

# African Swine Fever Virus load in hematophagous dipterans collected in outbreaks from Romania: risk factors and implications

Balmoş OM<sup>1</sup>, Supeanu A<sup>2</sup>, Tamba P<sup>3</sup>, Horváth C<sup>1</sup>, Luciana Cătălina Panait<sup>1</sup>, Cristina Daniela Cazan<sup>1</sup>, \* Ungur<sup>1</sup>, Motiu M<sup>3</sup>, Manita FA<sup>3</sup>, Ancuceanu BC<sup>3</sup>, Bărbuceanu F<sup>3</sup>, Sofie Dhollander<sup>4</sup>, and Andrei Mihalca<sup>1</sup>

<sup>1</sup>Universitatea de Stiinte Agricole si Medicina Veterinara din Cluj-Napoca

<sup>2</sup>National Sanitary Veterinary and Food Safety Authority Piaţa Presei Libere 1 Corp D1 Sector 1 Bucharest 013701 Romania

<sup>3</sup>Institute for Diagnosis and Animal Health Strada Dr Staicovici 63 Sector 5 Bucharest 050557 Romania

<sup>4</sup>European Food Safety Authority

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## Abstract

African swine fever (ASF) is a highly contagious viral disease of suids that causes significant economic damage. The summer peaks and river courses have triggered the hypothesis that vectors may be involved in the transmission of the virus. The aim of our study was to evaluate the risk factors associated with the presence of ASFV DNA in hematophagous dipterans and to analyze the relevance of Ct values of the positive samples in ASF outbreaks in Romania, as an indication for the viral load. The current study included 99 pools of stable flies (*Stomoxys calcitrans*) and 278 pools of biting midges (*Culicoides* spp.), collected in June-September 2020, from 30 outbreaks of ASF in domestic swine from backyard farms (BF), type A farms (TAF) and commercial farms (CF). All extracted DNA was tested for the presence of ASFV genome using a real-time PCR protocol. Ct values of 39.53 and below were considered as positive. The blood meal source was identified in the hematophagous insects by using a PCR protocol targeting the mitochondrial gene cytochrome c oxidase subunit 1. Data were analyzed using R software v. 4.0.5. In total, 2978 insects (*S. calcitrans* n= 198 and *Culicoides* n=2780) were collected in 19 outbreaks out of the 30 sampled farms. Ten species of biting midges were identified. The total number of insect pools showed significant differences according to the month of sampling, with a higher number of pools collected in August and September. Overall, 137 pools out of the 377 examined were positive for the presence of ASFV DNA. There was a higher viral DNA load in farms where pigs were present at the moment of sampling compared to farms where pigs were already culled, in *S. calcitrans* compared to *Culicoides* spp. and in CF and TAF compared to BF.

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