 2025]; 30(6):629-638. Available in: https://goo.su/lRHer

[40] Hernández A, Martín Vasallo P, Torres A, Salido E. Análisis del RNA: Estudio de la expresión génica. Nefrología. [Internet]. 1994 [cited 10 Jun 2025]; 14(2):145-162. Available in: https://goo.su/pkqyh

[41] Morgan-Morrow WE, Roberts J. PRRS fact sheet for animal science. Anim. Sci. Facts. [Internet]. 2001 [cited 10 Jun 2025]; 817S:1-2. Available in: https://goo.su/UksMkj

[42] Pitkin A, Deen J, Otake S, Moon R, Dee S. Further assessment of houseflies (*Musca domestica*) as vectors for the mechanical transport and transmission of porcine reproductive and respiratory syndrome virus under field conditions. Can. J. Vet. Res. [Internet]. 2009 [cited 10 Jun 2025]; 73(2):91-96. Available in: https://goo.su/zIA57L

[43] Benfield DA, Nelson E, Collins JE, Harris L, Goyal SM, Robison D, Christianson WT, Morrison RB, Gorcyca D, Chladek D. Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). J. Vet. Diagn. Investig. [Internet]. 1992; 4(2):127-133. doi: https://doi.org/dwrr3m

[44] Grübel P, Hoffman JS, Chong FK, Burstein NA, Mepani C, Cave DR. Vector potential of houseflies (*Musca domestica*) for *Helicobacter pylori*. J. Clin. Microbiol. [Internet]. 1997; 35(6):1300-1303. doi: https://doi.org/ghszj4

[45] Bloemraad M, de Kluijver EP, Petersen A, Burkhardt GE, Wensvoort G. Porcine reproductive and respiratory syndrome: temperature and pH stability of Lelystad virus and its survival in tissue specimens from viraemic pigs. Vet. Microbiol. [Internet]. 1994; 42(4):361-371. doi: https://doi.org/fqmr38

[46] Tousignant SJ, Perez AM, Lowe JF, Yeske PE, Morrison RB. Temporal and spatial dynamics of porcine reproductive and respiratory syndrome virus infection in the United States. Am. J. Vet. Res. [Internet]. 2015; 76(1):70-76. doi: https://doi.org/f65j5v