Microbiology Research International
Vol. 10(1), pp. 1-9, January 2022
DOI: 10.30918/MRI.101.21.021
ISSN: 2354-2128
Full Length Research Paper

Bacteria pathogens of the edible frog *Hoplobatrachus occipitalis* (Gunther, 1858) in the hydrographic basins of Benin

L. Codjo[1,2], E. Y. Attakpa[1,2,3]*, C. K. Boko[4], S. S. Fayalo[1,2], R. Tohozin[3], E.O.R Pelebe[2] and I. Imorou Toko[2]

[1]Laboratory of Ecology, Health and Animal Production (LESPA), Faculty of Agronomy, University of Parakou, Benin.
[2]Aquaculture and Aquatic Ecotoxicology Research Laboratory (LaRAEAq), Faculty of Agronomy, University of Parakou, Benin.
[3]Veterinary Diagnostic and Serosurveillance Laboratory (LADISERO) of Parakou, Benin.
[4]Communicable Disease Research Unit, Department of Animal Production and Health, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Benin.

Accepted 19 January, 2022

ABSTRACT

The consumption of the meat of certain animals from the natural environment with an unknown microbiological status exposes humans to more serious zoonoses caused by feared pathogens**.** The present study was carried out to identify the pathogenic microbiological flora of the edible frog, *Hoplobatrachus occipitalis*, used as a source of animal protein by several Beninese families, to assess the health quality of this meat. According to ISO 6887-2, the isolation and identification of bacterial species were carried out on a population of 135 specimens of *H. occipitalis* fished in three hydrographic basins of Benin (Niger, Mono and Ouémé basins). Their analysis revealed a positivity rate of 88.15%. Infection rates varied significantly ($p < 0.05$) from one basin to another, i.e. 77.78, 88.89 and 97.78% respectively in Ouémé, Niger and Mono. The respective proportions of the bacteria identified were: *Aeromonas hydrophila* (8.15%), *Bacillus anthracis* (1.48%), *Escherichia coli* (3.70%), *Enterobacter* sp. (21.48%), *Klebsiella* sp. (4.44%), *Proteus* sp. (6.67%), *Pseudomonas* sp. (11.85%), *Neisseria* sp. (2.22%), *Salmonella* sp. (0.74%), *Shigella* sp. (11.11%), *Staphylococcus* sp. (16.30%). Although the infection rate was higher in the Mono basin, no formidable species were observed in this basin. However, germs such as *E. coli* were observed in the Ouémé basin, and *E. coli*, *Salmonella* sp., and *Bacillus anthracis* in the populations of the Niger basin. These results showed that the safe consumption of the meat of this edible frog from the natural environment raises a real public health issue given the ever-increasing demand for this meat by consumers. To encourage the consumption of this meat, it is therefore urgent to promote the breeding of this frog in aquaculture operations close to the areas of consumption. The sensitization of potential consumers could limit the risks of food poisoning incurred especially in the event of poor cooking.

Keywords: Frog, *Hoplobatrachus occipitalis*, pathogenic germs, meat, Benin.

*Corresponding author. E-mail:attakpayae@yahoo.fr. Tel: (+229) 97 68 06 35.

INTRODUCTION

Human consumption of frog meat dates back centuries (Negroni and Farina, 1993). Frogs play an active role in feeding African populations (Onadeko et al., 2011). Like other meats, amphibians are an excellent source of dietary protein for consumers (Onadeko et al., 2011; Douglas and Amuzie, 2017). In dried or fried form, this meat is consumed in Benin, Nigeria, Burkina Faso and in many West African countries (Mohneke et al., 2010). According to these authors, the frog trade generates significant income for rural communities' subsistence.

According to Godome et al. (2018), Tohe et al. (2014), Mohneke et al. (2009), *Hoplobatrachus occipitalis* represents the most widespread frog species in West Africa and the most popular with consumers. However, amphibian populations are under threat from viral, bacterial, fungal and parasitic infectious diseases leading to their decline in several regions of the world (Culp et al., 2007). The orientation of research work towards the horizon of the determination of pathogenic bacteria to fight in *H. occipitalis* would be welcome for the intensification of the production of this meat and the protection of the health of consumers.

In the West African sub-region, research work has been carried out to determine the microbiological quality of *Hoplobatrachus occipitalis* (Douglas and Amuzie, 2017) as meat in Nigeria, but this work has not made it possible to know the microbial quality of living frogs originating from the natural environment in comparison with that raised in a controlled or semi-controlled system. This study provides valuable foundations for a new direction in raniculture of *Hoplobatrachus occipitalis* in the sub-region and particularly in the Republic of Benin.

MATERIALS AND METHODS

Study environment

The present study was carried out in three selected hydrographic basins in Benin (Figure 1): The basin of Niger located in the North, those of Mono and Ouémé in the South of the country. For the border basin of Niger, the samples were taken in the wetlands of the commune of Karimama, villages of Bello-Tounga (12°03'35.0" N and 003°11'54.0" E), from Bomi-Tounga (12°07'12.0" N and 003°08'51.0" E), from South-Touga (12°08'34.6" N and 003°07'14.1" E) and Goungoun Béri (12°10'15.7" N and 003°05'23.6" E); in Ouémé basin, they were carried out in the towns of Dangbo (Kessounou: 06°34'21.1" N and 002°31'16.4" E), Adjohoun (Agonli Lowe: 06°39'21.5" N and 002°28'28.6" E and Akpadanou: 06°45'54.8" N and 002°28'19.7" E), while the villages of Agbobada (06°33'46.1" N and 001°40'58.6" E), Athiéme Center (06°34'52.0" N and 001°39'45.2" E) in the town of Athiéme and that of Ouèdèmè (06°42'04.3" N and 001°41'12.8" E) in the commune of Lokossa served as sampling sites for the Mono Basin.

Animal material

The animal material consisted of a sample of 135 species of *H. occipitalis* collected in the hydrographic basins of Niger, Ouémé and Mono in Benin at the rate of 45 frogs per basin. The average weight of the subjects used was 49.812 ± 17.036 g for an average length of 8.582 ± 0.865 cm; 143.962 ± 31.448 g for a length of 11.338 ± 0.836 cm and 87.900 ± 20.457 g for an average length of 10.515 ± 0.760 cm respectively in the Niger, Ouémé and Mono basins. This frog is very common in West Africa, and it belongs to the order of Anurans and the family of Dicroglossidae. It is found in most of the hydrographic basins of Benin and feeds mainly on insects, molluscs and plant debris (Codjo et al., 2021).

Sample collection and organ retrieval

A total of 135 swab samples were taken from the frogs *H. occipitalis* in all of the three basins used for this study. A population of 45 specimens was collected in each of these three basins, regardless of sex and age. Identification of specimens of *H. occipitalis* was performed using description keys established by Rödel (2000), Rödel and Branch (2002), and Rödel et al. (2005). As soon as they were identified, the subjects were caught and placed in sterile jars then transferred to the laboratory for bacteriological analyses.

Swabbing and bacteriological analyses were carried out at the Veterinary Diagnostic and Serosurveillance Laboratory (LADISERO) of Parakou (Benin). The frogs were systematically anesthetized, then sacrificed by euthanasia. Organs removed for bacteriological analysis were skin, muscle, intestine and ulcerated snouts.

The sample identified was placed in sterile labeled aliquots containing 1 ml of distilled water and then kept at 4°C, until the moment of its treatment. All these protocols took place under a luminous flux hood, followed by the disinfection of all the materials (scissors, tweezers, etc.) with bleach and ethanol then the change of gloves and craps paper used.

Isolation and identification of pathogenic bacteria in *Hoplobatrachus occipitalis*

The organs removed, isolation and identification of bacteria in aquatic products were treated in accordance with ISO 6887-2 (ISO 2004):

Culture of bacteria

Culture of bacteria from organs was performed on Trypticasein Soy Agar (ISO 9308-1), Salmonella-Shigella and Nutrient Agar (plain or sheep blood 5%). The preparation of the culture media used was carried out in accordance with the dosages prescribed by the manufacturing laboratories. Each aliquot sample was grown in a separate, labeled petri dish. After inoculation of the culture media in "streak movements" on the agar, the culture of the bacteria was continued in an oven at 37°C for 24 to 72 h, after which the grown colonies are observed and described.

Isolated pure colonies were obtained after subculturing on nutrient agar for the identification of bacteria in each organ removed.

Gram's stain

The Gram stain is processed to know the morphological form of the isolated bacterium (rodshaped bacilli; Cocci of rounded shape; etc.) as well as the Gram-positive (purple) or Gram-negative (pinkish) type of said colony. It was performed according to the methodology described by Lachal and Bouchkima (2020), using the Gram-Nicolle Kit from the RAL Diagnostics laboratory. The bacterial colony was diluted and fixed, in distilled water on a slide. Carbolic Gentian Violet, stabilized Lugol, acetone and Ziehl's Fuchsin were successively poured onto the smears and rinsed with distilled water in accordance with the recommended latent time. The observation was carried out under a light microscope (objective 100 X).

Biochemical tests performed

Catalase and Oxidase were two biochemical tests performed on the basis of pure colonies. The bacterial colony was diluted in hydrogen peroxide and produced an effervescence reaction in the case of a positive catalase (Marchal et al., 1982). For the oxidase test, the colony produces a purple color on contact with an oxidase disc (soaked in distilled water) when the oxidase is positive (Flandrois

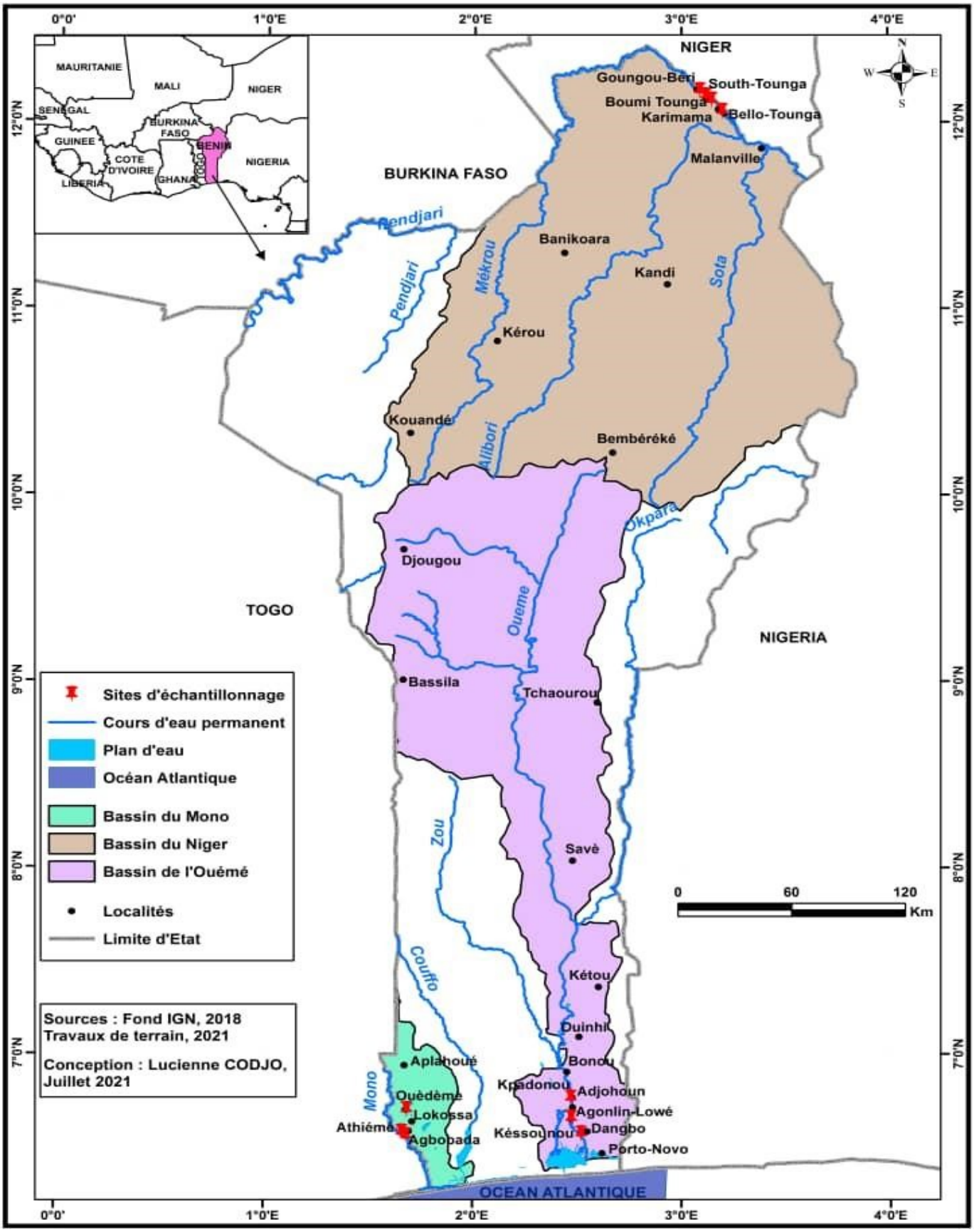

Figure 1. Sampling sites *H. occipitalis* in the three watersheds.

and Chomarat, 1988).

The identification gallery was used to determine the complementary biochemical properties of the bacteria to be identified: culture media such as Simone Citrate (ISO 10273), Mannitol, urea, Triple Sugar Iron Agar, Peptone water and Kovacs reagent from CONDA SA laboratory were prepared and used during the identification of bacteria. The methods described by Marchal et al. (1982), Frenry and Renard (2007) and Lanotte et al. (2016) have been used in the realization of bacterial identification galleries, with some modifications. Their preparations were made in accordance with the respective dosages and poured into macro-galleries then labeled. Seeding was carried out on the basis of the pure colony for incubation at 37°C. for 24 h followed by reading the reactions.

Calculated parameters

The parameters calculated in this study were: the infection rates of the biological materials sampled, the frequencies of the types of bacteria (according to the Gram stain and the shape), the frequencies of the frogs infected by sampling sites, the frequencies of the bacteria isolated by sampling site and throughout the study area, the positivity rates by organ type and by basin.

The frequency (F) of infested individuals was calculated by sampling site and the whole of the entire study area. This frequency was calculated through the percentage of the number of infected individuals compared to the number of the appropriate population (45 or 135 depending on whether it is respectively a sampling site or the whole of the study area).

$$F = \frac{Number\ of\ frogs\ hosting\ at\ least\ one\ bacterium}{Total\ number\ of\ the\ population\ concerned} \times 100$$

Frequencies (F_i) bacteria infection were also calculated for each of the bacteria isolated. The calculation formula was as follows:

$$F_i = \frac{Number\ of\ frogs\ hosting\ one\ bacterium\ i}{Total\ number\ of\ the\ population\ concerned} \times 100$$

Statistical analyses

The Excel spreadsheet (Office 2013) was used to calculate the descriptive statistics (frequencies). Pearson's Chi-square test was applied to measure the link between the different qualitative variables of the populations studied. The analysis of variance test (Anova 1) was performed to compare the positivity rates by organ type and by basin. All statistical analyses were performed using IBM SPSS Statistics 21 software, and the differences observed were compared at a 5% significance level.

RESULTS

Infection rate of biological materials collected

From the 135 samples collected and analysed, 119 samples were positive: with a positivity rate of 88.15%. Infectious status increases from bowel samples (83.33%) to ulcer swabs (95.65%), which are relatively the most infected (Table 1)

The infectious status did not vary significantly depending on the type of sample analysed in *H. occipitalis* ($p > 0.05$).

Table 1. Infectious status of the organs tested.

Sample	Number specimens	Infection rate (%)	
		Negative	Positive
Intestine	42	16.67	83.33
Muscular	37	13.51	86.49
Skin	33	9.09	90.91
Ulcerations	23	4.35	95.65
Total	135	11.85	88.15

Chi-2 = 2.51; dof = 3; p = 0.473.

Types and location of bacteria isolated

Gram-positive staining coccobacilli have not been encountered. Table 2 shows that Gram-negative bacilli are the most abundant in terms of microbiological diversity. The bacteria isolated from the species were: *Aeromonas hydrophila*, *Bacillus anthracis*, *Escherichia coli*, *Enterobacter* sp., *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Neisseria* sp., *Salmonella* sp., *Shigella* sp., *Staphylococcus* sp.

The list of the different genera (or species) of bacteria is presented in Table 2. The list of bacteria isolated from *H. occipitalis* (Table 2) indicates a diversity of bacteria of variable forms ranging from bacilli to coccobacilli.

Table 3 shows the distribution of these bacteria isolated in the different organs sampled. The bacteria isolated were significantly dependent on the organs analysed ($p=0.00$).

Frequencies of types of bacteria

Gram-negative bacteria were observed in high proportions in the Mono and Ouémé basins (79.55 and 100.00% respectively). Infection with Gram-positive bacteria was relatively high in the Niger basin (37.50%)

Table 2. List of bacteria isolated, by form and Gram stain, in *H. occipitalis*.

Form of bacteria	Gram stain	
	Negative	**Positive**
Bacilli	*Aeromonas hydrophila* *Escherichia coli* *Klebsiella* sp. *Proteus* sp. *Salmonella* sp.	*Bacillus anthracis*
Cocci	*Neisseria* sp.	*Staphyloccocus* sp.
Cocobacilli	*Enterobacter* sp. *Pseudomonas* sp. *Shigella* sp.	None

Table 3. Location of bacteria isolated from *H. occipitalis.*

Bacteria isolated	Organs analyzed			
	Intestine	**Muscular**	**Skin**	**Ulceration**
Aeromonas hydrophila	1 [a]	10 [b]	0 [a]	0 [a]
Bacillus anthracis	0 [a]	1 [a]	0 [a]	1 [a]
Escherichia coli	3 [a]	2 [a]	0 [a]	0 [a]
Enterobacter sp.	4 [a]	3 [a]	10 [b]	12 [b]
Klebsiella sp.	4 [a]	2 [a]	0 [a]	0 [a]
Neisseria sp.	0 [a]	3 [a]	0 [a]	0 [a]
Proteus sp.	2 [a,b]	6 [b]	0 [a]	1 [a, b]
Pseudomonas sp.	13 [a]	2 [b]	0 [b]	1 [b]
Salmonella sp.	1 [a]	0 [a]	0 [a]	0 [a]
Shigella sp.	7 [a]	1 [b]	4 [a, b]	3 [a, b]
Staphyloccocuss sp.	0 [a]	2 [a, b]	16 [c]	4 [b]
Absence of bacteria	7 [a]	5 [a]	3 [a]	1 [a]

Chi-2 = 124.529; dof = 33; p = 0.000
Values with the same letters in a column do not differ significantly at the 5% threshold.

but absent in the Ouémé basin. Thus, 79.83% of infected subjects are carriers of Gram-negative bacteria on analysis against 20.17% of Gram-positive (Table 4).

The frequencies of bacteria (according to the Gram stain) vary significantly from one pond to another ($p < 0.05$).

All the bacteria isolated are morphologically divided into three groups: bacilli, cocci and coccobacilli. The proportions of groups of bacteria (depending on their shape) vary significantly from one pond to another ($p < 0.05$). Frogs in the Ouémé basin were mainly infected with bacilli while the three bacterial forms appear more or less uniformly in the Niger basin. Coccobacilli were the most abundant in the Mono Basin. The bacilli were the most abundant in terms of frequencies in the infected population (43.70%) in all three basins (Table 4).

Epidemiological nature of the study

Frequencies of infection by sampling site

By sampling site, the infection rates were 77.78, 88.89 and 97.78% respectively for samples from the Ouémé, Niger and Mono basins. These values differ significantly from each other ($p = 0.013$). Infection of frogs, therefore, varies from one agroecological zone to another (Table 5).

Types of bacteria isolated by sampling sites

The bacterial flora isolated in frogs from the Niger basin is composed of nine genera of bacteria; while the populations originating from the Ouémé and Niger basins

Table 4. Bacteria frequencies according to Gram stain by sampling site in the population of *H. occipitalis* infected.

Sampling site	Gram bacteria (%)		Bacterial form (%)		
	(-)	(+)	Bacilli	Cocci	Coccobacilli
Mono basin	79.55[a]	20.45[a]	9.09[a]	27.27[b]	63.64[b]
Niger Basin	62.50[a]	37.50[b]	35.00[a]	32.50[b]	32.50[a,b]
Oueme basin	100.00[a]	0.00[b]	97.14[a]	0.00[b]	2.86[b]
Basins set	79.83	20.17	43.70	21.01	35.29
	Chi-2 = 16.307; *dof* = 2; *p* = 0.000		*Chi-2* = 66.856; *dof* = 4; *p* = 0.000		

Table 5. Frequencies of infected subjects per pelvis.

Basins	N	NP	FP (%)
Niger	45	40	88.89
Oueme	45	35	77.78
Mono	45	44	97.78
Total	135	119	88.15

N = Total workforce withdrawn; NP = Number of positive; FP = Frequency of infected.

presented only five and four genera of bacteria respectively (Figure 2). The infection of subjects in the Niger basin was mainly represented by Shigella and Staphylococci (33.33 and 28.89% respectively) while *Aeromonas* sp. and *Pseudomonas* sp. were represented in the Ouémé basin. The *Enterobacter* sp. were isolated in more than half of the subjects caught in the Mono basin (62.22%). The bacteria isolated were specific to the pool of origin of their hosts (p = 0.000).

In all three basins, the *Enterobacter* sp. and *Staphylococcus* sp. were frequently encountered (respectively isolated in 21.48 and 16.30% of the study population) while the *Salmonella* sp. were isolated from a single subject (Figure 3).

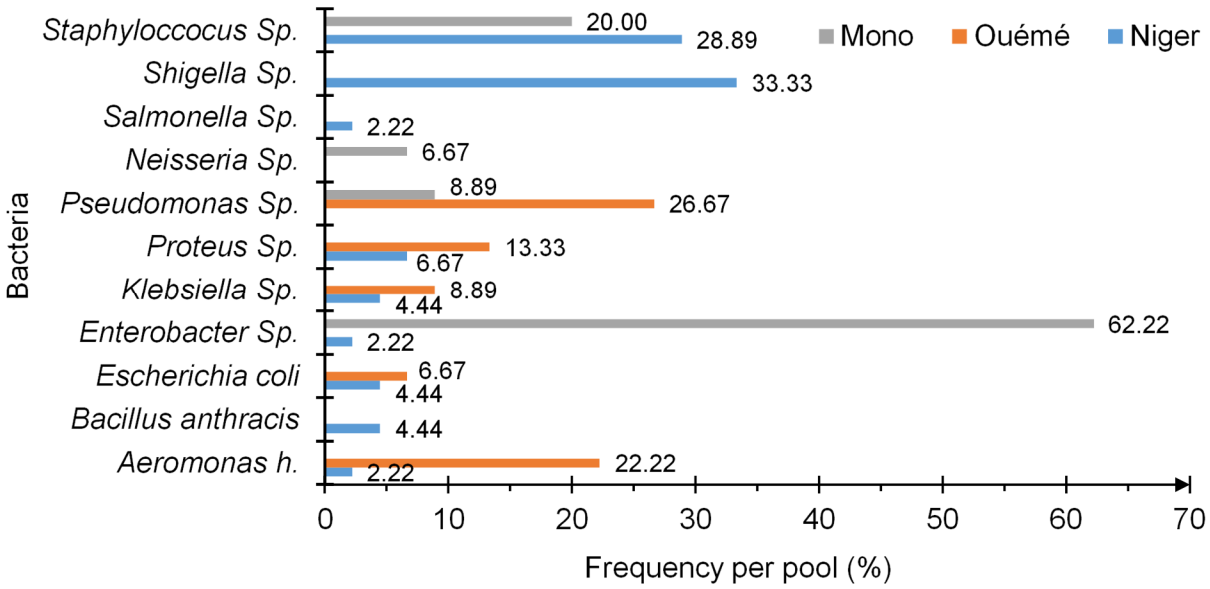

Figure 2. Types of bacteria and respective frequencies according to the pools.

Positivity rate by organ type and pelvis

According to Table 6, all of the intestine samples analysed were found to be infected (100%) with the exception of the Ouémé basin (68.18%). Likewise, apart from a few negative ulceration swabs, all bowel, muscle

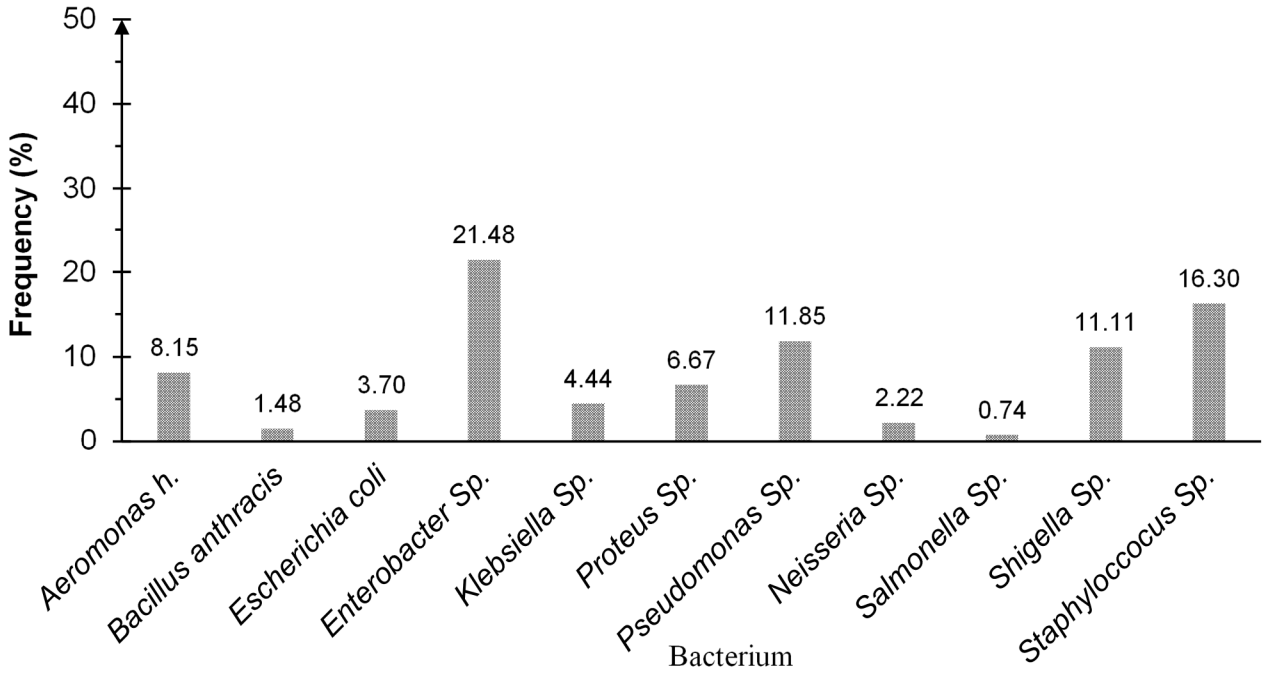

Figure 3. Prevalence of the different bacteria isolated in *H. occipitalis* in all 3 basins.

Table 6. Positivity rate by organ type and pelvis.

Organs	Basins					
	Niger		Ouémé		Mono	
	N	TP (%)	N	TP (%)	N	TP (%)
Intestine	13	100.00	22	68.18	7	100.00
Muscular	10	70.00	22	90.91	5	100.00
Skin	16	87.50	1	0.00	16	100.00
Ulceration	6	100.00	0	-	17	94.12
Total	45	-	45	-	45	-

N = number of samples; TP = Positivity Rate.

and skin samples were positive in the Mono's pelvis. Even if no ulceration samples were taken from the subjects coming from the Ouémé basin, note that a biological material did not give a positivity rate of 100% as opposed to the situation in the other two basins.

DISCUSSION

The present study led to the identification of bacterial species in the majority (85%) of specimens of *Hoplobatrachus occipitalis* collected in three hydrographic basins of Benin. This high rate of infection can be explained by the combined action of various factors facilitating the contamination and the spread of pathogenic germs in the aquatic environment. The sanitary quality of the supply of water, the nutritional conditions of the frogs, the climate and other environmental factors can determine the sanitary quality of these subjects as mentioned by Bartram and Craun (2004) and Gorski et al. (2011). Thus, the absence of a link between the infectious status of the frog and the organ which was used for the test indicates that all the organs removed were infected at the same intensity.

The list of bacteria isolated is consistent with the bacteria identified by Douglas and Amuzie (2017) in Nigeria among the same species with the exception of Vibrio cholerae, from *Serratia* sp. and *Aerobacter* sp., which were not encountered in our study. This similarity can be explained by the low variation in agroecological conditions between Benin and Nigeria and the sharing of the hydrographic network between these two border countries, in the West African sub-region. Among these isolated bacteria, *Pseudomonas* Sp., the *Aeromonas*

hydrophila, the *Bacillus* sp., the *Salmonella* sp. and the *Shigella* sp. were recognized as being typically aquatic (water from rivers, lakes, ponds) by Billon (1976). Blé et al. (2016) mainly met *A. hydrophila* when determining the prevalence of *Aeromonas* Sp. in samples of *H. occipitalis* fresh in Ivory Coast; which confirms our results.

Bacteria such as *Staphylococcus* sp., *Salmonella* sp., etc. isolated on the skin or in inedible parts could be eliminated through good hygienic practices and good cooking. In their work, Blé et al. (2016) in Côte d'Ivoire have shown the role of smoking and drying in the elimination of *Aeromonas* sp. in the meat of *H. occipitalis*. But those which sit in the muscle are the most dreaded especially *Bacillus anthracis* given its resistance to heat treatments.

The epidemiological study led to a significant difference in the rate of infection of frogs from one basin to another ($p < 0.05$). This same remark was made by Hird et al. (1981), then Blé et al. (2016) who reported that the rate of contamination of frogs from one collection site to another was significantly different. However, the two-by-two analysis only shows a significant difference in the rate of infection of frogs between the Ouémé and Mono basins, which are also both in South Benin ($p < 0.05$). It emerges from this analysis that frogs from Whedos are less infected than those from breeding ponds. In addition, the water in the ponds is less renewed, which generates a permanent contact of the frogs with germs and also with the possibilities of fertilizing the ponds with cow dung, a probable source of the coliforms. Our results agree with those of Douglas and Amuzie (2017).

Bacteria in *H. occipitalis* are predominantly Gram-negative; which is in agreement with the work of Gram and Dalgaard (2002) which revealed a predominance of these bacteria in temperate water fish. During the evaluation of the microbiological quality of *Hoplobatrachus occipitalis* meat, Douglas and Amuzie (2017) also isolated more Gram-negative bacteria in the species. The same observation was made by Culp et al. (2007) on three species of frogs with a predominance of Gram-negative bacteria. All this suggests that Gram-negative bacteria are more specific to aquatic animals such as fish, amphibians, etc.

The bacterial flora isolated in frogs from the Niger basin is composed of nine (9) genera of bacteria; while the populations originating from the Ouémé and Niger basins presented only five and four genera of bacteria respectively.

Frogs from the Niger Basin exhibited a very diverse range of bacterial genera compared to the other two basins. The most plausible explanation for this phenomenon is the abundant and continuous renewal of water in the tributary of the Niger River, exposing animals to the permanent risk of infection by new bacteria. In the other study environments, water renewal was seasonal (pond and whedo) limits the subjects' contact with new bacteria, hence low microbiological diversity.

Isolated in 11 frogs among the 135 analysed, *A. hydrophila* has a prevalence of 8.15% in the entire study area and 22.22% of subjects from the Ouémé basin. However, this bacterium has been associated with the worldwide decline of amphibians in several previous studies. It has been identified as the causative agent of the "red-leg disease" which is thought to be the cause of massive mortalities of frogs in the natural environment as well as in breeding (Hunsaker and Potter, 1960; Hawker and Linton, 1971; Hird et al., 1981; Forbes et al., 2004).

The presence of formidable bacteria such as *Bacillus anthracis*, *Escherichia coli*, *Salmonella* sp., *Enterobacter* sp., *A. hydrophila* and *Staphylococcus* sp. justify the health risk to which these animals are exposed in the natural environment both for their breeding and for human consumption. Our results agree with those of Douglas and Amuzie (2017) in Nigeria and Blé et al. (2016) in Côte d'Ivoire which showed that the consumption of this meat was not without risks for consumers. These isolated bacteria are responsible for septicemia, food poisoning, urinary and respiratory pathologies, etc. (Millemann, 1998; Gbané-Koné et al., 2015). The consumption of this meat, without minimum precautions, could be the basis of public health issues.

CONCLUSION

Bacterial flora identified in edible frogs *Hoplobatrachus occipitalis* is very diverse and mainly composed of Gram-negative bacilli. This study led to the isolation of pathogenic bacteria in *H. occipitalis*, probable sources of the decline of frog populations in the natural environment on the one hand and serious diseases among consumers of this meat on the other hand. However, a significant improvement was observed during the bacteriological analysis of frogs from ponds compared to those fished from rivers. Breeding in a controlled environment could therefore help in improving the microbiological quality of this meat. Strict compliance with the rules of hygiene and good cooking is also recommended for a beneficial operation for consumers of this meat.

ACKNOWLEDGEMENT

We (Authors) are grateful to all persons who have contributed to the data collection and analysis.

REFERENCES

Bartram J, **Craun** GF, **2004**. Waterborne zoonoses: identification, causes and control. World Health Organization.

Billon J, **1976**. Intérêt du froid dans la conservation du poisson et des crustacés. Aspects microbiologiques. Bulletin de l'Académie Vétérinaire de France, 129(3): 333-339. French

Blé CY, Dadié A, Cissé M, Yobouet BA, Djè KM, Fantodji A, **2016**. Prévalence et potentiel de virulence in vitro d'*Aeromonas* Sp. chez la

grenouille comestible *Hoplobatrachus occipitalis* (Ranidae) collectée dans le Centre Ouest de la Côte d' Ivoire. Int J Innovd Appl Stud, 18(2): 502. French

Codjo L, Attakpa EY, Fayalo SS, Massara ZSO, Imorou Toko I, **2021**. Germes Pathogènes Des Anoures d'Afrique De l'Ouest: Synthèse Bibliographique. Int J Progress Sci Technol, 28(2): 295-306. French

Culp CE, Falkinham JO, Belden LK, **2007**. Identification of the natural bacterial microflora on the skin of eastern newts, bullfrog tadpoles and redback salamanders. Herpetologica, 63(1): 66-71.

Douglas SI, **Amuzie** CC, **2017**. Microbiological quality of *Hoplobatrachus occipitalis* (Amphibia, Anura) used as meat. Int J Curr Microbiol Appl Sci, 6(6): 3192-3200.

Flandrois JP, **Chomarat** M, **1988**. L'examen cytobactériologique des urines. In: Bactériologie médical pratique, MEDSI / Mc GRAW-HILL, Paris; 21: 3-11. French

Forbes MR, Mcruer DL, Rutherford PL, **2004**. Prevalence of *Aeromonas hydrophila* in relation to timing and duration of breeding in three species of Ranid frog. Ecoscience, 11(3): 282-285.

Frenry J, **Renard** RP, **2007**. Précis de bactériologie Clinique. 2ème édition, paris, 1764. French

Gbané-Koné M, Koné S, Ouali B, Djaha KJ, Diomandé M, Eti E, Touré SA, Kouakou NM, **2015**. Tuberculose de l'épaule masque par une infection concomitante à *Enterobacter cloacae* : à propos d'un cas. Pan Afr Med J, 21(9): 5. French

Godome T, Tossavi E, Ouattara NI, Fiogbe ED, **2018**. Bibliographic synthesis on biology and ecology of *Hoplobatrachus occipitalis* (Günther, 1858). Int J Biol Chem Sci, 12(3): 1484-1493.

Gorski L, Parker CT, Liang A, Cooley MB, Jay-Russell MT, Gordus AG, **2011**. Prevalence, distribution, and diversity of Salmonella enterica in a major produce region of California. Appl Environ Microbiol, 77(8): 48-2734.

Gram L, **Dalgaard** P, **2002**. Fish spoilage bacteria–problems and solutions. Curr Opin Biotechnol, 13(3): 262-266.

Hawker LE, **Linton** AH, **1971**. Micro-organisms: function, form and environment. Micro-organisms: function, form and environment. Acad Press. Baltimore, New-York, 727.

Hird DW, Diesch SL, McKinnell RG, Gorham E, Martin FB, Kurtz SW, Dubrovolny C, **1981**. *Aeromonas hydrophila* in wild-caught frogs and tadpoles (*Rana pipiens*) in Minnesota. Am Assoc Lab Anim Sci, 31(2): 166-169.

Hunsaker D, **Potter** FE, **1960**. "Red leg" in a naturel population of amphibians. Herpetologica, 16(4): 285-286.

ISO 6887-2, **2004**. Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique. V 08-010-2:16. French

Lachal A, **Bouchkima** K, **2020**. Bactériologie médicale, Parasitologie et Mycologie. Institut Nationale des Professions Infirmières et Techniques de Santé, 32. French

Lanotte P, Isnard C, Garnier F, Mereghetti L, **2016**. Du prélèvement à la caractérisation des souches. In: Bactériologie médicale Techniques usuelles, 3ème édition Elsevier Masson SAS, 32-34. French

Marchal N, Bourdon JL, Richard C, **1982**. Les milieux de cultures pour l'isolement et l'identification biochimique des bactéries. Editeurs, paris, 482. French

Millemann Y, **1998**. Le pouvoir pathogène des salmonelles: facteurs de virulence et modèles d'étude. Vet Res, 29(5): 385-407. French

Mohneke M, Onadeko AB, Hirschfeld M, Rödel MO, **2010**. Dried or fried: amphibians in local and regional food markets in West Africa. Traffic Bull, 22(3): 117-128.

Mohneke M, Onadeko AB, Rödel MO, **2009**. Exploitation of frogs-a review with a focus on West Africa. Salamandra, 45(4): 193-202.

Negroni G, **Farina** L, **1993**. L'élevage de grenouille. Cahiers Agric, 2(1): 48-55. French

Onadeko AB, Egonmwan RI, Saliu JK, **2011**. Edible amphibian species: local knowledge of their consumption in southwest Nigeria and their nutritional value. West Afr J Appl Ecol, 19(1): 67-76. French

Rödel MO, **2000**. Herpetofauna of West Africa: Amphibians of the West African savanna. Chimaira, 7: 332.

Rödel MO, **Branch** WR, **2002**. Herpetological survey of the Haute Dodo and Cavally forests, western Ivory Coast, Part I: amphibians. Salamandra-Bonn, 38(4):245-268.

Rödel MO, Gil M, Agyei AC, Leaché AD, Diaz RE, Fujita MK, Ernst R, **2005**. The amphibians of the forested parts of south-western Ghana. Salamandra, 41(3): 107-127.

Tohe B, Kouame NG, Assemian NE, Gourene G, Rödel MO, **2014**. Dietary strategies of the giant swamp frog *Hoplobatrachus occipitalis* in degraded areas of Banco National Park (Ivory Coast). Int J Sci Res Rev, 3(2): 34-46.

Citation: Codjo L., Attakpa EY, Boko CK, Fayalo SS, Tohozin R, Pelebe EOR, Toko II, 2022. Bacteria pathogens of the edible frog *Hoplobatrachus Occipitalis* (Gunther, 1858) in the hydrographic basins of Benin. Microbiol Res Int, 10(1): 1-9.