Summary

Role of dipterans in the epidemiology of African swine fever in Romania

The first part (I) of the current thesis consists of four chapters, providing a comprehensive overview of the current scientific knowledge on the ASFV in the context of its entomological transmission. This section provides the state-of-play of the scientific information available in the literature on the ASFV (Chapter I.1). It also highlighted the morphological characteristics and roles of hematophagous (Chapter I.3) and non-biting (Chapter I.4) Diptera in the transmission of various pathogens, including bacteria, parasites, and viruses. Chapter I.2 focuses on the broader context of vector-borne diseases and their implications for the spread of infectious diseases through mechanical or biological transmission.

The second part (II) of the thesis proceeds by outlining the study's aims, which focus around four distinct investigations that assess the involvement of dipterans in the epidemiology of African swine fever (ASF). Subsequently, the sections containing the conclusions and recommendations are described, followed by an assessment of the thesis' originality. The thesis closes by listing a total of 242 titles from the cited references.

Chapter II.1. details a study that focuses on the involvement of various arthropods in the mechanical transmission of ASFV in Romania within and between domestic pig farms. The aim of the study was to analyse the risk factors that led to the summer peak in ASF outbreaks in domestic pigs in Romania and to investigate the potential involvement of specific arthropods in the transmission of the ASFV. The findings of this field investigation highlight the high rate of positivity to ASFV DNA (50.5%), collected from 30 outbreak farms. The prevalence of positive pools of ASFV DNA was highest in commercial farms, with 62.96% of positive pools in *S. calcitrans*. Moreover, the study concludes that *Culicoides* spp. and *Stomoxys* are the most common vectors found around pig farms, with ASFV DNA detection being more likely in insects collected when pigs weren’t culled at the time of the sampling.

Through the study presented in Chapter II.2. we aimed to evaluate risk factors associated with ASFV DNA in hematophagous dipterans and analyse the relevance of Ct values obtained following RT-PCR analysis of positive samples in ASF outbreaks in Romania. The study included 99 pools of stable flies and 296 pools of biting midges from 30 outbreaks in domestic swine from backyard farms (BF), type A farms (TAF), and commercial farms (CF). In total, 3,158 insects (*S. calcitrans* n = 198 and *Culicoides* n = 2960) were collected in 23 farms of the 30 outbreak 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 395 examined were positive for the presence of ASFV DNA. Ct values with a median value of 31.41 were obtained. 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.

The Chapter II.3. evidenced the study which aimed to analyse the risk factors associated with the presence of non-biting dipterans collected from African swine fever outbreaks in relation to the presence and load of viral DNA. The research involved sampling from backyard farms, type A farms, and commercial farms in 2020 and 2021, and identifying the collected flies to family, genus, or species level. The results showed that 201 of the 361 pools were positive for ASFV DNA, with lower CT values in Sarcophagidae (mean

value of 32.56) indicating a higher viral DNA load. A significantly higher number of positive pools were noticed in August, mean value = 33.12. This study highlights the importance of strengthening biosecurity measures and protocols for preventing the insect life cycle and distribution.

To conclude the thesis, Chapter II.4. presents a detailed study that is currently in progress and focuses on the role of mosquitoes in ASF epidemiology. Moreover, the research aimed to detect ASFV DNA in various mosquito genera from Romania that might be able to spread ASFV under field conditions. Out of the total of 175 pools, 88 were tested positive for the ASFV DNA (50.29%). The statistical analysis revealed significant variations in the insect's species. We obtained positive samples of ASFV DNA in all of the mosquito genera that were targeted, namely *Aedes*, *Culex*, *Anopheles*, and *Culiseta*. The *Anopheles* genus has been shown to have the highest prevalence of ASV-positive pools (61.82%). The positive samples for pig DNA (n = 11) were collected from farms where the pigs were still alive. Seven of these samples (63.64%) tested positive for ASFV DNA. These findings emphasised the putative role of Culicidae in ASF transmission.