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ORIGINAL ARTICLE

African swine fever in Benin and prevalence of the disease in Southern Benin: A retrospective study (2014–2018)

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ABSTRACT

Objective: This study aimed to assess the prevalence and risk factors of African swine fever (ASF) disease in Benin.

Materials and methods: A retrospective study was conducted on 70 pig farms from the Departement of Atlantique and Ouémé and also by using the data available from the Directorate of Livestock on the spread of ASF in Benin from 2014 to 2018. The prevalence of ASF was assessed with 106 nasal swabs from apparently healthy domestic pigs and 15 organ samples from dead ASF-suspected pigs. ASF virus detection was carried out by conventional polymerase chain reaction using Qiagen Kit for DNA extraction. Data recorded were processed with SAS software (2006).

Results: It appears that ASF is an endemic disease in Benin with the Department of Ouémé as the hotspot of dissemination of the virus in the country. The losses due to ASF recorded from 2014 to 2018 are evaluated to 884,850,000 CFA Franc by estimating the average cost of a pig at 25,000 FCFA. A prevalence of 1.89% (CI at 95%, 0.71–3.49) was recorded for live animals with a positive result in organs from all dead pigs suspected of ASF. Breeding practices related to the sharing of breeding males, scavenging pigs, and non-compliance with biosecurity measures were the risk factors identified.

Conclusion: The present study sheds light on the areas prone to the ASF virus in Benin. Moreover, the cross-sectional data recorded on the prevalence of ASF will help to better rule on the spread of the disease. It would be interesting for the Beninese Republic to increase its efforts for ASF control.

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Introduction

Swine production in West Africa is estimated at 13,678,363 pig heads or 1.43% of animal production [1]. Many diseases affect pigs, where some are zoonotic, whereas others are transboundary such as African swine fever (ASF) [2]. It is a deadly and highly contagious viral disease of domestic pigs and wild boars [3]. The continued spread of ASF from Africa to Europe and recently to high pork production countries in China and others in Southeast Asia threatens global pork production and food security [4]. Currently, ASF has expanded considerably in West Africa, with outbreaks in Ivory Coast, Benin, Nigeria, Togo, and Ghana [5]. The introduction of ASF in Benin in 1997 caused numerous economic losses as well as the profound disorganization

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of the pig sector although the primary source was never established [5]. To date, no vaccine or treatment exists to control ASF [6]. Thus, farmers and other actors in the swine sector continue to carry out their activities in an endemic situation of ASF. In this way, even sick pigs are slaughtered illegally, and their meat sold without much concern. These corrupt practices only maintain the existence of ASF, which, far from receding, only pass from country to country, especially in Africa, south of the Sahara. Some pigs are infected with the virus and, therefore, contaminants, but show little or no symptoms. Furthermore, acute and chronic forms of the disease are the most frequently encountered, leading to the almost impossible delimitation of the outbreaks [7]. Indeed, this pathology constitutes a significant threat to the development of the pork industry. The past 5 years have been marked by the episodes of ASF in Benin [8]. The present study aimed to reveal the risk factors of ASF based on retrospective data in Benin between 2014 and 2018 and estimate the prevalence of the disease in southern Benin.

Materials and Methods

The methodological approach initially consisted of a retrospective study on ASF with pig breeders in southern Benin and on the previous statistical data recorded by the Directorate of Livestock in Benin. Moreover, we collected nasal swabs from live pigs as well as organs from dead pigs suspected of ASF between April and May 2019 for laboratory diagnosis.

Study area

It takes into account the Departments of Atlantique and Ouémé in Benin. The two departments are located in the south of the country, constituted of eight and nine districts, respectively. This study takes into account the district of Sèmè-Kpodji, Porto-Novo, and Adjarra in Ouémé and that of Abomey-Calavi in Atlantique. Climates are subequatorial with four seasons: two rainy (one large from April to July and one small from October to November) and two dries (one large from December to March and one small from August to September). They present a rainfall between 900 and 1,500 mm, with temperatures varying from 25°C to 30°C. The chief crops are corn, cassava, peanuts, oil palm, vegetable crops, and cowpea. Livestock mainly includes cattle, especially in the Atlantic, fish farming, small farming, and pig farming [9].

A retrospective study on ASF in Benin

It consisted of an interview with 70 pig breeders from the districts of Abomey-Calavi, Adjarra, Sèmè-Kpodji, and Porto-Novo. The discussion was oriented toward the perception of ASF by breeders. Knowledge of the disease, its manifestation in farms, and the favorable conditions to the appearance of the disease were the data taken into account. Besides this, the data (Department affected by ASF and the number of cases reported each year) recorded at the Directorate of Livestock of Benin and reported in the annual reports for the years 2014 to 2018 have been used in this study. These data help to establish the economic incidence of ASF and the hotspot of the disease by identifying the department more affected during the 5 years, where the incidence of the disease was high.

Prevalence of ASF in South of Benin

This part of the study involved the collection of samples from live and dead pigs suspected of ASF (based on the clinical signs of ASF reported by the International Organization of Epizootie) followed by laboratory analysis of the polymerase chain reaction (PCR).

Sampling and data collection

The breeders were chosen according to the criteria of accessibility to the pig farms and their availability [10]. Samples were collected between April and May 2019 in pig farms taking into account the history of the farm against the ASF, the promiscuity of the farm to others having recorded ASF, the sanitary state of the pigs, and acceptability of the breeder. Thus, 121 samples (106 nasal swabs from live pigs and 15 organ samples including spleen, kidney, lung, liver, and gastric ganglion from dead pigs suspected of ASF) were collected to diagnose ASF by PCR. It should be noted that the animals suspected of ASF and dead were subjected to a clinical examination, including an extern appreciation of the animal until an autopsy. Concerning the swab, once made, the swab was cut out in a viral transport medium (phosphate-buffered saline [PBS] + penicillin + amphotericin B) contained in a cryotube and identified according to the sampling site and the district. As for the organs, they were placed in sterile bags. All the samples were kept in the field in a cooler containing cold accumulators at +4°C and then at −37°C once at the Research Unit in Applied Microbiology and Pharmacology of Natural Substances from the University of Abomey, Calavi.

Laboratory analysis

The samples (swabs and organs) collected from pig farms were subjected to diagnostic analysis following the PCR at the Laboratory of Veterinary Diagnosis and Sero-Surveillance at Parakou following the methodology of Office International des Epizooties (OIE) [11].

Extraction of nucleic acids from swabs and organs samples

The organs were previously cut into pieces and then ground in a buffer solution of PBS. DNA was extracted from 200 µl of clarified supernatant using the Qiagen red PCR kit according to the supplier's specifications. Concerning the swab, the extraction was carried out from 200 µl of the solution of the viral transport medium. The extracted and purified DNA was eluted in 50 µl of sterile ultrapure water and placed at −20°C until use or led directly to the amplification step.

Gene amplification

Gene amplification was carried out using DNA extracted from pigs' organs or nasal swabs. First, the master mix was prepared using sterile ultra-pure water, deoxyribonucleotides, gene expression master mix 5X, primer pairs PPA1 (5'-ATG GAT ACC GAG GGA ATA GC-3') and PPA2 (5'-CTT ACC GAT GAA AAT GAT AC-3'), and enzymes according to the supplier's instructions (Qiagen). Thus, the reaction mixture prepared for each sample was 23 µl, to which 2 µl of the extracted DNA was added. The mixture is then subjected to a polymerization reaction following a first denaturation step of 12 min at 95°C, followed by 40 cycles consisting of 30-sec denaturation at 95°C, 1 min of hybridization of the primers at 72°C, and a final elongation of 5 min at 72°C. The amplification products were visualized by illumination with UV radiation after electrophoretic migration in a 2% agarose gel in Tris Acetate Ethylenediaminetetraacetic acid (TAE) 1X. The size of the amplified DNA fragment was 257 bp.

Statistical analysis

The digital data were recorded in an Excel database and then analyzed with the SAS software (SAS, Cary, NC, 2006) using the bilateral *Z*-test.

Results and Discussion

Retrospective study on ASF in Benin

The results of the retrospective study take into account data on the incidence of ASF from 2014 to 2018 in Benin. Similarly, the perception of ASF by pig breeders in Southern Benin and the risk factors linked to the disease emergence were taken into account.

Areas affected by ASF, cases reported, and incidence of the disease between 2014 and 2018

The evaluation of statistics from the Directorate of Livestock in Benin between 2014 and 2018 reveals that the ASF circulates in many areas in Benin. In 2014, six departments of Benin were affected by ASF. These are the Departments of Borgou with the district of Parakou; Zou with the districts of Bohicon and Zogbodomè; Collines with the district of Savalou; Ouémé with the districts of Adjarra, Adjohoun, Akpro-Missérété, Dangbo, and Sèmè-Kpodji; Plateau with the districts of Ifangni, Kétou, and Sakété; and the Atlantique Department with the districts of Abomey-Calavi, Allada, Kpomassè, and Tori-Bossito. A total of 14,737 cases of ASF were recorded during this period (Fig. 1).

In 2015, two departments were identified as being affected by the disease. These are Ouémé with the districts of Adjohoun and Dangbo and the Department of Plateau with the district of Ifangni. The total number of cases was estimated as 152 (Fig. 1).

In 2016, five departments of Benin were reported for the ASF. We noticed Atacora represented by the District of Natitingou; Ouémé represented by the districts of Adjohoun, Avrankou, Dangbo, and Sèmè-Kpodji; Plateau represented by the districts of Sakété and Ifangni; Atlantique represented by the districts of Allada and Toffo, and the Departement of Mono represented by the district of Grand-Popo. During this period, 721 cases were recorded (Fig. 1).

For 2017, Benin was affected at the level of four departments. These are Atacora with the district of Boukoumbé; Ouémé with the districts of Aguégués, Avrankou, and Dangbo; Atlantique with the districts of Allada, Ouidah, and Toffo; and then, the Department of Mono represented by the districts of Athiémé, Bopa, and Houeyogbé. For this year, 7,478 cases of ASF outbreaks were recorded (Fig. 1).

Recently, in 2018, six departments were identified as being affected by ASF. These were the Departments of Zou with the districts of Bohicon; Collines with the districts of Dassa-Zoumé, Ouèssè, Savalou, and Savè; Ouémé with the districts of Adjarra, Adjohoun, Aguégués, Avrankou, Bonou, Dangbo, and Sèmè-Kpodji; Plateau with the districts of Sakété and Kétou; Atlantic with the district of Abomey-Calavi, and the Departement of Mono represented by the district of Grand-Popo. A total of 16,729 cases of ASF outbreaks were recorded during this period (Fig. 1).

Figure 2 shows the incidence of ASF that occurs between 2014 and 2018. We note that the Department of Ouémé registered the high number (36) followed by Atlantique (22), Plateau (10), Mono (6), Atacora (5), Zou (7), Collines

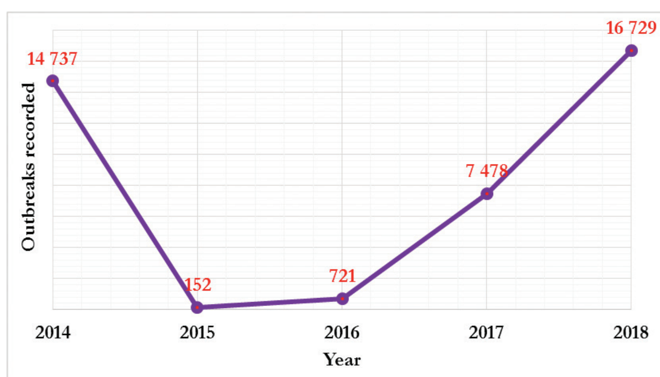

Figure 1. Cases of ASF recorded from 2014 to 2018 in BENIN.

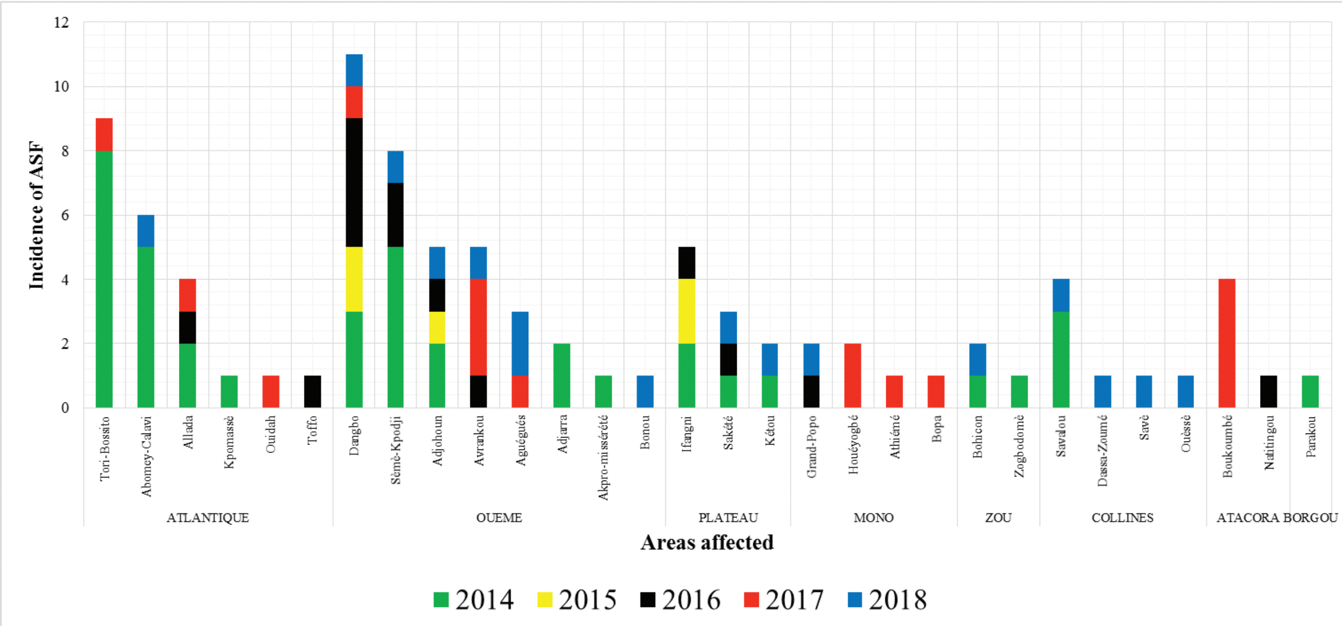

Figure 2. Incidence of ASF between 2014 and 2018 in Benin.

(3), and Borgou (1). In the same way, the District of Dangbo registered the higher level of ASF incidence compared to the other district in the same department, even those of the other departments. The analysis of retrospective data shows that ASF has been recorded every year from 2014 to 2018 only in the district of Dangbo of the Department of Ouémé. The confirmation of ASF in this area during these 5 years can lead to the declaration of this district as endemic to ASF. As a result, the episodes of ASF recorded in Benin every year from 2014 to 2018 and in several districts, therefore, in several departments of the country, allowed us to declare the state of endemicity of Benin toward the ASF.

In 2014, the high number of cases recorded in Benin can be explained by the non-control of the source of infection and the lateness of delimitation of the extent of the manifestation of the disease in pig herds. Indeed, the declaration of the disease gives a right to a prefectural order carrying the declaration of infection, which leads to the application of defensive measures essentially characterized by the systematic slaughter of all the animals present in the area (stamping out) of the outbreak and the establishment a seat belt to prevent the virus from spreading to other territories. Unfortunately, the insufficiency of the measures accompanying these decisions leads the breeders not to collaborate by systematically declaring the suspect animals. Thus, the virus takes the time to settle down by devastating almost all of the pig herds surrounding the area of the first infection. This may be the reason for the lower ASF cases recorded in 2015 and 2016, where the pig populations took time to rebuild, leading to an epidemic outbreak in 2017 and 2018. All of these had the direct consequence of a significant economic loss for breeders in the first place.

Perception of pig farmers on ASF in Southern Benin and economic impact of the disease

At the end of the interview with pig breeders in southern Benin, we note that the majority of breeders know ASF versus a minority of breeders who have no idea of the disease or ignore it ($p < 0.001$). However, around two-third of the farmers interviewed did not record any case of ASF on their farm ($p < 0.05$). The results are shown in Table 1. It appears that ASF constitutes a disease well known by the majority of pig breeders in Benin because of its recurrence and the damage caused by its passage through farms. Indeed, although this dreaded disease is decimating pig

Table 1. ASF perception by pigs breeder interviewed in south of Benin.

Variable	Frequency ± Confidence interval (%)	*Z* test
Recognition of ASF		
Yes	$82.86^{a} \pm 8.8$	***
No	$17.14^{b} \pm 8.8$	
ASF cases		
Yes	$38.57^{b} \pm 11.4$	*
No	$61.43^{a} \pm 11,4$	

Frequencies of the same column follow with differenletterser (a, b) show significant difference at the rate of 5%.
***$p < 0.001$; *$p < 0.05$.

Table 2. Economic incidence of ASF in BENIN pigsty from 2014 to 2018.

Years	2014	2015	2016	2017	2018	Total
Swine population	431,000	448,000	466,000	414,387	504,000	2,263,387
Mortalities due to ASF	12,782	117	544	6,000	14,179	33,622
Stamping out	1,285	21	37	418	11	1,772
Economic losses (FCFA)	351,675,000	3,450,000	14,525,000	160,450,000	354,750,000	884,850,000

populations in Benin, many pig farmers continue production. This determination is explained on the one hand by the direct income, in which these breeders record since the output is of a short cycle, but also the cultural character of the breeding of pigs, which would be tarnished if they gave up production.

Table 2 shows the animal losses recorded in Benin related to ASF from 2014 to 2018. We noticed that during these 5 years, ASF had caused mortalities up to 33,622 heads with stamping out of pigs estimated at 1,772 heads, so a total of 35,394 pigs. If the cost of an adult pig is estimated as 25,000 CFA Franc (FCFA) [12], the losses recorded from 2014 to 2018 should be evaluated to 884,850,000 FCFA.

Although the economic losses recorded between 2014 and 2018 in Benin are less than those reported by Randriamparany [13] estimated at 6 million US dollars during the epidemic which occurred between 1997 and 1998, they are no less significant or negligible as constituting a shortfall for producers and even the loss of investments with the direct consequence of neglecting to farm. The work of Saka et al. [14] on the incidence of ASF after spreading through several farms in Lagos (Nigeria) reported an economic loss of up to 485,853.91 Naira or approximately 797,000 FCFA. The generalization of this situation throughout the country would lead to a great loss, which would have a significant impact on the economy of the country, because animal production contributes significantly to the Gross Domestic Product of the country. Faced with all these, the producers, the first victims of the ASF epidemic crisis, affirm that they are left to their own devices without any support measures.

Risk factors associated with the emergence of ASF in southern Benin

The survey carrying out in the south of Benin allowed us to record farming practices that could enhance the spread of ASF virus in farms. This concerns the use of a breeding male from a pigsty to carry out coupling in another one, the promiscuity of pig breeding, the use of plants to feed animals, the wandering of pigs whose owners are directly unknown, and the absence of an effective disinfection system at the level of raw material sources supply for food preparation. Besides this, there is the non-compliance with animal transport rules at the border. Farmers reported that given the high

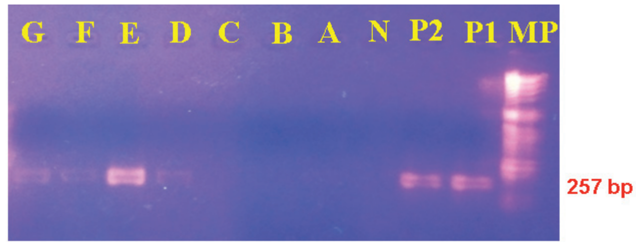

Figure 3. PCR-based detection of ASF virus. MP = 100-bp DNA ladder; P1 = DNA extraction positive control; P2 = Gene amplification positive control; N = Negative control; A-B-C = Negative samples; D-E-F-G = Positive samples.

cost of animals offered for sale in Benin, some traders export pigs from Nigeria and Togo not only to submerge the market and this in clandestine fashion, which exposes Benin to the risks linked to the spread of multiple diseases including ASF.

Attakpa et al. [3] justified the emergence of ASF in northern Benin by the type of farming dominated by the wandering of animals on the one hand and the non-compliance with hygienic and sanitary measures. These various findings are, therefore, generalized in the country, given the results recorded in this study. However, the study covered such few farms, where the animals are in temporary confinement. Not only taking into account repressive measures against wandering animals but also sensitize farmers' awareness about the importance and respect of biosecurity measures coupled with the application of defense rules by the Directorate of Livestock in ASF outbreaks must constitute guidelines to be followed to restore peace in pig farming in the country. Kouakou et al. [15] reported the same risk factors in their studies. These authors justify the persistence of the ASF virus in the countries of the South Sahara by the only epidemiological model consisting of the wandering of pigs, followed by the insufficient level of biosecurity required in rearing as well as the control of animal movements.

Prevalence of ASF virus in pig farms in South of Benin

From the analysis of the results, it appears that two of the samples consisting of swabs were found to be favorable for the PCR, a percentage of 1.89%. Similarly, all the organs from the 15 dead pigs tested were positive in PCR (Fig. 3). It should be added that these many positive results were obtained in the Department of Ouémé (Fig. 4).

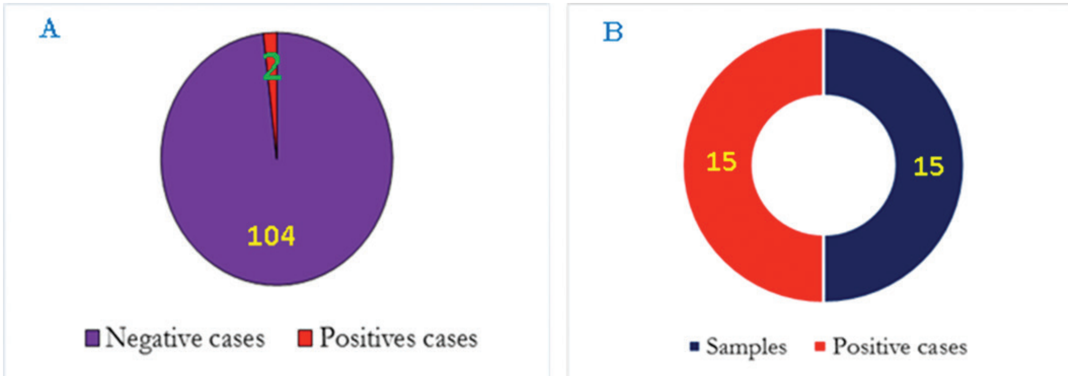

Figure 4. Prevalence of ASF for nasal swabs from live pigs (A) and organs from suspected dead pigs (B) in southern Benin.

The positive results recorded from the laboratory diagnosis confirm the various clinical signs observed in farms suspected of manifesting ASF. In addition, these results justify the effective circulation of the virus in the Department of Ouémé, where all the positive cases have been registered. Furthermore, the frequency of 1.89% obtained for nasal swab samples may be explained by the nature of the analysis carried out, which is PCR. Indeed, this test consists of detecting only the actual presence of the virus DNA or viral proteins in the animal organism, therefore in animals indeed carrying the ASF virus. This could justify the difference in prevalence recorded on living animals sampled during this study compared to that of the work of Attakpa et al. [3] in Parakou in the north of Benin. In their research, these authors employed Enzyme-linked Immunosorbent Assay (ELISA) techniques to titrate the ASF viral antibody. Nevertheless, the organs such as the liver, spleen, kidneys, spleen, and lungs detected positive by these same authors are consistent with the organs incriminated during the tests on the organs of dead pigs suspected of ASF. In this case, we use the same methodology for sample analysis with Attakpa et al. [3].

However, few studies have reported the prevalence based on the actual presence of the ASF virus in pig farms in Benin. The majority of the results published so far show the seroprevalence of the disease after the virus has spread through farms. Although the ASF virus causes a lot of damage, there are nevertheless 24 genotype characteristics of the disease [16]. Thus, immunocompetent pigs resist to the outbreak of less virulent strains, which explains the different results presented by the authors. These claims are justified by the results reported by Atuhaire et al. [17] with an estimated ASF seroprevalence of 53.59% in apparently healthy domestic pigs in Uganda. These same authors found a prevalence of 0.55% of the ASF virus in apparently healthy domestic pigs in the surveillance zone. The difference recorded with the prevalence can be justified by the sample size because it influences the prevalence rate calculated. Furthermore, the high viral prevalence of 92% reported by Kouakou et al. [15] can be explained by the nature of the samples which come from a suspect or dead ASF animals but also by the more extended study period (5 years), where several episodes of the disease are encountered.

Conclusion

The present study sheds light on the hotspot of the ASF virus in Benin. Thus, the Department of Ouémé has been identified as being riskier in the spread of the ASF virus. In addition, certain farming practices by breeders support the favorable conditions for the spread of the ASF virus in farms in the same area and throughout the country. The prevalence recorded also justifies the nucleus providing homes of the virus in the country, causing significant economic losses for the breeders who find themselves almost without means to revive activities. It would be interesting for the Beninese Republic to increase its efforts through available services to sensitize farmers about the risks involved in non-compliance with biosecurity measures, but also the fraud of not reporting animals suspected of suffering from ASF. To the scientific community, research should focus on finding an endogenous alternative in the treatment of the disease, given the lack of vaccine and treatment to eradicate the disease.

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Conflict of interest

The authors declare that they have no conflict of interest

Authors' contribution

OOFH, DTJ, and GM designed the study. OOFH, KK, and DTJ did the actual works. OOFH, KK, and DTJ drafted the manuscript. FS, YAKI, AA, and GM critically checked and improved the manuscript. All the authors read the manuscript and approved the final version for publication.

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