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Since 2014, African swine fever virus (ASFV) has been spreading within Eastern Europe. Within affected regions, the virus has infected some farms with high biosecurity and a marked seasonality of outbreaks in domestic pigs has been observed. ASFV transmission from stable flies, Stomoxys calcitrans, has previously been shown both mechanically and via ingestion of whole flies. Hence, blood-feeding flies may offer one explanation for the introductions into high biosecurity farms and for the observed seasonality. The aim of this study was to further elucidate the potential role of stable flies in ASFV transmission. Different parts of flies were analyzed for the presence of viral DNA and infectious virus at different time points following in vitro feeding of the flies on blood from an ASFV-infected pig. Using qPCR, ASFV DNA was detectable in mouth parts of flies for at least 12 h and remained in head and body samples from the flies for up to three days following feeding. Infectious virus was detected in fly body samples prepared at 3 h and 12 h after feeding. The presence of infectious ASFV in stable flies following feeding on viremic blood means that such flies are capable of transporting infectious virus. The detection of ASFV DNA in the flies for up to three days following feeding suggests that qPCR analysis of blood-feeding flies during ASFV outbreaks could be a useful method to elucidate the role of these flies in ASFV transmission under field conditions.

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