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Use of conventional PCR for the detection of the African Swine Fever virus in apparently healthy traditional and semi-modern pig farms in Chad

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Abstract

Objective: The objective of this work is to detect the ASF virus genome in the carcasses and viscera of apparently healthy pigs slaughtered at different slaughter sites in Chad.

Material and method: The study was conducted over a period of 12 months from September 2021 to September 2022 in the provinces of Chari Baguirmi, Mayo Kébbi East and N'Djamena. It began with two censuses. A first census among 492 farmers who had already recorded ASF and then a second census on 26 slaughter areas in the study area. A total of 50 organs were collected and analyzed by the conventional PCR technique for the detection of viral DNA. The data from the interviews as well as the molecular biology results were analyzed with the R Studio software.

Results: The results of this study revealed a prevalence viral which varies from 13.33% to 52.17% from one province to another. In cities and sub-prefectures, the prevalence was between 22.22% to 57.14% and 16.66% to 40% varied from one city to another. The prevalence in the arrondissements was between 38.46% to 70% as well as in the cantons and it was from 0% to 50%. The study also revealed that variations in the detection of ASF virus DNA are linked to types of breeding, age groups of pigs, gender of animals, breed, general and physiological condition of animals before slaughter. The prevalence of viral genome detection in organs ranged from 25.19% to 50.19%

Conclusion: This work has shown that the ASF virus replicates quietly in some pig farms in Chad, despite the absence of apparent clinical signs. The presence of this virus in farms is maintained by the inadequacy of biosecurity measures and poor farming practices, but above all the lack of a slaughter area dedicated to this sector.

Keywords: Prevalence; African Swine Fever virus; Conventional PCR; Pig farming; Traditional and semi-modern; Chad.

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1 Introduction

African Swine Fever (ASF) is a very contagious infectious disease classified in the list A of World Organisation for Animal Health (WOAH) diseases. It is caused by a DNA virus, Icosaedrical symmetry, wrapped, the size of which is around 200 nanometers. The ASF virus comes from the *Asfarviridae* family in which it is the only representative and included in the new of the genus *asfivirus*. This virus constitutes an obstacle to the economic development of the pig sector in many regions of the globe (FAO, 2010; Carrascosa and al., 1984). The disease is endemic in most African countries. Recent declarations came from East and West Africa: Uganda in 2012 and then in 2013, in the Kilimandjaro region of Tanzania in 2013 and in the state of Taraba in Nigeria in 2013 (Atuhaire and al. 2013; Abwage and al., 2015; Swai and Lyimo, 2014). It has been reported in East and Southern Africa, thus on the island of Madagascar where the ASF virus is recognized to have been present in warthog, *hylocherus* and wild boars for a very long time. In Central Africa, there were incursions in Sao Tomé and Principe in 1979, in the South Kivu province in the Democratic Republic of Congo in 2016 and in Cameroon in 2018 were officially reported (Accra, 2017; Ravaomanana, 2011; Bisimwa and al., 2020; Ngu-Ngwa and al., 2020; Thomson, 1980).

In Chad, the ASF was reported for the first time in October 2010 and receiving in less than four months, four provinces, namely the Mayo-Kébbi East, Mayo Kébbi West, Tandjilé, Western Logone and Chari Baguirmi (Ban-Bo and al., 2012). In 2012, a first home for the ASF was reappeared among the warthog in the province of Mandoul and Batha (DGSV, 2018a). In 2018, the ASF was once again reporting in the capital N'Djamena and touching, the first arrondissement, seventh and ninth arrondissement with a mortality rate of 89.72% (DGSV, 2018b; Ban-Bo and al., 2012; Bidjeh and al., 2015).

The virological diagnosis of ASF is based on molecular biology techniques called Polymerase Chain Reaction (PCR). The PCR is a highly sensitive and specific technique that confirms the presence of the virus by amplification of viral DNA present in samples. The PCR technique uses primers corresponding to a highly preserved region of the genome in order to detect a wide variety of known isolates from the ASF virus, including the blood and non-hemadsorbent strains. This technique is currently used by several reference laboratories in order to make an effective virological diagnosis that can confirm the presence of the ASF virus. It can be used both on fabric samples and serum samples taken from animals with clinical signs of the disease, as it prolongs the viremic. The PCR can therefore be used to detect the presence of the virus in the blood from the second day of the infection and up to several weeks. This test is also used to analyze poorly preserved samples where the virus isolation would be impossible. This is a very fast, high specificity and sensitivity but expensive test in terms of equipment (Wilkinson, 1996; Sánchez-Vizcaíno, 2010; Gonzague and al., 2001).

Deception failures, mismanagement of diseases and the lack of pig slaughter areas as well as bad farm practices would be the first factors influencing the circulation of the ASF virus in the various provinces and cities of Chad and its persistence in households. The purpose of this work is to use the conventional PCR to detect the genome of the ASF virus in the apparently healthy carcasses and viscera shot in the various slaughter sites in Chad in order to sequence the genome of the different circulating viral strains in Chad in order to eradicate it.

2 Material and method

2.1 . Study zones

The study was carried out in four areas namely:

The Rigaza sub-prefecture, located 50 km from the city of Bongor. The geographic coordinates of this area carried out by Global System Position (GPS) are: 10 ° 91'604 '' of the Northern attitude and 15 °19'604 '' of East-West.

The city of Bongor and its surroundings, chief town of the province of Mayo Kébbi East, located 240 km from N'Djamena, capital of Chad. The geographical coordinates made by GPS: are 10 °16'29 '' of Northern latitude and 15 ° 22'39 '' East longitude.

The city of N'Djamena whose geographic coordinates made by GPS are: 12°6'47 '' of Northern latitude and 15 ° 2'57 '' East longitude.

The cities of Mandalia and Mailao, respectively prefecture and sub-prefecture of the province of Chari Baguirmi. The geographic coordinates of this area produced by GPS are: 11 ° 43'37 '' of Northern latitude and 15 ° 14'52 '' longitude is

for the prefecture of Mandalia; 8°31'0 '' from Northern latitude and 15 ° 46'0 '' Longitude is for the sub-prefecture of Mailao.

These four areas correspond to porcine farming areas, the most important in Chad, and are often struck by strong waves of the ASF.

2.2 Sampling

The sampling was done voluntarily. He started with two censuses. A first census from 492 farmers who had already recorded the ASF. Then a second census on 26 slaughtering areas of the study area was carried out. Not having an official fellowship area for pigs, the bodies were collected in the slaughtering areas of certain districts and others on weekly markets or a large number of pigs were shot down on this occasion. The bodies withdrawn were, the ganglia, lungs, hearts, livestock kidneys and rats on pigs aged 06 months to 48 months according to the slaughtering areas of weekly districts and markets. The quantity of slaughtered pigs depends on attendance and consumption in porcine meat.

2.3 Method of collecting organs and epidemiological data

The collection of epidemiological data and bodies were carried out from September 2021 to September 2022. The team was on the slaughtering areas of the weekly districts and markets at 5:30 am to collect and record epidemiological information relating to slaughtered pigs. These data were: type of breeding, season, race, age and sex, the general condition of animals before slaughter, their physiological statutes before the slaughter, presence or absence of moles ticks on the animal, farming system and geographic information (provinces, departments, sub-prefectures, cities, cantons and arrondissements). The slaughter of animals begins at 6:30 am and ends at 8:30 am. The team inspected animals slaughtered in the morning.

Organs such as lungs, liver, kidneys, spleen, heart and precurals ganglia, prescapillary ganglia are taken.

Being the target organs of the ASF virus, the kidneys and rats are systematically taken. In the event of reluctance or opposition by certain butchers and collectors, the organs are purchased at a variable price between 750 to 1500 CFA FRANCs with the latter.

2.4 The technique of organs of the organs

The organs taken are deposited in the waterproof and labeled Zip® sachets and then deposited in a cooler containing Ice-Pack, then transported to the Virology Laboratory at IRED. Once arrived in the laboratory, the organs were cut into pieces using a scalpel and the pieces are placed in stomachers sachets and there are 16ml of distilled water passed in the autoclave then the pieces of organ mixed with distilled water are crushed in a device called stomacher for two minutes. The juice thus obtained is centrifuged 15.000rpm/Min and minutes for 10 minutes. The supernatant is taken to be save at -20°C for subsequent use.

2.5 Conventional PCR protocol

The viral genome was highlighted using a PCR test according to the protocol recommended by WOAH (WOAH, 2012). The viral DNA extraction in organ grinding (in organ juice) was carried out with Qiaamp® DNA Mini Kit, marketed by Qiaigen. The extraction technique used is that recommended WOAH (WOAH, 2012). For amplification, the Aguero and al., (2004) protocol was used. Primer ASF-1 Sense (20µm): 5'-AGT-TAT-GGG-AAA-CCC-GAC-CC-3' and Primer Antisens ASF-2 (20µm): 5'-CCC-TGA-ATC-GGA-GCA-TCC-3' and Tag Gold DNA Polymerase (Applied Biosystem Ref.N °: N: 808-0245).

Take: 17.375µl of H_2O PCR, 2.5µl of buffer 10X II, 2µl of Cl_2Mg (25mM), 0.5µl of dNTP's (10mM), 0.25µl of ASF-1 (20µl), 0.25µl of ASF-2 (20µl), 0.125 µl of Amplitag Taq Gold (5U/µl). Place all the tubes in the thermocycler and program the polymerization in three stages: a cycle of 10 minutes at +95°C, comes the denaturing stage of 40 cycles at 95°C for 15 seconds, followed 30 seconds of hybridization of primers at +62°C and 30 seconds of elongation at +72°C then the final elongation from 72°C. Amplification products are visualized by Illumination with ultraviolet (UV) radiation after electrophoretic migration in an Agarose gel (Biotium, USA) 1.5% in 1X TAE (amplification of the expected fragment: 257bp) (Aguero and al., 2004).

2.6 Data processing and analysis

The data from the interviews as well as the result of the organs tested, were entered in an Excel spreadsheet of Microsoft Office 2007 then converted to CSV then exported to the R Studio Software Version 4.0.4.2021 for the analyzes. Regarding

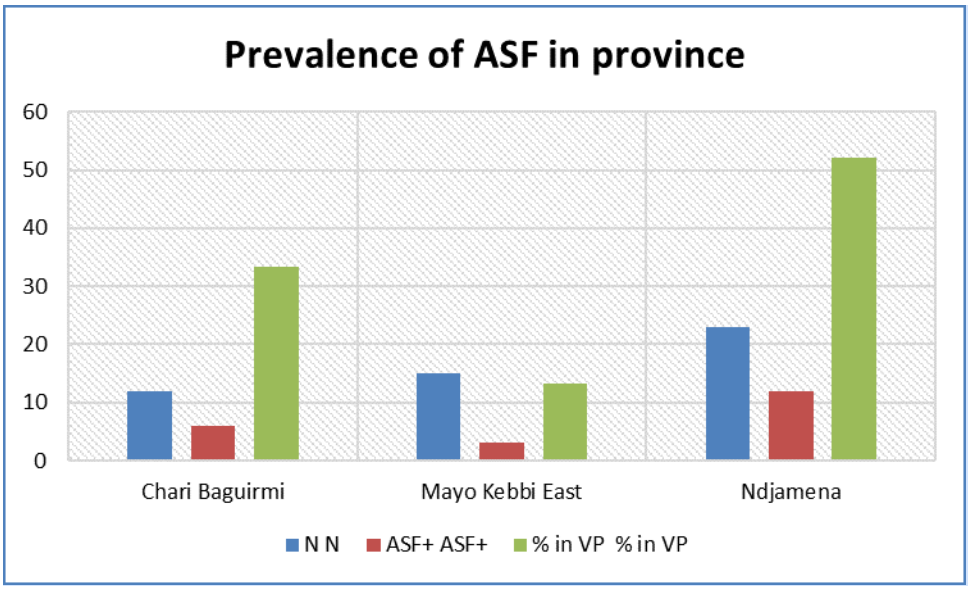
analytical statistics, the Chi-square test and the Exact Fisher test were used to compare proportions (provinces, prefecture, sub-prefecture cities, neighborhoods and villages) and variations in the prevalence linked to the detection of the viral genome as well as for their significations. The significance threshold was set at 0.05 and the P-Value calculated from the Exact Fisher test.

3 Results

After laboratory analyzes, the results below were obtained:

3.1 The prevalence of ASF virus DNA detection in geographic areas

In the three provinces of study, the prevalence of the ASF virus was significant and varied at the 5% threshold (P-Value= 0.03302) : It was 52.17% in N'Djamena province, 33.33% in Chari Baguirmi and 13.33% in Mayo Kébbi East.



On the x-axis the provinces and on the y-axis the prevalence of the virus in percentage.

Figure 1 Prevalence of ASF virus in provinces

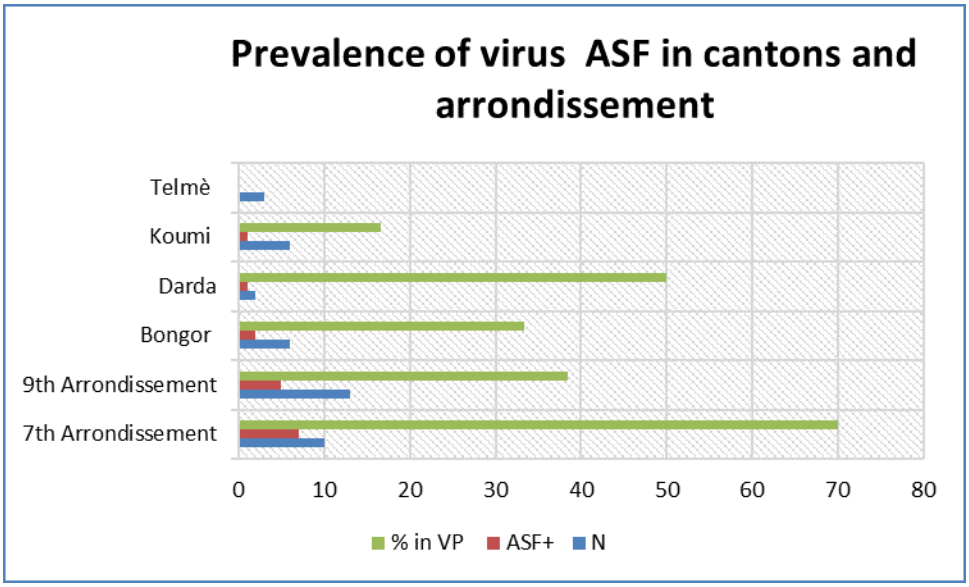
The prevalence of the ASF virus in cities and sub-prefecture was 57.14%, 52% ; 40%, 22 ; 22% and 16.66% respectively in the cities of Mandalia, N'Djamena, Mailao, Bongor and Rigaza. This prevalence was significant at the rate of 5% (P-Value = 0.03774)

Table 1 Prevalence of ASF virus detection in cities and sub-prefectures

Sub-prefecture and town	N	ASF+	% in VP	CI at 95%	P-value	Interpretation
Bongor	09	02	22.22	[18.30 ; 26.14]	0,03774	Significant
Mandalia	07	04	57.14	[40.51 ; 73.77]		
Mailao	05	02	40	[30.34 ; 49.66]		
N'Djamena	23	12	52	[37.59 ; 66.41]		
Rigaza	06	01	16.66	[14.16 ; 19.16]		

Comment : N = number of organs tested; ASF+= Organs Tested positive for ASF virus; VP = prevalence rate of DNA detection of ASF virus in the organs; CI = confidence interval.

The prevalence detections of the genome of ASF virus in the districts and cantons are highly significant at the rate of 5% (P-Value=0.00000078779). In the districts or districts, they are 70% in the 7th districts and 38.46% in the 9th districts, then 50% in Darda, 33.33% in the Canton Bongor and 16.66% in the Canton Koumi.



Comment: P-value 7.879= 0.000007879. On the x-axis the viral prevalence in percentage and on the y-axis the different arrondissement and cantons.

Figure 2 Prevalence of ASF virus in cantons and arrondissements

3.2 Variations in the prevalence linked to the detection of the viral genome of the ASF

In Table 2, the prevalence of the ASF virus were linked certain variations:

The prevalence of ASF viruses had exceeded 50% in the rainy season and 35.89% in the dry season. This result is statistically non-significant at the rate of 5% (P-Value = 0.06126).

The prevalence was 42.51% in farming of traditional and significant types (95% CI = [31.90;53.12] with a P-Value = 0.05 which significant at the 5% threshold).

The prevalence according to the age groups was heterogeneous. The age group of 6-12 months had a prevalence of 46.15%than those of 13-18 months (40.15%) and ≥ 19 months (29.36%). These prevalence are significant at the 5% threshold (P-Value = 0.01668).

Boars had a prevalence of 41.52% and sows 33.33%. This two prevalence were highly significant at the 5% threshold (P-Value = 0.00216).

Table 2 Variations related to the seasons type of breeding ages and sex of the slaughtered animals

Variations		N	ASF+	% in VP	CI at 95%	P-value	Interpretation
Season	Dry	39	14	35.89	[27.70 ; 44.08]	0.06126	Not significant
	Rainy	11	06	54.54	[39.04 ; 70.04]		
Type of farming	traditional	40	17	42.51	[31.90 ; 53.12]	0.05187	Significant
	Semi-modern	10	03	30.10	[24.40 ; 36.36]		
Age	6-12 months	13	06	46.15	[34.13 ; 58.17]	0.01668	Significant
	13 -18 months	30	12	40.15	[30.43 ; 49.87]		
	≥ 19 months	07	02	29.36	[23.34 ; 35.37]		
Sex	Sow	09	03	33.33	[26.02 ; 40.64]	0.00216	Very significant
	Boars	41	17	41.52	[31.28 ; 51.76]		

The local breed had a prevalence of 40.49% and the mixed breed of 33.33%. These results are statistically non-significant at the 5% threshold (P-Value = 0.126).

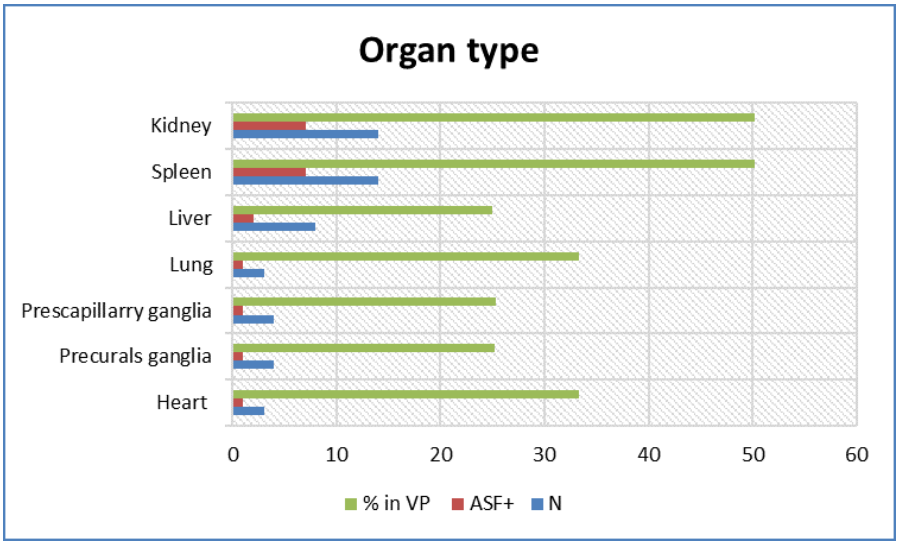
The physiological state of animals before slaughter had a relatively very variable prevalence. The strongest prevalence is 69.04% with 95% CI [46.88; 91.20]. In whole and weakest boars of 32.25% with 95% CI = [22.29; 42.21]. In empty sows. This prevalence was highly significant at the rate of 5% (P-Value = 0.00616).

The general condition of the animals has a prevalence of the virological of 50%, 42.85% and 33.33% respectively in animals with petechiaes and dejection, anorexic and asthenic and at the apparently healthy end which are highly significant at the rate of 5% (P-Value = 0.00160).

Table 3 Variations related to the race, the physiological state and the general condition of the slaughtered animals

Variations		N	ASF+	% in VP	CI at 95%	P-value	Interpretation
Breed	Local	41	41.46	40.49	[30.64 ; 50.34]	0.12610	Not significant
	Mixed	09	03	33.33	[26.02 ; 40.64]		
Physiological state	Whole boars	10	07	69.04	[46.88 ; 91.20]	0.00616	Very significant
	Castrated boars	31	20	32.25	[22.29 ; 42.21]		
	Empty sows	06	01	17.12	[14.50 ; 19.74]		
	Pregnant sows	03	02	67.06	[-45.85 ; 88.26]		
General condition	Well	27	09	33.33	[26.02 ; 40.64]	0.00160	Very significant
	Anorexic and asthenic	07	03	42.85	[32.11 ; 53.59]		
	Petechiae and dejection	16	08	50	[36.42 ; 63.58]		

The organs taken and analyzed were the rats, kidneys, livers, lungs, precurals ganglia, precapillary ganglia and in the pre -concrete nodes and hearts. The DNA of the virus was detected with a rate of 50.19% in the kidneys, 33.33% in the lungs and cores lives, 25% in the precurals ganglia, prescapillary ganglia and liver nodes which are highly significant at the 5% threshold (P-Value=0.0066).



Comment : P-value=0,0066. On the x-axis the viral prevalence in percentage and on the y-axis the different types of organs analyzed.

Figure 3 Detection of ASF virus in organs types

4 Discussion

4.1 The prevalence of detection of the ASF virus genome in geographic areas.

It determines the prevalence of the viral genome in the different study areas.

In the three provinces of study, the prevalence of the ASF virus was significant and varied at the 5% threshold (P-Value = 0.03302): it was 52.17% in the province of N'Djamena, 33.33% in Chari Baguirmi and 13.33% in Mayo Kébbi East. This high prevalence could be explained by the establishment of a commercial circuit by collectors and holders of restaurants who buy pigs in the various provinces of Chad and keep these animals in the various sub-prefectures of the province of Chari Baguirmi and then transported to N'Djamena. However, these animals which are discreetly transported with doubtful health statutes and completely escape the control of health agents of the Ministry of Livestock and Animal Productions.

The prevalence of the ASF virus in cities and sub-prefecture was 57.14%, 52%; 40%, 22; 22% and 16.66% respectively in the cities of Mandalia, N'Djamena, Mailao, Bongor and Rigaza. This prevalence was significant at the rate of 5% (P-value = 0.03774). These results show a relatively very high prevalence in N'Djamena and Mandalia. The prevalence increases increasingly each time you approach N'Djamena. This increase is explained by the importance of demand in porcine meat. Farmers and collectors transport the animals generally at night to keep them in Mandalia and then transported them to N'Djamena to sell to restaurant holders mainly located in N'Djamena.

The prevalence of detections of the genome of the ASF virus in the districts and cantons are highly significant at the rate of 5% (P-Value = 0.000007879). In the districts, they reached 70% in the 7th districts and 38.46% in the 9th districts, then 50% in Darda, 33.33% in the Canton Bongor and 16.66% in the Canton Koumi. The very high rate in the 7th districts could be explained by the size of this district. It is one of the largest districts in the city of N'Djamena and is full of a plethoric number of restaurants to which porcine meat is popular with customers. In this districts, most restaurants are located close to drinking flows (Dancing bar, wine cellar and liqueurs as well as drinking club (cabarets). In addition to this, this districts are subject to strong waves of ASF in case of appearance.

4.2 Variations in the prevalence linked to the detection of the viral genome of the ASF

The general condition of the slaughtered pigs was highly significant and variable. A rate of 50% of the slaughtered pigs had petechiaes and excrement, 42.85% were anorexic and asthenic and 33.33% of pigs only were well. These results are higher than those obtained in the DRC by Bisimwa and al., 2020 which detected the presence of DNA in 22.8% of apparently healthy pigs. These results show that the ASF virus is endemic in certain areas of Chad. Just by the fact that it is even detected in the carcasses of slaughtered animals with no symptoms of the disease.

The organs taken and analyzed were the rats, kidneys, livers, lungs, precapillary ganglia and in the precurals ganglia and hearts. The DNA of the ASF virus was detected with a prevalence of 50.19% in the rats and kidneys, 33.33% in the lungs and cores, 25% in the precurals ganglia, precapillary ganglia and liver. These results are higher than those obtained in the carcasses of the pigs slaughtered at the slaughterhouse of Ambatondrazaka in the Republic of Madagascar by Randriamparany and al., 2005 (78%) in the kidneys and ganglia. This strong DNA detection of the ASF virus in the organs of animals slaughtered in this study is explained by the fact that in Chad there are not slaughtering areas dedicated to pigs. Pigs are often slaughtered, either in the farmer is courtyard and sold to wholesalers or sometimes to butchers and transported to weekly markets or in most pigs are purchased in weekly and slaughtered markets on site without inspection of the veterinary services. These results show that the ASF virus is replied to low noise in these animals

5 Conclusion

The objective of this work is the use of conventional PCR to the detect the genome of the ASF virus in the carcasses and viscera of apparenttly healthy pigs slaughtered in the different slaughter areas in Chad. Overall, the prevalence in this study is 40%. This prevalence of the ASF virus varies from 13.33% to 52.17% from province to another. In cities and sub-prefecture, the prevalence varied from 16.66% to 57.14% and varied from one city to another and in the districts prevalence varies from 38.46% to 70% as well as in the cantons, they varies from 16.66% to 50%. Varaiations in the DNA detection of the ASF virus were related to the types of breeding, age groups, the sex of the animals slaughtered, the breed, general status and physiological of the animals before slaughter. The prevalence of the detection of the viral genome in the organs varies from 25% to 50.19%. This DNA detection of the ASF virus in the organs of apparently

healthy slaughtered pigs shows a low noise replication of the virus or a residual replication of the ASF virus in some pig farms in the Chad that apparently have the era are healthy.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

The all authors declare that they have no conflicts of interest related to this study.

Statement of authors contributions

NKA contributed to the design of the protocol, to the writing of the first draft of the manuscript, BBA, NBNR, and RLG in the development of the study protocol and its critical review. AAB, AAI, AD, BBA, DJS, MH, MFA, RLG, NBNR, SL and TS supervised data collection and sample sorting. NBNR and DJS supervised the laboratory analyses. NKA and RLG performed the statistical analyzes of the data. All authors proceeded to the proofreading, correction and validation of the manuscript.

References

[1] Carrascosa JL, Carazo JM and Carrascosa AL,. General morphology and capsid fine structure of African Swine Fever virus particles. *Virology*. 1984 (132): 160-172.

[2] Atuhaire, D.K., Afayoa, M., Ochwo, S., Mwesigwa, S., Mwiine, F.N., Okuni, J.B., Mukani, W.O. and Ojok, LM, . Prevalence of African Swine Fever virus in apparently healthy domestic pigs in Uganda. *BMC Vet. Res*. 2013 (9), 263. https://doi.org/10.1186/1746-6148-9-263.

[3] Abwage SA, Umaru GA, Musa YB, Adamu Z, Akensire UA, Njobdi AB & Bello OA. Detection of African Swine Fever virus (ASFV) antibodies in pigs in Taraba state, north east Nigeria. *Sokoto Journal of Veterinary Sciences,* 2015 13 (2): (P-ISSN 1595-093X/E-ISSN 2315-6201).

[4] Swai, E. S., & Lyimo, C. J. Impact of African Swine Fever epidemics in smallholder pig production units in Rombo district of Kilimanjaro, Tanzania. Livestock Research for Rural Development, 2014 26(2), Article #32. Retrieved from http://www.lrrd.org/lrrd/26/2/SWAI26035.html

[5] Accra. Regional strategy for the control of African Swine Fever in Africa. 2017 56p. ISBN 978-92-5-209384-8 (FAO).

[6] Food and Agriculture Organization of the United Nations/FAO. The State Capacities of Animal Genetic Resources Management, Rome. *Manuel Production et Santé Animale FAO* .2010.

[7] Ravaomanana J. African Swine Fever in Madagascar: assessment of the potential existence of wild compartments and their epidemiological impacts. Antananarivo University/Madagascar. Thesis in SVT. 2011 24-70, 251p.

[8] Bisimwa NP, Eunice MM, Dedan G, Gedeon B, Joshua OA, Juliette RO, Charles M, Richard PB, Lucilla S, Appolinaire D and Roger P,. Evidence for the presence of African Swine Fever virus in apparently healthy pigs in South-Kivu Province of the Democratic Republic of Congo *Veterinary Microbiology.*2020 (240):108521

[9] Ngu Ngwa V, Abouna A, Pagnah ZA and Anna-Rita A, 2020. Epidemiology of African Swine Fever in Piggeries in the Center, South and South-West of Cameroon *Vet. Sci.* 2020 (7), 123.

[10] Thomson GR, Gainaru MD and Van Dellen AF,. Experimental infection of warthog (*Phacocherus aethiopicus*) with African Swine Fever virus. Onderstepoort. *J Vet Res*, .1980 (47): 19-22.

[11] Ban-bo BA, Idriss OA and Squarzoni CD. Control of African Swine Fever (ASF) in traditional pig farms in Chad star_border. *Journal of Animal &Plant Sciences*, .2012 15(3): 2261-2266.

[12] Directorate General of Veterinary Services (DGSV). Provisional report on the epidemiological situation of African Swine Fever in the commune of N'Djamena, *Field activity report*, Chad, 2018a 10p.

[13] Directorate General of Veterinary Services (DGSV). Information on African Swine Fever (ASF) in Chad, *Account information*, Chad, 2018b 3p.

[14] Bidjeh K, Ban-Bo BA and Mopaté LY. Factors contributing to the introduction and the spread of African Swine Fever Virus in Chad. *IJCMAS*.20154(8): 607-613.

[15] Wilkinson P J. Epidemiology of African Swine Fever. *Rev. Sci. Tech. Off. Int. Epiz.* 1986 (5): 487–493.

[16] Sánchez-Vizcaíno JM. Early detection and contingency plans for African Swine Fever. *Conf. OIE* 2010, 149-158

[17] Gonzague, M., Roger, F., Bastos, A., Burger, C., Randriamparany, T., Smondack, S and Cruciere, C. Isolation of a no-haemadsorbing, no-cytopathic strain of African Swine Fever virus in Madagascar. *Epidemiol. Infect*. 2001 (126): 453–459.

[18] World Organisation for Animal Health/WOAH, Section 2.8.1. African Swine Fever. In: Manual of diagnostic tests and vaccines for terrestrial animals. Paris, France, *World Organisation for Animal Health*. 7th Edition.2012 http://www.oie.int/eng/normes/MMANUAL/A_00035.htm

[19] Agüero, M., Fernández, J., Romero, L.J., Zamora, M.J., Sánchez, C., Belák, S., Arias, M. and Sánchez- Vizcaíno, J.M. A highly sensitive and specific gel-based multiplex RT-PCR assay for the simultaneous and differential diagnosis of African Swine Fever and Classical swine fever in clinical samples. *Vet. Res.* 2004 (35) : 551–563.

[20] Randriamparany, T., Grenier A., Tourette T., Maharavo Rahantamalala C., Rousset D. and Lancelot R. Epidemiological situation of African Swine Fever in the Lake Alaotra region (Madagascar) and possible consequences for the organization of control and surveillance. *Revue Élev. Méd. vét. Pays trop.* 2005 58 (1-2) : 15-20.