



No evidence of African swine fever virus replication in hard ticks

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Introduction

African swine fever (ASF) is a highly contagious haemorrhagic disease of swine, caused by the African Swine Fever Virus (ASFV). ASF is a vector borne disease for which no vaccine is available.

Soft ticks of the genus *Ornithodoros* are known to be competent vectors, while hard ticks are not. However, common European hard ticks such as *Ixodes ricinus* and *Dermacentor reticulatus* have never been tested for their capacity to replicate ASFV.

Objective

Determine if ASFV can replicate in *Ixodes ricinus* and *Dermacentor reticulatus*, ticks frequently found in Europe.

Methods

Three tick species were used:

- *Ixodes ricinus*, nymphs (hard tick)
- *Dermacentor reticulatus*, adults (hard tick)
- *Ornithodoros moubata*, nymphs (soft tick, known vector)

Six ASFV isolates were used (with titres between 5.4-5.9 log₁₀TCID₅₀/ml):

- OURT88/1 – isolated from infected *O. erraticus* ticks
- LIV13/33 – isolated from infected *O. moubata* ticks
- Georgia 2007, Malta'78, Netherlands'86 and Brazil'78 - isolated from infected pigs

ASFV acquisition through in vitro feeding

Defibrinated fresh pig blood was collected from a slaughterhouse and spiked (1:10 dilution) with each of the six ASFV isolates.

Every tick species was fed in vitro with the spiked blood (Figure 1), until ticks were fully engorged.

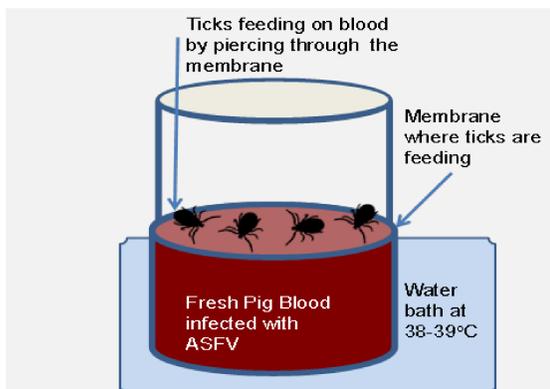


Figure 1. In vitro feeding of ticks. Hard ticks: lasted for a maximum of 1 week; blood was changed every 12 hours. Soft ticks: lasted approximately 5 hours. Soft ticks were subsequently fed after each moulting with blood not containing any ASFV.

Sampling and testing of ticks

Table 1. Sampling and testing scheme of hard and soft ticks

Tick species	Sampled	Tested
<i>I. ricinus</i>	After feeding, every week up to 6 weeks	Individual ticks were tested in quantitative real-time polymerase chain reaction (qPCR)
<i>D. reticulatus</i>	One week after feeding, every week up to 8 weeks	
<i>O. moubata</i>	After feeding, 1 week after feeding, one week after 1 st moult (corresponding to weeks 2-4 after feeding) and 7 weeks after feeding	

ASFV titres found on ticks fed on blood infected with different ASFV isolates tested by qPCR

Table 2. ASFV titre in log₁₀TCID₅₀/tick after feeding on blood with ASFV OURT88/1

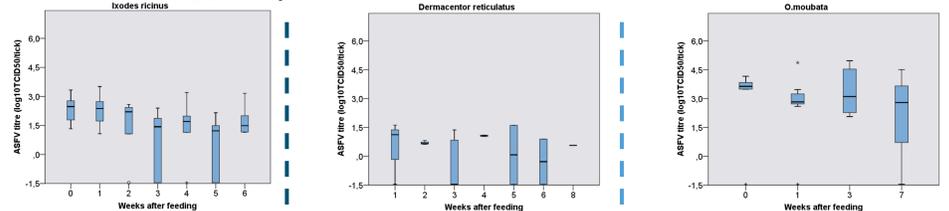


Table 3. ASFV titre in log₁₀TCID₅₀/tick after feeding on blood with ASFV LIV13/33

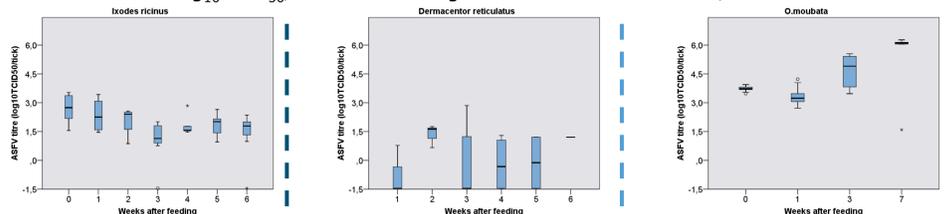


Table 4. ASFV titre in log₁₀TCID₅₀/tick after feeding on blood with ASFV Georgia 2007

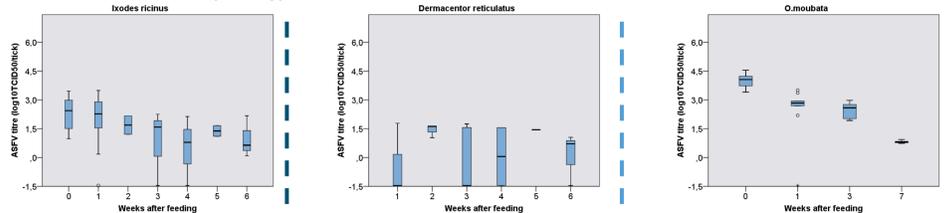


Table 5. ASFV titre in log₁₀TCID₅₀/tick after feeding on blood with ASFV Malta'78

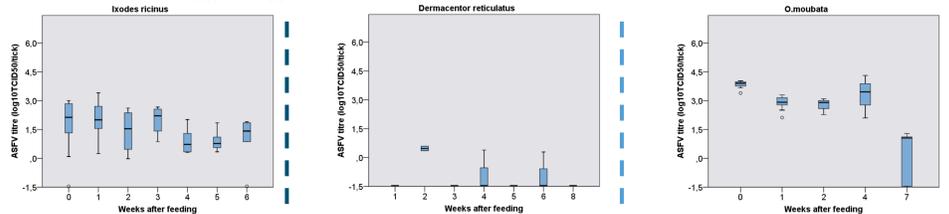


Table 6. ASFV titre in log₁₀TCID₅₀/tick after feeding on blood with ASFV Netherlands'86

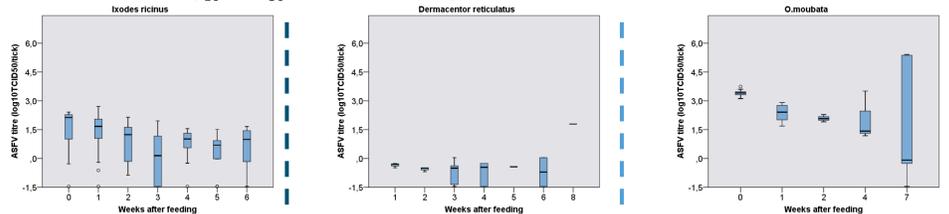
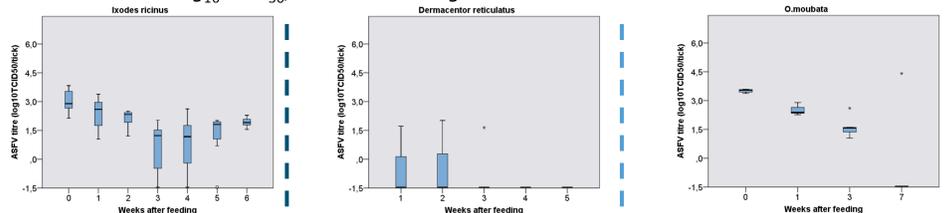


Table 7. ASFV titre in log₁₀TCID₅₀/tick after feeding on blood with ASFV Brazil'78



Tables 2-7: negative results in qPCR tests are represented as -1.5 log₁₀TCID₅₀ (detection threshold)

Results and Conclusions

- Quantities of ASFV DNA in *Ixodes ricinus* nymphs decrease in time, but DNA remains detectable in most ticks until 6 weeks after feeding.
- Quantities of ASFV DNA in *Dermacentor reticulatus* adult ticks were very low after feeding and remained low or disappeared after 6-8 weeks.
- Not all viruses replicate equally well in *O. moubata*. In these ticks, the ASFV isolate LIV13/33 could replicate, while the ASFV isolate Georgia 2007 could not.
- In conclusion, there is no evidence from this experiment that ASFV replicates in hard ticks, even though ASFV DNA can still be detected for at least 6 weeks after the acquisition feeding.



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