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Epizootic hemorrhagic disease virus oral infection affects midge reproduction and is vertically transmitted to offspring in *Culicoides sonorensis*

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Epizootic hemorrhagic disease virus (EHDV: Reoviridae: *Orbivirus*) is a *Culicoides*-borne pathogen that affects a variety of ruminants, causing significant economic losses and/or ecological impacts in animal agriculture/wildlife populations worldwide. In this study, we examined the effect of EHDV serotype-2 oral infection on the survival and reproduction of *Culicoides sonorensis* Wirth and Jones (a confirmed vector of EHDV in North America), and the potential vertical transmission of EHDV-2 (from infected female to its offspring) in this midge species. *Culicoides sonorensis* females were fed on defibrinated bovine blood mixed with EHDV-2 (5.5 $\log_{10}$ PFU/ml) or without EHDV-2 (control). Adult survival/longevity, oviposition rates, number of eggs deposited, egg hatch rates (fertility), larval survival, larval stage duration, eclosion rates, and sex-ratios of the progeny were recorded and compared between the two groups. In addition, the progeny (eggs and F_1 generation adults) of EHDV-2 fed females were processed for viral detection through RT-qPCR and plaque assays. Survival/longevity of the blood-fed adults, oviposition rates, number of eggs deposited, larval stage duration, eclosion rates, and sex-ratios were not significantly different between the two groups. However, egg hatch rates were significantly lower in the EHDV-2 fed group (35.8 ± 5.2%) than the control group (74.5 ± 6.8%), but larval survival rates were higher in the EHDV-2 fed group (59.8 ± 4.9%) compared to the control group (34.1 ± 6.5%). EHDV-2 (Ct < 35) was detected in the eggs (3.4%, 1/29 females tested, Ct = 22.1 [4.9 $\log_{10}$ PFUe/ml]) and F_1 adult progeny (1.7%, 1/58 adults tested, Ct = 23.5 [4.5 $\log_{10}$ PFUe/ml]) of the orally exposed females through RT-qPCR as well as through plaque assays. Our findings suggest that EHDV-2 infection has no major impact on *C. sonorensis* survival/longevity or oviposition but has a significant negative effect on midge fecundity/fertility. Our study also provides evidence for the vertical transmission of EHDV-2 from an infected adult female to its offspring in *C. sonorensis*. However, salivary transmission of EHDV-2 from the vertically infected progeny and its significance in the epidemiology of hemorrhagic disease are currently unknown and remain to be examined in further studies. Overall, these findings collectively indicate that *Orbivirus* infection can negatively affect vector reproduction, and that vertical transmission is a probable mechanism of overwintering of EHDV in North America.

Keywords *Culicoides sonorensis*, Biting midges, Survival, Longevity, Reproduction, Fecundity, Fertility, EHDV, BTV, Vertical transmission, Overwintering

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Culicoides (Diptera: Ceratopogonidae), a genus of biting midges sometimes called no-see-ums, transmit numerous disease-causing agents to humans and animals worldwide[1]. In North America, *Culicoides* species are mainly important in the transmission of epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) that cause hemorrhagic disease in susceptible hosts such as sheep, cattle, and white-tailed deer, and result in significant economic losses in animal agriculture. In the United States (US), *Culicoides sonorensis* Wirth & Jones and *Culicoides insignis* Lutz are the only confirmed vectors of EHDV/BTV[2–4]. However, other species such as *Culicoides stellifer* Coquillett, *Culicoides venustus* Hoffman, *Culicoides debilipalpis* Lutz, and others are likely involved in virus transmission in sylvatic cycles and in areas outside the geographic range of the primary vectors, particularly in the southeast[5,6].

Arboviral infections alter several life history traits of biting insects, influencing their overall vectorial capacity. For example, *Aedes aegypti* Linnaeus mosquitoes infected with dengue[7] or chikungunya virus[8] show reduced survival and fecundity, *Ae. aegypti* infected with Zika virus show reduced survival but no change in fecundity[9], while *Ae. aegypti* infected with Mayaro virus show reduced fecundity but no change in survival[10]. Similarly, *Culex tarsalis* Coquillett females infected with West Nile virus show reduced fecundity but no change in survival[11], *Cx. tarsalis* infected with western equine encephalitis virus show reduced survival and fecundity[12], while *Culiseta melanura* Coquillett mosquitoes infected with eastern equine encephalitis virus show reduced survival and fecundity[13].

Arboviruses are usually maintained in their natural transmission cycles through horizontal transmission between the arthropod vectors and the vertebrate hosts. However, during cold/dry seasons when the vector population densities are low or when herd immunity of the vertebrate hosts is high, alternate mechanisms such as vertical transmission (from infected arthropod vectors to their offspring) may drive the persistence of arboviruses in nature[14]. In the US, hemorrhagic disease is endemic with several serotypes of EHDV and BTV circulating in domestic and wild ruminants, particularly in the southeast[15]. However, the mechanisms by which these orbiviruses overwinter in the US remain uncertain to date. Previous studies concluded that vertical transmission of BTV is unlikely[16–18], and suggested that long lived *C. sonorensis* females, infected during the previous year, are more likely to contribute to the interseasonal maintenance of BTV in California[19]. However, the overwintering mechanisms of BTV outside California have received little attention to date[20].

In general, the vectorial capacity parameters of *Culicoides* species associated with EHDV/BTV transmission have not been studied comprehensively. In addition, the potential overwintering mechanisms of EHDV in North America have received little attention to date, collectively representing important knowledge gaps in our understanding of the overall transmission dynamics of orbiviruses in North America. In this study, we examined the survival and reproduction of *C. sonorensis* orally challenged with EHDV serotype-2 and examined the possibility of vertical transmission of EHDV-2 from an infected adult female to its offspring in this midge species.

Materials and methods

Oral virus exposure

The EHDV-2 strain used in this study was initially isolated from the spleen of a dead white-tailed deer from a commercial cervid facility in Quincy, Florida, US in 2016 (NCBI Accession #MF688816-MF688825). The virus was passaged twice on African green monkey kidney (Vero) cells maintained on Medium 199 (HyClone Medium 199, GE Healthcare Life Sciences, Logan, UT, US) containing 10% fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA, US), 2% penicillin/streptomycin and 0.2% Amphotericin B (Thermo Fisher Scientific, Waltham, MA, US). *Culicoides sonorensis* females Ausman strain[21] (48 h post adult emergence) were allowed to feed for 1 h on defibrinated bovine blood (HemoStat Laboratories, Dixon, CA, US) inoculated with EHDV-2 in cell culture media (5.5 $\log_{10}$ plaque forming unit equivalents [PFUe]/ml final concentration of blood + virus) or with clean media without EHDV-2 (control group) using a Hemotek artificial feeding system (Hemotek, Blackburn, United Kingdom) with a stretched Parafilm® M membrane (Amcor, Zurich, Switzerland). The viral titers of infectious blood fed to the midges are representative of viremia levels experienced by infected deer[22].

Assessment of midge life history traits

After feeding, blood-engorged females in each group were sorted using a CO_2 pad into individual 175 ml cardboard containers fitted (hot glued) with an oviposition substrate (Petri dish [60 × 15 mm] with moist cotton + filter paper) on the bottom. All females were provided with 10% sucrose solution on moistened cotton pads that were replaced daily, and individuals were monitored daily for survival and oviposition. The eggs deposited by females in each group were placed on 0.3% agar slants ($n = 10$ eggs/dish) and the hatched larvae were reared on a diet of *Panagrellus redivivus* nematodes (Carolina Biological Supply Company, Burlington, North Carolina, US), according to previously established methods[23]. Developmental life history traits of the larvae were monitored and recorded every other day. Overall, several life history traits of *C. sonorensis* were recorded and compared between the two groups. These included: blood-fed adult survival/longevity, percentage of females that oviposited, number of eggs deposited, egg hatch rates, larval survival to pupal stage, larval stage duration, eclosion rates, and sex-ratios of the emerging adults. Environmental conditions throughout the experiments were 26 ± 1 °C, 60–80% RH, and 16:8 (L:D) h cycle.

Virus detection from EHDV-2 fed females and their progeny

All EHDV-2 fed females were processed for the detection/presence of EHDV-2 in their bodies (infection) and legs (dissemination) at the time of their death. In addition, a randomly selected subset of the egg batch deposited by EHDV-2 fed females ($n = 29$) and F_1 generation adults reared from the other subset of eggs ($n = 58$; 32 males and 26 females) were processed for the detection/presence of EHDV-2. All EHDV-2 fed females, partial egg batches from each female, and F_1 generation adults were individually placed in 200 µl of Medium 199 (HyClone

Medium 199, GE Healthcare Life Sciences, Logan, UT, US) in 1.5 ml microcentrifuge tubes containing 5–10 borosilicate glass beads (2 mm; Sigma-Aldrich, St. Louis, MO, US). This was followed by homogenization for 5 min using a Bullet Blender Storm homogenizer (Next Advance, Troy, NY, US). Viral RNA was then extracted from 150 µl of the homogenate using the QIAamp Viral RNA Mini Kit (Cat#52906, Qiagen, Hilden, Germany) using manufacturer's protocol. The remainder of the homogenate (50 µl) was stored at −80 °C for later use as inoculum for culturing virus, if the sample was determined RT-qPCR positive. RT-qPCR was run on the extracted RNA using SuperScript III One-Step qRT-PCR kit (Thermo Fisher Scientific) using previously established protocols[24] employing primer and probe sequences from Wernicke et al.[25] and PCR conditions from Wilson et al.[26], modified slightly (25 min at 55 °C, 2 min at 95 °C, and 45 cycles of 10 s at 95 °C and 1 min at 57 °C). All RT-qPCR assays were run with positive (EHDV-2 stock) and negative (water) controls. The RNA samples of egg batches and F1 adults that were EHDV-2 positive through RT-qPCR were further processed using plaque assays to quantify replication competent virions. Briefly, the remainder of homogenates were added to 6-well plates of confluent Vero cells, incubated for an hour at 37 °C, and covered with an agarose gel overlay. One week post infection, overlays were removed, and crystal violet stain was used to view plaque formations. Each plaque was assumed to have originated from a single infecting virus. The samples showing plaques were again processed for EHDV-2 detection through RT-qPCR in the same manner as described above. The quantification cycle values (Ct) and standard curves for the EHDV-2 Florida strain used in the study were reported in our previous study and its detection limit was determined to be at Ct value 35[24].

Statistical analysis

Variation in the survival/longevity of adults between the two groups was analyzed using Kaplan–Meier survival curves and log rank (Mantel-Cox) tests. Variation in the percentage of females that oviposited, number of eggs deposited per female, egg hatch rates, larval survival to pupal stage, larval stage duration, eclosion rates, and sex-ratios of the emerging adults between the two groups were analyzed using generalized linear models (GLM) under Poisson or negative binomial distributions. All data were analyzed using R statistical software v.3.6.1[27] using packages *Mass*[28], *car*[29], and *lsmeans*[30].

Results

In total, there were 56 individual blood-fed females in the EHDV-2 fed group and 50 in the control group.

Life history traits of adults

The longevity of females in the EHDV-2 fed group (13.7 ± 1.2 days post blood feeding [pbf]) (mean ± SE) was slightly, but not significantly longer than that in the control group (11.8 ± 1.2 days pbf) ($\chi^2_1 = 3.32$, $P = 0.0685$) (Fig. 1A). The percentage of females that oviposited in the EHDV-2 fed group was 57.1% (32/56; 95% CI 43.2–70.3%), which was not significantly different from that in the control group (52.0%, 26/50; 95% CI 37.4–66.3%) ($\chi^2_1 = 0.28$, $P < 0.5954$) (Fig. 1B). The number of eggs deposited per female in the EHDV-2 fed group (89.1 ± 10.4 eggs/female) was not significantly different from that in the control group (73.7 ± 9.1 eggs/female) ($\chi^2_1 = 0.72$, $P < 0.3964$) (Fig. 1C). The egg hatch rate in the EHDV-2 fed group was 35.8 ± 5.2%, which was significantly lower than that in the control group (74.5 ± 6.8%) ($\chi^2_1 = 87.8$, $P < 0.0001$) (Fig. 1D).

Life history traits of progeny

The rate of larval survival to pupal stage in the EHDV-2 fed group was 59.8 ± 4.9%, which was significantly higher than that in the control group (34.1 ± 6.5%) ($\chi^2_1 = 18.0$, $P < 0.0001$) (Fig. 2A). Larval stage duration in the EHDV-2 fed group (29 ± 0.9 days) was not significantly different from that in the control group (27.3 ± 1.1 days) ($\chi^2_1 = 2.0$, $P = 0.1526$) (Fig. 2B). The eclosion rate of adults in the EHDV-2 fed group (44.6 ± 5.4%) was not significantly different from that in the control group (57.6 ± 8.8%) ($\chi^2_1 = 0.01$, $P = 0.9334$) (Fig. 2C). The sex-ratio of F_1 adults in the EHDV-2 fed group (1.1:1 [M:F]) was not significantly different from that in the control group (0.9:1) ($\chi^2_1 = 0.21$, $P = 0.6490$) (Fig. 2D).

Virus detection from EHDV-2 fed females and their progeny

A high percentage of the EHDV-2 fed females were positive through RT-qPCR in their bodies at the time of death, showing high infection rates (94.4%, 51/54 [two midge samples were accidentally lost]) (Ct < 35). Among these, a high percentage of females were positive through RT-qPCR in their legs, showing high dissemination rates (84.3%, 43/51). Partial egg batches from 29 different females were tested for EHDV-2. Infection rates and dissemination rates in these 29 females were 100% (29/29) and 86.2% (25/29) respectively (Ct < 35). Partial egg batches from four females (4/29, 13.8%,) were positive through RT-qPCR. The partial egg batch of one female was positive through plaque assays as well (1/29, 3.4%, Ct = 22.1 [4.9 $\log_{10}$ PFUe/ml]). All four females whose egg batches were RT-qPCR positive were also RT-qPCR positive in their bodies (Ct = 24.0 ± 1.8 [mean ± SE]) and legs (Ct = 29.0 ± 1.4 [mean ± SE]) (these females were not processed for live virus through plaque assays). A total of 58 F_1 adults (32 males and 26 females) were tested individually for EHDV-2. Among these, one male (1/32, 3.1%) and 0 females (0/26, 0.0%) were positive through RT-qPCR. The F_1 adult male was positive through plaque assays as well (1.7%, 1/58 adults tested, Ct = 23.5 [4.5 $\log_{10}$ PFUe/ml]). All samples positive through plaque assays were subsequently re-confirmed as EHDV-2 through RT-qPCR (Table 1).

Discussion

Overall, our findings demonstrate that EHDV-2 oral infection does not have a major impact on *C. sonorensis* survival/longevity or oviposition. However, EHDV-2 infection does have a significant negative effect on *C. sonorensis* fecundity (fertility) as it reduces the egg hatch rates in EHDV-2 fed females. The lower egg hatch rates

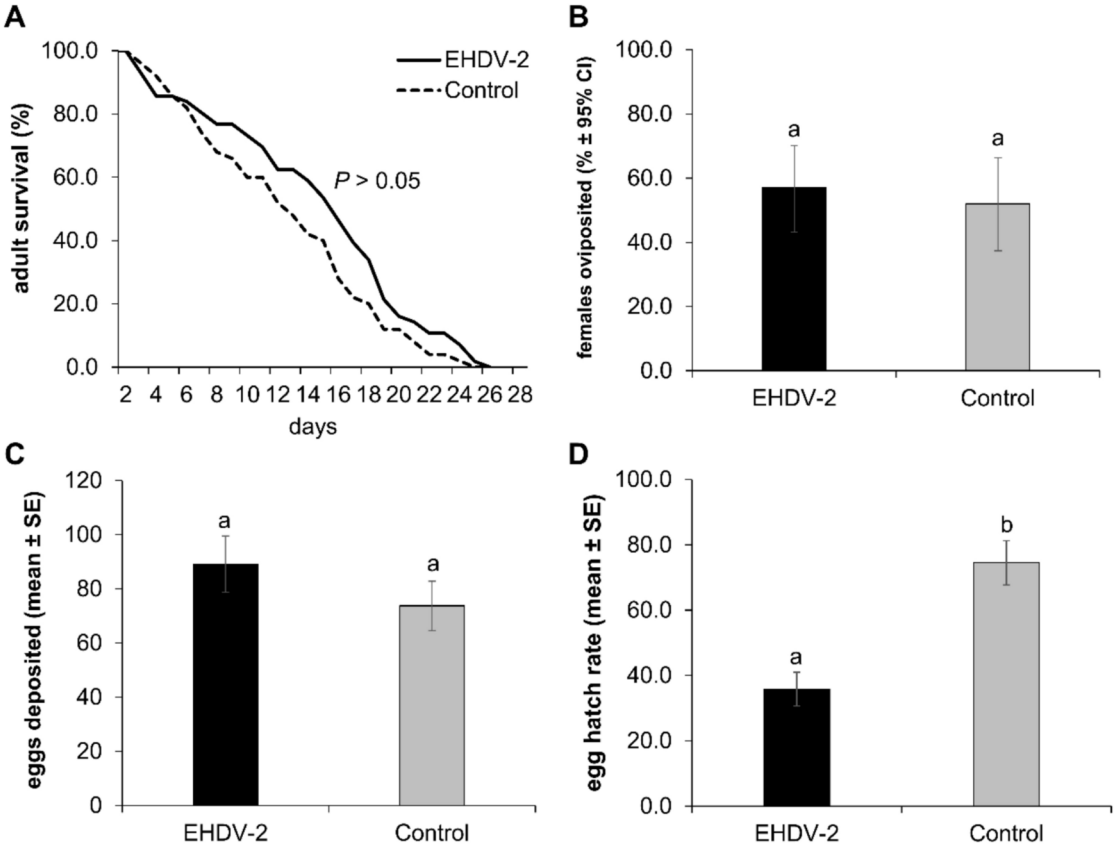

Fig. 1. Life history traits of *C. sonorensis* adults in the two groups, (**A**) longevity, (**B**) percentage of females that oviposited, (**C**) number of eggs deposited, and (**D**) egg hatch rates. Letters above bars represent significant differences ($P < 0.05$).

are possibly a cost incurred by *Culicoides* species for harboring EHDV-2, which may also be a fitness cost of the coevolution between the virus and the vector. Interestingly, the larval survival rates to pupal stage were higher in the EHDV-2 fed group than in the control group, which may be an attribute crucial for virus transmission by the adults they become. These tradeoffs possibly offset the lower egg hatch rates of EHDV-2 fed females and balance the overall reproductive output of the two groups. Nonetheless, although they denote transgenerational virulence with consequences for offspring life history, the reasons behind these outcomes are currently unknown. Further studies will be needed to examine the impact of EHDV-2 on the overall reproductive output of *C. sonorensis* and to examine the physiological mechanisms by which EHDV-2 infection increases survival rates in the larvae. The reasons behind the reduced egg hatch rates in EHDV-2 fed *C. sonorensis* females are currently unknown as well. However, it is possible that EHDV infection alters the expression of certain genes in the midge ovaries that are important for egg development/maturation. Previously, *Ae. aegypti* mosquitoes infected with dengue-2 virus showed reduced fecundity compared to their uninfected counterparts[31]. Subsequent transcriptomic profiling of the ovaries of virus-infected mosquitoes showed the upregulation of several genes, which were suggested to interfere with egg production[31]. Similarly, *Ae. aegypti* infected with chikungunya virus also showed reduced fecundity compared to uninfected mosquitoes[8]. Subsequent gene expression analyses on virus infected mosquitoes showed the downregulation of six transcripts in the egg laying pathway of *Ae. aegypti*[8], a reduction in fecundity was also reported for *Culex pipiens* Linnaeus mosquitoes infected with Rift Valley fever virus[32]. A second possible reason for the reduced fecundity found in *C. sonorensis* in this study is that the transfer of sperms from spermathecae into the micropyle of the eggs during oviposition (fertilization) is affected due to EHDV infection of certain tissues in the female reproductive tract (see further discussion below). Previously, EHDV-2 was detected in the epithelial cells of the spermathecae and the ovarian sheath in *C. sonorensis*[33]. Another possible reason is that EHDV infection causes unknown pathogenesis in the midges reducing their ability to produce fertile eggs or causes direct/indirect mortality in the eggs/embryos. Previous studies have shown that chikungunya virus infection caused reduced egg hatch rates in *Ae. albopictus*[34] and West Nile virus infection caused reduced egg hatch rates in *Cx. tarsalis* mosquitoes[11]. However, further studies will be needed to test these hypotheses.

EHDV-2 was detected in the eggs and F_1 generation adults of virus fed *C. sonorensis* females through RT-qPCR as well as plaque assays, thus suggesting that vertical transmission of EHDV may occur in nature. It was previously thought that EHDV and BTV are unlikely to be transmitted vertically in biting midges. This was because EHDV/BTV infections in the midge reproductive tissues in previous studies were detected essentially

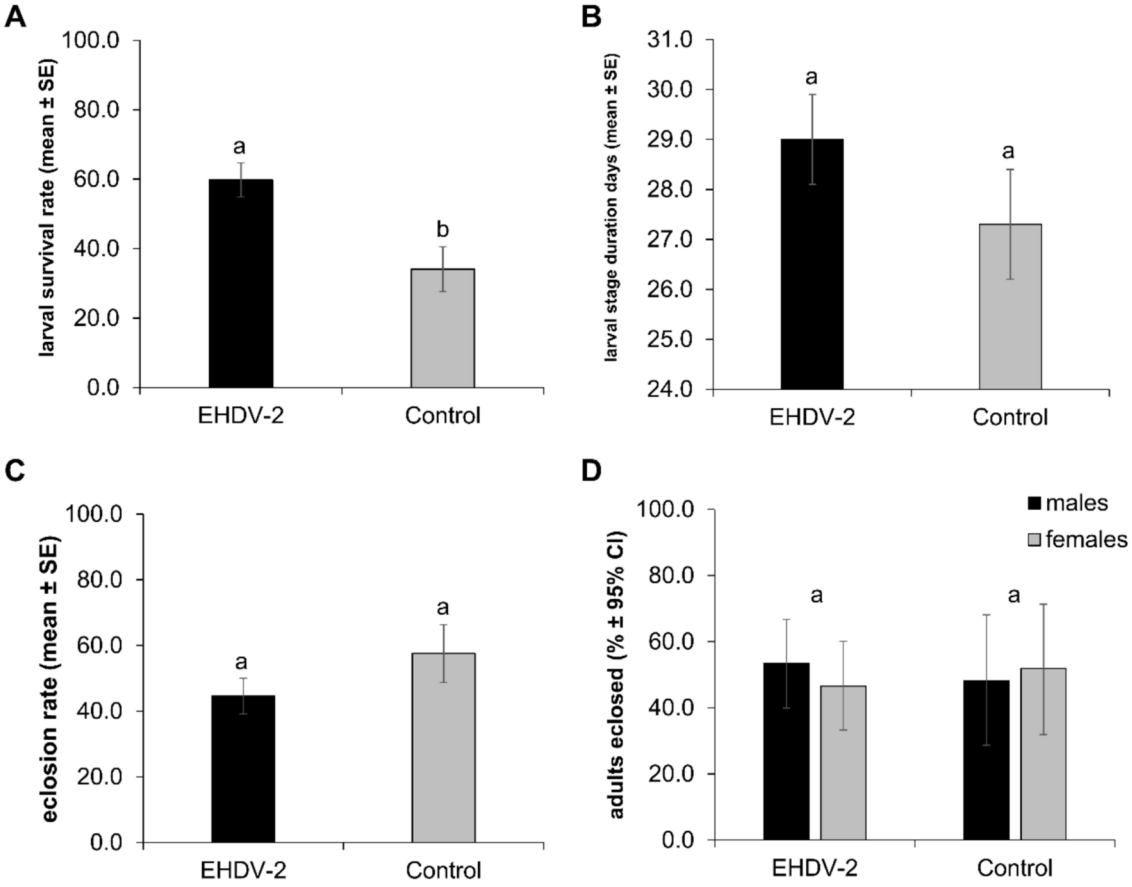

Fig. 2. Life history traits of *C. sonorensis* progeny in the two groups, (**A**) larval survival rate to pupal stage, (**B**) larval stage duration, (**C**) eclosion rate, and (**D**) sex-ratios. Letters above bars represent significant differences ($P < 0.05$).

Life stage	Sex	# Tested	# Positive through RT-qPCR[a] (%)	Mean Ct values (± SE)	# Positive through plaque assays (%)	Ct values (PFUe/ml)
Eggs		29[b]	4 (13.8%)	31.9 ± 1.2	1[c] (3.4%)	22.1 (4.9 $\log_{10}$)
F1 adults	Male	32	1 (3.1%)	31.1 ± 0.0	1 (3.1%)	23.5 (4.5 $\log_{10}$)
	Female	26	0 (0.0%)	NA	0 (0.0%)	NA

Table 1. Virus detection from the progeny of EHDV-2 fed females. [a]Ct < 35. [b]Number of females from which eggs were tested. [c]Number of females from which live virus was detected in the eggs.

only along the outer layers, but not in the gametes. For example, BTV was detected only within the ovarian sheath, immature yolk bodies and vitelline membrane of the oocyte in *C. sonorensis*, but not in the oocytes or nurse cells[17,35]. Subsequent studies found no evidence for the vertical transmission of BTV under laboratory or field conditions[16–18]. Similarly, EHDV-2 was detected only within the spermathecal epithelium and the ovarian sheath in *C. sonorensis*, but not in the ovariolar sheath, ovarioles, follicular epithelium, oocytes, or nurse cells[33]. Therefore, it was suggested that vertical transmission of EHDV is unlikely as well. However, our findings contradict previous notions and demonstrate clearly that EHDV-2 can be vertically transmitted in *C. sonorensis*. It is possible that these discrepancies are due to differences in the EHDV-2 strains used in the studies. Mills et al.[33] used an EHDV-2 strain isolated from a white-tailed deer from Kansas, while we used an EHDV-2 strain isolated from a white-tailed deer from Florida in this study. It is possible that the EHDV-2 Florida strain infects different reproductive tissues/cells of *C. sonorensis* than the Kansas strain, which enable the virus to be vertically transmitted in the midge (transovarial route). Alternately, the virus may enter the fully developed egg later during oviposition (transovum route). Currently, variations in the midge reproductive tissues/cells infected by the EHDV-2 Florida strain and other geographic strains (and serotypes) of EHDV/BTV are unknown. Further studies will be needed to examine the tissue specific infections caused by different serotypes/strains of orbiviruses in biting midges, which may provide clues towards the mode of vertical transmission of EHDV-2 found in *C. sonorensis* in this study. In mosquitoes, arboviruses are known to be vertically transmitted through the transovarial or the transovum routes[36,37]. In a previous study, we found that the EHDV-2 Florida

strain exhibited higher infection and dissemination rates than the EHDV-2 Can-Alberta strain in *C. sonorensis*, suggesting that the consequences of EHDV infection in biting midges vary with the geographic strain of the virus[24]. Therefore, it is likely that different serotypes/strains of EHDV and BTV influence the vector competence and other life history traits/vectorial capacity parameters of *Culicoides* species differently as well.

The overall vertical transmission rate in this study was low (3.4%, 1/29 partial egg batches examined). Notably, our observations were made only during the first gonotrophic cycle of *C. sonorensis*. As such, it is possible that these vertical transmission rates may persist or even increase during the subsequent gonotrophic cycles of *C. sonorensis*, as reported for other arboviruses in mosquitoes[38]. The filial infection rates in this study were low as well (1.7%, 1/58 F_1 adults tested). These low filial infection rates possibly influenced the larval stage duration of *C. sonorensis*, which was found to be not significantly different between the two groups. Previously, mosquito larvae vertically infected with various arboviruses took significantly longer time to develop than uninfected larvae[39–42]. Interestingly, the F_1 generation adult from which EHDV-2 was detected in this study was a male (3.1%, 1/32) and EHDV-2 was not detected from any of the females examined (0.0%, 0/26). This is likely an artifact of the relatively small sample size of the females examined and may not represent any sex-specific vertical transmission of the virus. Nonetheless, the presence/detection of EHDV-2 in the F_1 males of *C. sonorensis* suggests the possibility of sexual transmission of EHDV-2 from infected males to females as well. Previously, sexual transmission of vesicular stomatitis virus was reported in *C. sonorensis*[43], in addition to many other arboviruses in their insect vectors[44–47]. However, further studies will be needed to test these hypotheses.

Hemorrhagic disease is known to be endemic in the US with several serotypes of EHDV and BTV frequently detected in a variety of domestic and wild ruminants, particularly in the southeast[15]. However, the potential mechanisms by which these orbiviruses overwinter in temperate regions such as North America remain enigmatic to date. In the past, several studies have concluded that vertical transmission of BTV is unlikely[16–18,35]. However, Mayo et al.[19] found BTV RNA in *C. sonorensis* parous females collected during the early part of the interseasonal period (Feb–Mar) of 2013 and 2014 in California, suggesting that in the absence of vertical transmission, long lived females infected the previous year may contribute to the interseasonal maintenance of BTV in temperate regions. Along similar lines, our current findings demonstrating the vertical transmission of EHDV-2 in *C. sonorensis* offer a strong explanation for one of the potential overwintering mechanisms of EHDV in North America and provide a plausible explanation for the endemic nature of hemorrhagic disease in the US. On a per capita basis, the vertically infected midges that are infectious will likely contribute strongly to EHDV transmission because, (1) they can transmit the virus to vertebrates upon their first bite, and (2) they will be infectious for a longer period as adults compared to midges that acquire EHDV by bite through horizontal transmission. It is possible that the low vertical transmission rates found in our study are adequate to sustain the virus between years while still requiring several months of amplification within host populations before outbreaks are observed, typically during late summer to fall. Moreover, considering the high densities in which *Culicoides* species occur in the field, these low vertical transmission rates could become highly efficient at the population scale. However, it is currently unknown whether the vertically infected progeny of orally infected midges can transmit EHDV-2 through their saliva/bite. Therefore, further studies will be needed to demonstrate the salivary transmission of EHDV-2 from the vertically infected progeny to susceptible ruminant hosts and to determine its significance in the epidemiology of hemorrhagic disease. Moreover, further studies will also be needed to gather evidence from field-collected *C. sonorensis* to ascertain whether these findings are biologically significant. However, it is to be noted that *C. sonorensis* is scattered/rare in the southeastern US[48]. Therefore, further studies will be needed to examine whether any of the endemic EHDV and BTV serotypes or strains in the US are vertically transmitted through other species associated with EHDV/BTV transmission in the southeast such as *C. insignis, C. stellifer*, *C. venustus*, *C. debilipalpis*, or others. However, the current lack of colonies of *Culicoides* vector species other than *C. sonorensis* may pose a challenge to such studies.

Conclusions

In conclusion, our study demonstrates that EHDV does not have a major impact on the survival/longevity, or oviposition of *C. sonorensis*, but has a significant negative effect on midge reproduction as it reduces the egg hatch rates in EHDV fed females (but increases larval survival rates). In addition, our findings demonstrate that EHDV-2 can be vertically transmitted in *C. sonorensis*, a phenomenon that was previously thought unlikely. These findings collectively improve our understanding of the vectorial capacity parameters of *C. sonorensis*, provide valuable insight into the potential overwintering mechanisms of EHDV in North America, and offer a plausible explanation for the endemic nature of hemorrhagic disease in the US. Further studies will be needed to understand the physiological mechanisms by which EHDV-2 infection causes reduced fecundity/fertility and increased larval survival rates in biting midges and to determine the mode of vertical transmission of EHDV-2 in *C. sonorensis*. More importantly, further studies will be needed to examine the salivary transmission of EHDV-2 from the vertically infected progeny to susceptible ruminant hosts and to evaluate its significance in the epidemiology of hemorrhagic disease. In addition, further studies will also be needed to gather evidence from field-collected *C. sonorensis* to ascertain whether these findings are biologically significant and to determine whether any of the endemic (or novel) serotypes/strains of EHDV and BTV in North America are vertically transmitted through other species associated with *Orbivirus* transmission such as *C. insignis*, *C. stellifer*, *C. venustus*, *C. debilipalpis* or others.

Data availability

All data generated or analyzed during this study are included in this published article.

Received: 3 May 2024; Accepted: 30 April 2025

Published online: 08 May 2025

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Acknowledgements

We thank Lee Cohnstaedt and William Yarnell (USDA, Manhattan, KS, US) for the maintenance and provision of colonized midges for this study. Agustin Quaglia provided help with statistical analyses of the data. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Author contributions

D.E.: conceptualization, methodology, data curation, formal analysis, visualization, validation, investigation, resources, project administration, writing—original draft preparation. B.M.: methodology, writing—review and editing. C.A.: methodology, writing—review and editing. B.A.: resources, writing—review and editing. N.B.C.: funding acquisition, supervision, writing—review and editing. All authors have reviewed and agreed to the final version of this manuscript.

Funding

This study was funded by the Cervidae Health Research Initiative (CHeRI) sponsored by the State of Florida Legislature. NBC is partially supported by NIFA Project FLA-FME-006106.

Declarations

Competing interests

The authors declare no competing interests.

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