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Raport de cercetare intermediar 2

Aferent proiectului de cercetare intitulat : Optimizarea analizei datelor RT-PCR și modelarea epidemiologică a potențialilor vectori mecanici ai pestei porcine africane prin Inteligență Artificială

Noiembrie, 2025

Director (semnătura)

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Noiembrie, 2025

Titlul proiectului de cercetare

Optimizarea analizei datelor RT-PCR și modelarea epidemiologică a potențialilor vectori mecanici ai pestei porcine africane prin Inteligență Artificială

Noiembrie, 2025

Cuprins

Introducere

Pesta porcină africană (PPA) reprezintă o amenințare majoră la adresa sănătății suinelor și a securității alimentare în Europa și la nivel global [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12].

Transmisia virusului poate fi influențată de vectori mecanici, precum insectele hematofage și nehematofage. Interpretarea corectă și rapidă a datelor RT-PCR este esențială pentru diagnostic și răspuns epidemiologic.

Acest proiect își propune utilizarea Inteligenței Artificiale (AI) pentru automatizarea și îmbunătățirea analizei acestor date, dar și pentru modelarea riscului vectorial asociat răspândirii virusului PPA.

Obiective

- Automatizarea interpretării valorilor Ct din RT-PCR pentru detecția eficientă a ADN-ului viral.
- Modelarea riscului epidemiologic asociat vectorilor mecanici (insecte hematofage și nehematofage).
- Dezvoltarea unui model AI de alertă timpurie bazat pe date moleculare și de mediu.
- Compararea performanței modelului AI cu metodele tradiționale de analiză PCR.

Rezultate (preliminare – etapa 1 (iulie, 2025))

- A fost realizată curățarea și integrarea datasetului provenit din focare PPA (anii 2020, 2021, 2023).

- Directorul de proiect a participat la programul de training organizat de Imperial College London (One Health - Vector-Borne Disease Hub), în perioada 4-6 iunie 2025, câștigând un fellowship internațional.
- Directorul de proiect a participat la prezentarea rezultatelor preliminare (etapa 1) în cadrul sediul Academiei Oamenilor de Știință din România, în data de 15 iulie 2025.
- Au fost inițiate activitățile de dezvoltare și testare ale algoritmilor de Machine Learning pentru interpretarea valorilor Ct.

Rezultate (preliminare – etapa 2 (noiembrie, 2025))

Articol pregătit pentru submitere până la sfârșitul anului 2025 (Anexa 1) sau în prima parte a lunii ianuarie 2026 (în funcție de disponibilitatea co-autorilor pentru revizuire).

Articolul are ca scop întărirea bazei de date, pentru a facilitata obiectivele proiectului, respectiv etapele III și IV.

Abstract

Studiul de față investighează prezența genomului virusului pestei porcine africane (ASFV) în mai multe genuri de țânțari colectați din ferme afectate de focare de boală din România. Un total de 1750 de indivizi, grupați în 175 de pool-uri, aparținând genurilor *Aedes*, *Culex*, *Anopheles* și *Culiseta*, au fost analizați prin Real-Time PCR. ASFV ADN a fost detectat în 50,29% dintre pool-uri, cu cea mai mare prevalență în genul *Anopheles*. Analiza statistică a evidențiat diferențe semnificative în funcție de tipul fermei, luna și anul recoltării, precum și de prezența porcilor la momentul recoltării. Valorile CT au indicat încărcături virale mai ridicate în pool-urile colectate în luna iulie și în fermele în care porcii erau încă prezenți. ADN de porc a fost identificat în 11 pool-uri, dintre care 63,64% au fost concomitent pozitive pentru ASFV, sugerând

posibilitatea achiziției virusului în timpul hrănirii. Rezultatele susțin ipoteza că țânțarii pot participa la epidemiologia ASF, probabil prin transmitere mecanică, deși demonstrarea vectorialității rămâne încă neconcludentă. Studiul subliniază necesitatea unor cercetări suplimentare privind competența vectorială și importanța consolidării măsurilor de biosecuritate și a managementului integrat al vectorilor în fermele suine.

Concluzii

Proiectul este în desfășurare conform calendarului propus. Primele rezultate susțin fezabilitatea abordării AI pentru analiza RT-PCR și sugerează valoarea adăugată a corelării entomologice și climatice.

S-a adăugat o nouă funcție de variabilitate – parametri de climă (umiditate, temperatura medie, precipitații); foarte relevanți pentru îndeplinirea modelelor AI (date solicitate ANM, în curs de primire și integrare).

Mai mult, s-a realizat un manuscris care întărește numărul total de vectori introduși pentru studiu – țânțarii. Se preconizează publicarea articolului științific în următoarea perioadă (Anexe – Anexa 1).

Etapa următoare va include validarea modelelor AI și generarea de hărți de risc predictiv. Activitățile derulate contribuie semnificativ la obiectivul general al proiectului și la consolidarea cercetării interdisciplinare în domeniul sănătății animalelor (Tabel 1).

Obiectiv asumat în propunerea de proiect	**Status (noiembrie 2025)**	**Observații / Detalii**
1. Automatizarea analizei RT-PCR (valori Ct)	În curs	Algoritmi ML testați pe probe din 2020–2023. S-a adăugat o nouă funcție de variabilitate – parametri de climă (umiditate, temperatura medie, precipitații); foarte

		relevanți pentru îndeplinirea modelelor AI (date solicitate ANM, în curs de primire și integrare).
2. Model AI pentru vectori mecanici	În curs	Grupare vectori în funcție de specie și localizare; analiză preliminară vectori semnificativi
3. Sistem de alertă timpurie AI	Nu a început	Etapă programată pentru 2026, după validarea modelelor
4. Comparare AI vs. metode tradiționale	Nu a început	Etapă programată pentru 2026. Se va realiza după calibrare completă modelelor AI
5. Hărți AI de risc epidemiologic	Nu a început	Preconizat pentru semestrul III 2026
6. Publicare și diseminare	Parțial	Manuscrise în pregătire, participare la training internațional (Imperial College London). Preconizare participare la conferință internațională (SVEPM 2026, Londra).

Tabel 1: Progresul realizat față de obiectivele propuse

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Anexe

Anexa 1

African Swine Fever virus DNA is present in mosquitoes collected from outbreaks farms in Romania

1. Introduction

The African swine fever virus (ASFV) is a DNA virus that is recognised as a member of the Asfarviridae family. The ASFV is unique characteristic is its ability to be transmitted via arthropods, which distinguishes it from other DNA viruses (COSTARD et al., 2009).

ASFV is a highly contagious and lethal virus affecting domestic and wild pigs, causing significant economic losses in the swine industry globally (WOAH, 2019). ASFV is known for its ability to persist in various environments and hosts, contributing to its widespread distribution and outbreaks (NETHERTON et al., 2019). Historically, the transmission of ASFV has been primarily attributed to direct contact with infected animals, ingestion of contaminated feed, and through soft tick vectors of the genus *Ornithodoros* (COSTARD et al., 2009; DIXON et al., 2020). However, recent studies have suggested the potential role of mechanical vectors, including mosquitoes, in the spread of ASFV (EFSA, 2021).

Mosquitoes, belonging to the family Culicidae (BECKER et al., 2010), are well-known vectors of numerous viral pathogens affecting both humans and animals, including arboviruses such as West Nile virus, Dengue virus, and Zika virus (GUBLER, 2002, TURELL et al., 2005). Mechanical transmission of viruses by mosquitoes occurs when the pathogen is transferred from an infected host to a susceptible host via contaminated mouthparts, without the need for biological replication within the vector (FOIL & GORHAM, 2000, POWERS et al., 2008). This mode of transmission can be particularly relevant in environments where mosquitoes feed on multiple hosts in close succession, such as farms and wildlife settings (CARN, 1996, POWERS et al., 2008).

Several factors contribute to the plausibility of mosquitoes as mechanical vectors for ASFV. First, the feeding behaviour of mosquitoes, which involves probing multiple hosts (MARTINA et al., 2017) during a single gonotrophic cycle, increases the likelihood of virus transfer (TEMPELIS, 1975). Second, the robust nature of ASFV allows it to remain viable on mosquito mouthparts for extended periods (BONNET et al., 2020). Finally, the presence of high-density pig farming systems in endemic areas with mosquito populations creates optimal conditions for potential mechanical transmission events (LINDAHL et al., 2012).

Scientific evidence supporting the possible role of mosquitoes in the mechanical transmission of ASFV includes very few studies that have detected ASFV DNA in mosquito species. Moreover, no studies have validated any possible biological or mechanical transmission (BONNET et al., 2020).

From our knowledge, only PLOWRIGHT et al., 1994 mentioned a study that failed to demonstrate the vectorial competence of mosquitoes and horse flies for the viral genome transmission.

For instance, a study conducted by QIN et al., 2021 wasn’t successful to detect ASFV DNA in *Culex*, *Aedes*, *Anopheles* mosquitoes collected from ASFV-affected pig stables. Similarly, negative results were obtained in Estonia in a research where only one mosquito pool was tested (HERM et al., 2019).

Despite the hypothesis supporting the vectorial competence of mosquitoes in the mechanical transmission of ASFV, comprehensive evidence remains limited. These research studies demonstrate only that mosquitoes can acquire the virus from infected hosts (BONNET et al., 2020). The isolation of the virus wasn’t successful until now (HERM et al., 2019).

Our study aims to investigate the detection of ASFV DNA in various mosquito genera from Romania that might be able to transmit ASFV mechanically under field conditions. Additionally, this study intends to assess and analyse the risk factors. By supporting the role of mosquitoes in ASFV epidemiology, we aim to enhance our understanding of the virus's transmission dynamics, inform interested parties about the development of more effective biosecurity measures, and mitigate the spread of this devastating disease.

2. Materials and methods

Mosquito trapping was conducted on a total of 25 outbreak farms, which corresponded to ASF-positive case farms, between the months of June and September 2020 and August and September 2021. To select these farms, we used a score system and a set of inclusion criteria, prioritizing the communes based on various factors. The publication by BALMOȘ et al., 2021 provides a comprehensive description of all the sampling details, methods, and methodologies involved. The planned sampling for the year 2020 targeted backyard farms (BF), type A farms (TAF), and commercial farms (CF). In the year 2021, no samples were collected from the BF. One single sample was taken from each farm, and it was collected soon after the ASF outbreak was officially confirmed. For a period of twenty-four hours, each of these farms was subjected to the sampling process.

2.1. Insect collection, morphological identification and pooling

The mosquito specimens analysed in the current study were by-catch pools from mini-CDC traps, trapped previously (BALMOȘ et al., 2021, BALMOȘ et al., 2023, BALMOȘ et al., 2024). We separated the Culicidae individuals from biting flies and non-hematophagous insects, then preserved them at -20 degrees Celsius for further analyses (morphological identification and DNA extraction).

We identified the collected mosquitoes at the genus level by examining their morphological characteristics using detailed keys and descriptions (BECKER et al., 2010). Only females, respectively blood-fed specimens were prepared for further investigations.

Prior to DNA extraction, pools were established. We pooled the mosquitoes based on their genus and the specific farm. We tested the mosquitoes in groups of ten individuals per pool. We formed pools alone when the required number of insects per pool (10) was available. The study only incorporated a maximum of five pools per group and farm. The study included a total of 1750 insects, dispersed across 175 pools.

2.2. *DNA extraction and Real-Time PCR*

A DNeasy Blood & Tissue Kit manufactured by Qiagen was utilised in order to extract the genome of the ASF virus. All of the DNA samples were kept at a temperature of -20 degrees Celsius until they were processed. At the National Reference Laboratory (IDAH-Institute for Diagnosis and Animal Health), a real-time PCR technique was employed in order to determine whether or not the ASFV was present in any of the pools. In addition, the DNA that was extracted was examined in accordance with the Standard Operating Procedure (SOP) Identification of the ASFV genome, which was based on the SOPs of the European Union Reference Laboratory for African Swine Fever (ASF EURL).

The CT values obtained throughout RT-PCR analysis represents quantitative information regarding the amount of ASFV DNA (viral load). For this purpose, we considered each and every sample to be positive if its CT value was not higher than forty.

2.3. *Blood meal analysis*

The analysis of the blood meal was carried out in order to determine whether or not the mosquitoes feed on pigs or on other hosts, as well as to detect whether or not the ASFV DNA-positive mosquitoes have fed on pigs. Amplification of the cytochrome c oxidase subunit 1 (COI) gene region (~758 bp) was used in the PCR process. The reaction was carried out similarly as in the previous studies (BALMOȘ et al., 2021, BALMOȘ et al., 2023). All positive sequences were analyzed and edited by the use of Genius 4.85 software (KEARSE et al., 2012).

2.4. *Statistical analysis and mapping*

For the purpose of conducting the statistical analysis, a tool known as Epi Info 7 (CDC, USA) was utilised. Calculations were made regarding the presence of insects, as well as the observed PCR positivity of infection concerning each category, as well as the 95% confidence interval (95% CI) for each category.

The Pearson's chi-square test was performed with the aim of evaluating the differences that existed across categorical variables, which included the presence or absence of pigs, the insect genus, the farm type, the sampling year, and the collection month.

The Kruskal-Wallis H Test was conducted in order to investigate the type of association that exists between the mean CT values and the categorical variables.

The odds ratio was established by logistic regression. The statistical significance of all the results was determined to be at a level of $P<0.05$.

The calculation of the Phi (Φ) coefficient was applied to evaluate the correlation between two dichotomous variables, particularly PCR positivity and the presence or absence of pigs or the sampling year.

The Point-Biserial coefficient was employed in order to evaluate the degree of correlation that exists between a dichotomous variable and a group of variables (insect genus, farm type, and month). Furthermore, the Spearman's rank correlation test was used in order to assess the degree of relationship that exists between CT values and categorical variables.

The values of the correlation coefficients were interpreted as follows: 0.01–0.20 was considered to be none to slight, 0.21–0.40 was considered to be fair, 0.41–0.60 was considered to be moderate, 0.61–0.80 was considered to be large, and 0.81–1.00 was considered to be almost perfect.

We generated A multiple regression model was generated with the presence of ASFV DNA as a dependent variable. The predictors included pig DNA, sampling year, sampling month, insect genus, farm type, and the presence of pigs presence as categorical factors. The model was then used to estimate the relationship between the two variables.

The maps were generated by using the ArcGIS Pro software (ESRI, 2021).

3. Results

3.1. Mosquitoes identification

Aedes, *Culex*, *Anopheles*, and *Culiseta* were the four genera that were identified within the mosquitoes that were collected for this study. In 2020, a total of 110 pools, consisting of 1110 insects, were collected from 17 outbreaks. These pools were then prepared and tested for ASFV DNA presence. Eight outbreak farms were sampled in 2021, and 650 insects were sampled from those farms and divided among 65 pools. Table 1-4 illustrates the distribution of pools according to the year, the month of collection, the type of farm, and whether or not the pigs were present at the time of sampling.

Table 1. Distribution of mosquitoes by year.

Insect	**Year**			
	2020		**2021**	
	n	**%**	**n**	**%**
Aedes spp.	31	28.18	26	40
Culex spp.	47	42.73	9	13.85
Anopheles spp.	25	22.73	30	46.15
Culiseta spp.	7	6.36	0	0
TOTAL	110	100	65	100

Table 2. Distribution of mosquitoes by month.

Insect	**Month**							
	June		**July**		**August**		**September**	
	n	%	n	%	n	%	n	%
Aedes spp.	5	20	5	62.5	43	40.57	4	11.11
Culex spp.	15	60	3	37.5	23	21.7	15	41.67
Anopheles spp.	5	20	0	0	38	35.85	12	33.33
Culiseta spp.	0	0	0	0	2	1.89	5	13.89

TOTAL	25	100	8	100	106	100	36	100

Table 3. Distribution of mosquitoes by month.

Insect	**Farm type**					
	BF		**CF**		**TAF**	
	n	**%**	**n**	**%**	**n**	**%**
Aedes spp.	11	21.15	36	40.45	10	29.41
Culex spp.	25	48.08	17	19.1	14	41.18
Anopheles spp.	11	21.15	36	40.45	8	23.53
Culiseta spp.	5	9.62	0	0	2	5.88
TOTAL	52	100	89	100	34	100

Table 4. Distribution of mosquitoes by month.

Insect	**Pigs present**			
	yes		**no**	
	n	**%**	**n**	**%**
Aedes spp.	46	43.4	11	15.94
Culex spp.	23	21.7	33	47.83

Anopheles spp.	37	34.91	18	26.09
Culiseta spp.	0	0	7	10.14
TOTAL	106	100	69	100

3.2. PCR positivity

Out of the total of 175 pools, 88 were tested positive for the detection of ASFV DNA (50.29%, 95% CI 42.64-57.92).

The statistical analysis revealed significant variations in the insect's species. *Anopheles* had the highest percentage of positive pools (61.82%, 95% CI 47.73-74.59), while *Culiseta* had the lowest. There was no statistically significant association seen between the presence of ASFV DNA and the insect genus (r=0,005; P=0,93).

Based on the farm type, out of the total of 175 pools that were collected, 52 pools derived from BF, 89 from TAF, and 34 from CF. However, the majority of positive samples were obtained from the BF pool (65.38%, 95% CI 50.91-78.03). The statistical analysis found that the results were significant based on the farming system (Chi-square test, X2=10.93; d.f.=2; P=0,004). No correlation was noted between the farm type and PCR positivity (r=0,08; P=0,28).

Out of the total number of pools collected, a higher percentage of positive pools were found in farms where the pigs had already been culled (65.22%, 95% CI 79-76.29) compared to the farms where the pigs were still present at the time of sampling. These results were statistically significant (Chi-square test, X2=9.19; d.f.=1; P=0.001). No correlation was observed (φ=0,06; P=0,36). A statistically significant difference was observed between the years of sampling (Chi-square test, X2=17.02; d.f.=1; P<0.0001).

The difference between the years of sampling and PCR positivity was not statistically significant (φ=0.32; P=0.99).

There was a significant difference in the prevalence of ASFV DNA positive pools based on the month of sampling (Chi-square test, X2=15.89; d.f.=3; P=0.001), with the highest prevalence seen in August (41.51%, CI 95% 32.02-51.49).

Logistic regression analysis was conducted to examine the combined effect of various predictors. The results revealed that only the presence of pigs and the farm type were found to be significant predictors of ASF DNA positivity.

3.3. CT values

Out of the 175 pools, 88 were considered positive, as they had a CT value < 40. We determined the positivity degree based on the CT values, labeling values greater than 30 as weakly positive, values between 30 and 24 as positive, and values less than 24 as strongly positive. The CT values of this study were within the range of 20.39 to 39.17, with a median value of 32.08 and a mean value of 31.89.

The assessment of CT values by insect genus did not indicate a significant overall difference (Kruskal-Wallis test, H = 4.21; d.f. = 3; P = 0.23). Aedes had the lowest mean CT value (29.74), indicating a higher viral DNA load. However, the correlation between insect genus and CT values was not significant (r =0,01; P=0,86).

There was no significant difference of the mean CT values between different types of farms (Kruskal-Wallis test, H = 2.8; d.f. = 2; P = 0.24), However, the lowest mean value of 31.32 was observed for BF. There was a slight correlation between farm type and CT value (r=0,18; P=0,09).

Furthermore, the presence of pigs in the farms was linked to significantly lower mean CT value (Kruskal-Wallis test, H = 1.01; d.f.=1; P=0.31). A significant correlation was observed (r=-0,25; P=0,01).

The lowest mean CT values (corresponding to higher ASFV DNA load) were noticed in July. Higher CT values, corresponding to a weaker positivity (above 33.46) were predominant in pools from June, August and September (Kruskal-Wallis test, H = 9,99; d.f.=3; P=0,01). A slight correlation was observed (r=0,16; P=0,13).

A significant mean value (24.33) of the CT values of the pig DNA was obtained where the pigs were still present at the time of sampling (Kruskal-Wallis test H = 9,13; d.f.=1; P=0,002).

The multiple regression model highlighted the insect genus, farm type, presence of pigs, sampling month, and sampling year as statistically significant predictors (P<0.008), as shown in Table 5.

3.4. Blood meal

Out of the total pools examined (n=175), in 11 pools pig DNA was detected. The positive samples for pig DNA were collected from farms where the pigs were still alive. Out of this, 7 were positive for ASFV DNA (63.64%, 95% CI 30,79-89,07).

Table 5. The insect genus, farm type, presence of pigs, sampling month, and sampling year as statistically significant predictors.

Factors	**Odds ratio**	**95%**	**CI**	**Z**	**p**
Insect genus	1.21	0.84	1.74	1.04	0.29
Farm type	0.79	0.49	1.27	-0.97	0.33
Pigs presence	0.74	0.33	1.68	-0.69	0.48
Sampling month	0.7	0.47	1.03	-1.79	0.07
Sampling year	0.32	0.14	0.75	-2.62	0.008

4. Discussion

The study we conducted provides new insights into the role that mosquitoes play as possible vectors of the African swine fever virus. 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.

Moreover, the overall prevalence of ASFV DNA in mosquito pools was 52.9%, and positive samples had been obtained from each of the four genera that were investigated. The results we obtained are in alignment with the study carried out by HERM et al., 2020, where the viral DNA was detected in both non-biting flies and mosquitoes on ASF positive farms in Estonia. However, the CT values for the mosquitoes were significantly higher (36.98). These findings are consistent with the observations of previous investigations by various researchers.

The results of our research indicate that mosquito populations contain a higher ASF viral genome than was previously reported. This suggests that there may be a greater possibility for mechanical transmission than was recorded. According to the findings of our research, the temporal analysis showed that the month of August had the highest prevalence of ASFV DNA-positive pools, whereas the month of July had the lowest mean CT values, which indicated that the viral loads were higher. It has been observed in studies such as those conducted by YOON et al. (2021) and TURČINAVIČIENĖ et al. (2021) that there is a correlation between the seasonal pattern and the increased mosquito activity that occurs during warmer months.

Additionally, our research, which was based on the spatial analysis, revealed that backyard farms had the highest number of positive pools, which was 65.38 %. This highlights the potential risk that is posed by situations that are more dependent on biosecurity management.

It is noteworthy to point out that in our research, pig DNA was found in 11 out of the 175 pools that were analysed, and more than 66% percent of these pools also tested positive for ASFV DNA. This finding provides further evidence that there is a direct connection between the presence of pigs and the virus load in mosquitoes, which further strengthens the possibility of mechanical transmission within and between farms that are neighbouring to one another. The finding brings evidence to the necessity of implementing complex biosecurity measures in order to reduce the possibility of ASFV being transmitted through mosquito vectors, as BLOME et al.,2024 have proposed.

The findings that we obtained help to a better understanding of the possible role that mosquitoes play in the spread of ASFV infections.

QIN et al., 2021 conducted a similar study in China from where it were collected mosquitoes and other Dipterans. Negative results were obtained for ASFV detection in mosquitoes collected from five pig farms in China. This result stands in contrast to the high prevalence rates that we obtained.

SAEGERMAN et al., 2021 highlighted the importance of conducting additional research on the vectorial capacity of blood-feeding arthropods, which is a gap that our study helps to address. There is still a lot of uncertainty around the role that mosquitoes play as vectors for ASFV. HAKOBYAN et al. (2022) conducted an experimental study in order to assess the vertical transmission of ASFV in *Aedes aegypti*. Negative results were obtained. Moreover, they also highlighted that even though mosquitoes might contribute to the spread of the virus, it remains highly unlikely that they are the main vectors.

Our finding that we detected a significant prevalence of ASFV in mosquito pools, notably in *Anopheles*, suggests that these insects are capable of carrying and possibly transmitting the live virus.

To successfully achieve this goal, additional research is required in order to have a better understanding of the precise mechanism and effectiveness of the transmission process.

There is a need for integrated vector management techniques in ASF control programmes, as evidenced by the connections that exist between the presence of pigs and increased ASFV DNA loads in mosquitoes.

When it comes to reducing mosquito numbers and the likelihood that they would act as mechanical carriers, some measures, such as the control with insecticides, the modification of habitats, and the use of repellents, could be of critical importance. In addition, it is fundamental to implement increased biosecurity standards, particularly on backyard farms, in order to reduce the possibility of the ASFV virus being transmitted by mosquito vectors.

5. Conclusion

Our study emphasises the detection of ASFV DNA in multiple mosquito genera found in farms experiencing ASF outbreaks. These data suggest that Culicidae insects may have a role in ASF transmission, either through mechanical or biological routes.

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