African swine fever virus DNA in stable flies and biting midges collected from swine farms during outbreaks in Romania

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INTRODUCTION

African swine fever virus (ASFV) represents a serious global threat with major economic implications for the pig industry. Recently, epidemiological research raised questions about the involvement of hematophagous arthropods in spreading the disease. Mechanical transmission has been studied on several occasions, with Stomoxys calcitrans confirmed in experimental studies as transmitting ASFV to domestic pigs following a bite or ingestion. Moreover, ASFV was found in S. calcitrans for up to three days after experimental infection (Olesen et al. 2018). Our aim was to perform field investigations to demonstrate the presence of viral DNA in blood-feeding arthropods collected during ASF outbreaks.

METHODOLOGY

30 farms (16 backyard farms, 9 type A farms and 5 commercial farms (CF)) were selected for sampling between June and September 2020. A method of risk scoring was used to determine which farms would be sampled. At the time of sampling, pigs had been present at nine farms, while in the others they had already been culled after an ASF outbreak was confirmed on the farm. Standard entomological techniques were used to trap Culicoides spp. and S. calcitrans. The morphological identification of insects was followed by DNA extraction. PCR was used to evaluate the interpretation of Ct (cycle threshold) values that are generated during qPCR, testing the presence of ASFV DNA and the presence of pig DNA. In our study, we considered samples with Ct values of 39.53 and below as positive. A Ct value between 39.53 and 30 was considered weakly positive, a value between 30 and 24 positive, and values below 24 were strongly positive. A total of 383 vector pools (291 composed of Culicoides spp. from 20 farms and 92 composed of S. calcitrans from 15 farms) were analysed.

RESULTS

In total, 35.77 % of the tested pools were positive for ASFV DNA. The prevalence of infected pools was highest in CF. From the total number of Culicoides pools, 25.48 % were found positive. S. calcitrans positive pools (63.04 %) were found in the sampled farms. A Ct value under 24 was considered intensely positive. Most of these intense values were obtained during August (31.03 %) in S. calcitrans positive pools. Moreover, there was a significant difference between the prevalence of infected pools in Culicoides and S. calcitrans. If Ct values <24 were considered, the obtained prevalence was significantly higher in farms where sampling was carried out when pigs were still present (S. calcitrans – 44.82 %). Our research has shown that ASFV DNA-positive Culicoides and S. calcitrans vectors are commonly observed near pig farms and both groups are highly frequent in CF when pigs are present.

DISCUSSION

A similar study has been recently performed in Lithuania when insects of the families Muscidae, Calliphoridae, and Tabanidae were analysed for the presence of ASFV DNA in farms with ASF outbreaks and without ASF outbreaks. The DNA of ASFV was detected in 7 individual insects out of the 42 tested. In S. calcitrans, the prevalence was 1/29 (Turčinavičienė et al. 2020). In 2016, a study examined the ASFV status in a Polish population of wild boars. A Ct value lower than 37 was considered a positive result (Woźniakowski et al. 2016).

Our research is the first to demonstrate the presence of ASFV DNA in Culicoides. The detection of ASFV DNA in vectors is not an indication of their vectorial role. To demonstrate the vectorial role, it would be necessary to isolate the living virus in such arthropods and experimental transmission studies.