Scientific Research Publishing

Journal of Agricultural Chemistry and Environment, 2024, 13, 223-234
https://www.scirp.org/journal/jacen
ISSN Online: 2325-744X
ISSN Print: 2325-7458

# Identification of Microorganisms in Poultry Farms in N'djamena and the Border Areas of Hadjer-Lamis and Chari-Baguirmi Chad

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**How to cite this paper:** Zakaria, A.A., Ban-Bo, B.A., Terei, N., Gandolo, B.N.R. and Doutoum, A.A. (2024) Identification of Microorganisms in Poultry Farms in N'djamena and the Border Areas of Hadjer-Lamis and Chari-Baguirmi Chad. *Journal of Agricultural Chemistry and Environment*, **13**, 223-234.
https://doi.org/10.4236/jacen.2024.132015

**Received:** February 22, 2024
**Accepted:** May 28, 2024
**Published:** May 31, 2024



## Abstract

**Introduction:** On the outskirts of Ndjamena, semi-industrial poultry farming and traditional poultry farming are practised informally on almost all poultry farms in Chad. This type of poultry farming is faced with real health problems attributable to a lack of monitoring of the vaccination schedule, inadequate compliance with biosecurity measures and poor application of the Ichikawa rule based on the 5 M's. **Objective:** The aim of this article is to identify the microorganisms responsible for contamination of poultry farms in the study area. **Method:** The study was carried out from 28/04/2022 to 31/01/2023 on the basis of 300 samples taken from feed, drinking water, droppings and scrapings from poultry housing surfaces in the 30 farms that served as a framework for our research. Sampling was of the simple random type, and farms were selected on the basis of the farmers' consent. The data were recorded on pre-established survey forms. Our study was cross-sectional, descriptive and prospective. Bacteria were isolated using the reference method NF EN ISO 6579 for *Salmonella spp.* and cultured on the specific medium eosin methylene blue (EMB) for *Escherichia coli*, *Pseudomonas* and *Citrobacter freundii*. **Results:** The following results emerged from this study: *Escherichia coli* (5.33%), *Pseudomonas* (1.33%), *Citrobacter freundii* (12%) and *Salmonella paratyphi* (21.68%). **Conclusion:** Of the 300 samples analysed, 121 (40.33%) were contaminated with pathogens. This high level of contamination is a health problem. The study shows that biosecurity is less satisfactory on the farms visited. Nevertheless, farms with a very satisfactory level of biosafety ensure food safety and variety for the population.

**Keywords**

Microorganisms Identification, Poultry Farms, N'Djamena, Hadjer-Lamis, Chari-Baguirmi (Chad)

## 1. Introduction

Chad, a Sahelian country at the heart of the African continent, has a diverse agroecological profile, making it an agropastoral country (52% food crop production, 9% cash crops and 39% livestock and fishing) [1]. The livestock sector provides direct or indirect income for 40% of the rural population. It plays a significant role in income redistribution in rural areas, where it is sometimes the only source of income for the most disadvantaged sections of the population, and the only form of farming in semi-arid regions [2]. The livestock sector, which accounts for 53% of rural GDP and provides a livelihood for around 40% of the rural population, is a sector capable of boosting the national economy thanks to the large number of livestock [3]. In 2021, the livestock population is estimated at more than 137,664,217 head, including 36,650,145 poultry, almost exclusively from traditional farms. These figures are updated by the General Livestock Census Office [4]. In a rural context, poultry can be used to repair damage, and the number of poultry required depends on the seriousness of the offence committed [5]. Modern poultry farming in Burkina Faso is rather unusual in that the sector is still relatively undeveloped but is undergoing strong growth [6]. The recent review of the poultry sector in Chad, validated in 2010 by the Food and Agriculture Organisation of the United Nations (FAO), estimated the number of poultry at 47.8 million head. This livestock, organised into semi-industrial and traditional family farms, is dominated by the domestic chicken Gallus-gallus [7]. Village rearing improves the protein level of the population and generates cash, which is managed by the women who are more involved in this activity [8]. Poor biosecurity practices on family poultry farms and on some poorly maintained commercial farms, together with poor access to veterinary services and medicines, contribute to the persistence and spread of certain poultry diseases [9]. Very little reliable statistical data is available at national level on the prevalence of contamination by *Salmonella paratyphi* and *Escherichia coli.* However, there are disparate reports of a worrying spread of salmonellosis in semi-industrial farms [10]. In Chad, poultry houses are often polluted by corpses, droppings and other poultry waste, which are dumped in the vicinity of farms [11]. In addition, factors such as feed, watering, housing conditions and farm management can have an impact on the health status of poultry [12]. The biosecurity of poultry markets helps to reduce the risk of diseases spreading between birds, or from birds to humans [13]. Every time poultry arrive or leave the farm or are moved between buildings or rearing areas, there is a risk of introducing and spreading

infectious diseases [14]. Biosecurity is a set of measures and effective means used on farms to control poultry health and improve farm profitability and product quality [15]. Farms are not immune to bacteriological contamination if biosecurity measures are poor. They could be contaminated by visitors or staff moving from one farm building to another [16]. Biosecurity measures are "safeguards" designed to prevent the introduction and spread of diseases or pathogens on poultry farms [17]. Although poultry farming is practised informally in Chad, it contributes to food self-sufficiency and reduces poverty. It was this important research that attracted our attention.

- ✓ **General objective**: To identify the microorganisms responsible for the contamination of poultry farms in our study area.
- ✓ **Specific objectives**:
- Collect samples of feed, drinking water, droppings and surface scrapings from poultry habitats;
- Analysing samples collected in the field at IRED;
- Knowing which germs have a negative impact on the health and profitability of poultry.

## 2. Methodology

### 2.1. Study Area and Research Framework

The study took place in N'Djamena and in the border areas of Hadjer-Lamis and Chari-Baguirmi. The latitude of the study area is between 11˚47 and 12˚25 and its longitude between 14˚56 and 15˚18. To identify the pathogens responsible for avian diseases, the IRED laboratory was used for the microbiological analysis of our samples.

#### 2.1.1. Duration of the Study

The study was carried out from 28/04/2022 to 31/01/2023 in the above-mentioned study area. During this period, we took 300 samples from 30 farms selected by simple random sampling (**Figure 1**, **Figure 2**).

#### 2.1.2. Hardware

- ✓ **Usual equipment:** gowns, gloves, boots and muffler.
- ✓ **Survey equipment:** The equipment consists of the survey protocol, sampling sheets, GPS, digital camera, USB keys, computer, mobile phone, pencil, felt-tip pens and ballpoint pens.
- ✓ **Laboratory equipment:** bags, aluminium foil, fridge, microscope, autoclave, stomacher, balance, benzene burner, hot plate, ovens, tubes, flasks, erlen and beakers.
- ✓ **Reagents and disinfectants:** Distilled water, alcohol, EPT (buffered peptone water), RV (Rappaport-Vassiliadis), XLD (Xylose lysine desoxycholate), GVB (brilliant green agar), H (Hektoen), GN (nutrient agar), and MKTTN (Muller-Kaufmann tetra thionate-novobiocin).

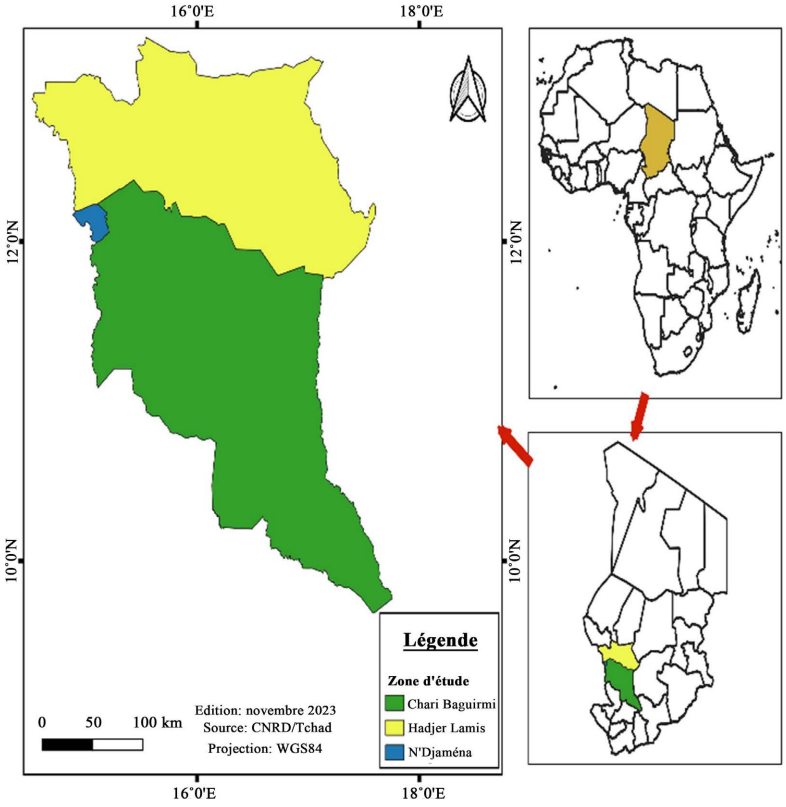


**Figure 1.** Map of the study area.

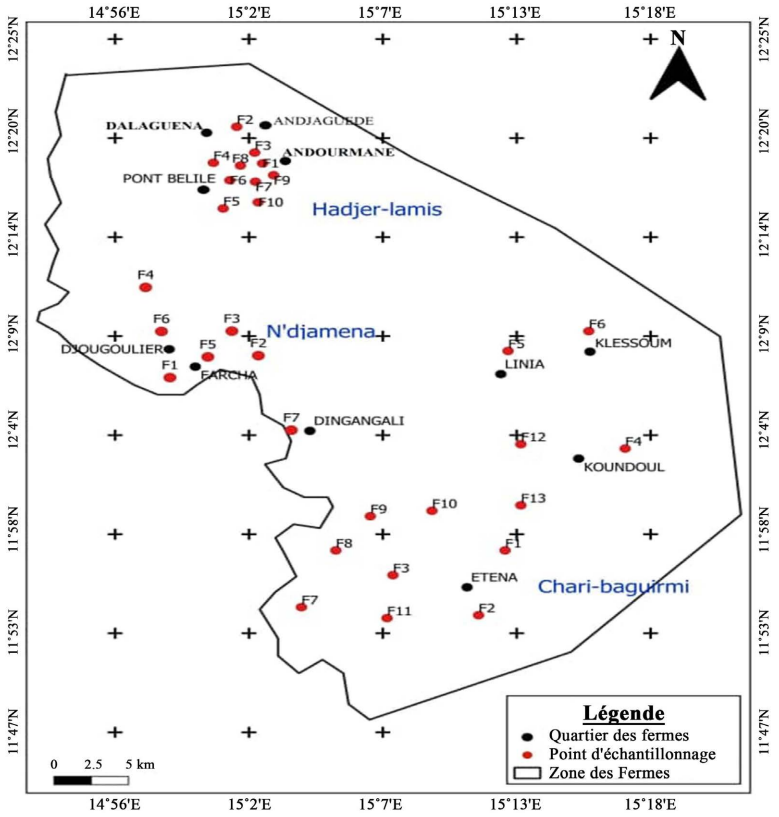


**Figure 2.** Area of farms dedicated to taking samples.

## 2.2. Methods

### 2.2.1. Sampling and Data Collection

**1) Sampling method**

This is simple random sampling.

**2) Sample sizes**

- Farms: 30 samples;
- Food: 60 samples;
- Drinking water: 60 samples;
- Droppings: 60 samples;
- Scraping of habitat surfaces: 120 samples (taken from the 4 corners of the habitat).

### 2.2.2. Conduct of the Study

**1) Conduct of the survey**

Poultry farmers were contacted directly in advance to facilitate access to their farms and authorise interviews with staff. The questionnaire, introduced to the farmers, was completed on the spot as the survey progressed.

The notion of ethics is observed during the interview: farmers are interviewed on their consent and are under no obligation or pressure from us.

**2) Sample collection procedure**

Sample collection has begun on the target farms, as sampling sheets, protective equipment and laboratory materials have been made available.

- **Sampling droppings**

Hygiene conditions and the Ishikawa rule based on the 5Ms were respected before any access to the poultry buildings. Protective equipment was worn. Sampling tools, ice, antiseptics, sterile plastic bags and packaging were available. The spatula was disinfected with bleach and the droppings were collected in the middle and at the bottom of the poultry house.

Two samples of droppings (F1 and F2) per farm were taken and placed in two different sterile bags, then labelled, coded, placed in insulated ice chests and deposited at the IRED before 4 hours for better preservation in a cool place. Sufficient time was set aside for weighing, centrifugation and homogenisation. At the end of these operations, all the samples were placed one after the other in other sterile bags to be kept in a cool place until they were processed.

- **Sampling water from drinking troughs**

Hygiene conditions were met for taking water from the troughs. Protective equipment was worn. Sampling equipment and plastic bags were provided. The water was put directly into sterile bags. Two water samples were taken from each farm (E1 and E2), placed in sterile bags, labelled, coded and placed in insulated ice chests for transport to the laboratory before 4 a.m. so that they could be kept cool. Time is then set aside for the progressive renewal of the bags, followed by weighing, centrifugation and homogenisation. After this operation, the samples are kept in a cool place until they are analysed.

- **Food sampling**

Hygiene measures were observed when the feed was taken from the troughs. Protective equipment was worn first. The sampling materials and sterile plastic bags were prepared. The feed was taken directly from the feed trough using a spatula that was disinfected at all times. The feed samples were placed in pre-prepared sterile bags. Two samples (A1 and A2) per farm are taken and placed in two different sterile bags. The samples were then labelled, coded, placed in insulated ice-boxes and transported to IRED before 4 a.m. to ensure that they were kept cool. After this operation, the samples are weighed, centrifuged, homogenised and stored in a cool place until they are ready for analysis.

- **Sampling of scrapings from building surfaces**

Hygiene measures are observed in advance when collecting scrapings from the habitat surfaces. Protective equipment is worn. Sampling tools and sterile plastic bags are prepared. The samples were scraped directly from the poultry housing surfaces using sterile cloths removed from their packaging. The wipes were soaked in distilled water to encourage rapid fixation of the scrapings on the cloths.

Four surface scraping samples (S1, S2, S3 and S4) per farm were collected and packaged in four (4) different sterile bags. The samples were labelled, coded, placed in insulated ice chests and sent to IRED within 4 hours for storage in a cool place. The wipes were wetted with EPT (buffered peptone water) and the solution obtained was then placed in a new sterile bag. After measurement, centrifugation and homogenisation, the samples are kept in a cool place until they are analysed.

- **Bacteriological analyses**

Bacteria were isolated as follows:

✓ The NF EN ISO 6579 reference method for the detection of *Salmonella spp*;

✓ Culture on a specific methylene blue eosin medium (EMB) to detect *Escherichia coli*.

- **Testing for *Salmonella spp***

**Using the** NF EN ISO 6579 **reference method**, we went through the following four (4) phases:

1) A pre-enrichment phase in a non-selective medium in which samples taken from the refrigerator are pre-enriched in buffered peptone water, homogenised using a vortex for 2 minutes, left to revive at room temperature for 30 minutes and then incubated at 37˚C for 18 to 20 hours;

2) Enrichment in Rappaport broth at 42˚C for 18 to 24 hours;

3) Isolation on selective medium;

4) Identification in which suspect colonies on Hektoen medium, lactose-negative with a black centre, are transferred to Kligler medium in a tube and incubated at 37˚C for 24 hours.

In our case, we carried out biochemical identification of suspected salmonella strains using the conventional gallery and the API 20 gallery[E].

- **Testing for *Escherichia coli*, *Citrobacter* and *Pseudomonas***

Samples removed from the refrigerator were pre-enriched 1:10 with buffered

peptone water, homogenised with a vortex for 2 minutes and left to revive at room temperature for 30 minutes, then incubated at 37˚C for 18 to 24 hours. Next, a TBX agar plate was inoculated with the pre-enriched solution using the quadrant method and incubated at 37˚C for 24 hours. Five specific colonies (dark purple, 2 to 3 mm in diameter, with a black centre and a greenish metallic sheen in reflected light) were then picked and plated on nutrient agar for purification. The colonies obtained are confirmed by biochemical tests (Kligler-Hajna slant agar then$^{R}$ API 20$^{E}$ galleries).

#### 2.2.3. Type of Study

This is a cross-sectional, descriptive, prospective study.

The research was conducted as a cross-sectional survey. The questionnaire was administered by direct contact. The study focused on the survey protocol, bibliography and field surveys. The questionnaire wastested and validatedbefore use.

### 2.3. Data Analysis

Laboratory results were recorded in an Excel spreadsheet and all data were analysed using SPSS version 23 statistical software.

## 3. Results

The identification of microorganisms in water, droppings, feed and poultry housing surfaces enabled us to isolate four (4) microorganisms from the three hundred target samples.

The results in **Figure 3** show that:

- ✓ Of the 300 samples analysed, 121 (40.33%) were positive for microorganisms:
- ✓ 65 samples positive for *Salmonella paratyphi*;
- ✓ 36 samples positive for *Citrobacter freundii*;
- ✓ 16 samples positive for *Escherichia coli*;
- ✓ 04 samples positive for *Pseudomonas*.

The results shown in **Figure 4** enable us to identify the microorganisms in the water in the poultry troughs:

- Of the 60 drinking water samples taken in the study area, we detected:
- ✓ 11 samples positive for *Citrobacter freundii*, including 7 from Hadjer-Lamis and 4 from Chari-Baguirmi;
- ✓ 08 samples positive for *Salmonella paratyphi*, including 2 in Hadjer-Lamis, 5 in Chari-Baguirmi and 01 in N'Djamena;
- ✓ 03 positive samples for *Escherichia coli*, including 2 from Chari-Baguirmiand 01 from Hadjer-Lamis but in N'Djamena, there was no *Escherichia coli* contamination;
- ✓ 01 sample positive for *Pseudomonas* at Hadjer-Lamis).

The results shown in **Figure 5** enable us to identify the microorganisms in the feed at the poultry feeders:

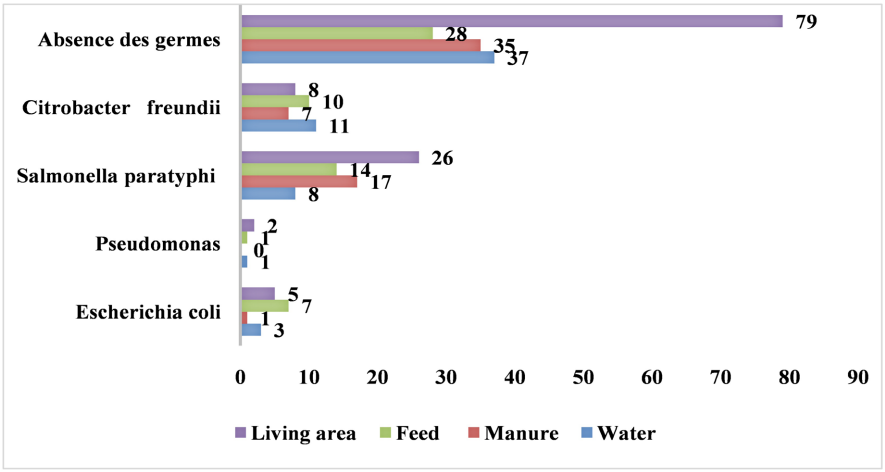


**Figure 3.** Microorganisms identified in water, droppings, feed and poultry habitat surfaces.

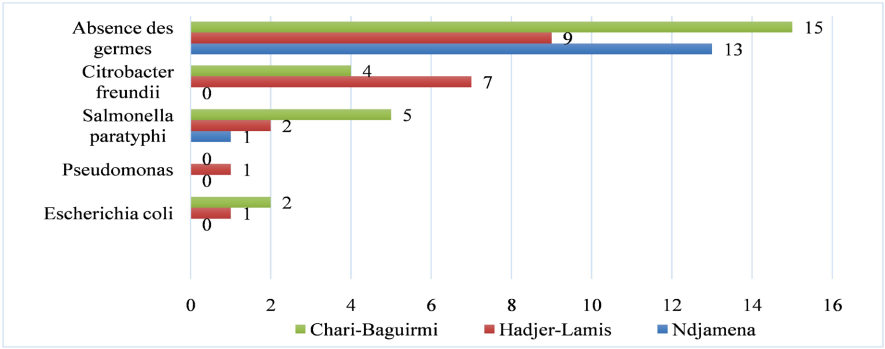


**Figure 4.** Microorganisms identified in water from poultry troughs in the study area.

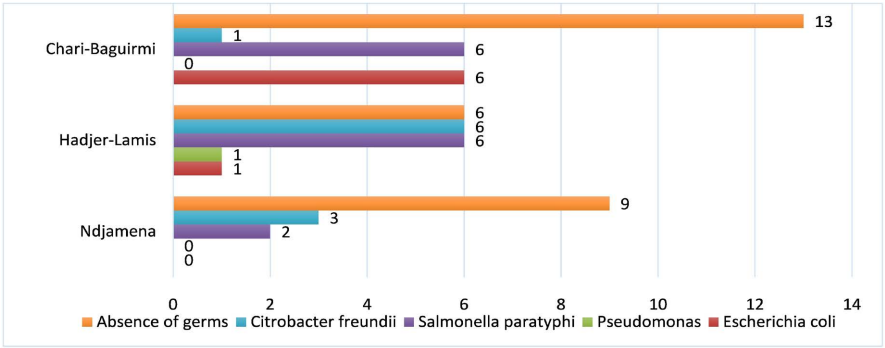


**Figure 5.** Microorganisms identified in feed from poultry feeders in the study area.

- Of the 60 food samples taken in the study area, we detected:

✓ 14 samples positive for *Salmonella paratyphi*, including 6 in Hadjer-Lamis, 6 in Chari-Baguirmi and 02 in N'Djamena;

✓ 10 samples positive for *Citrobacter freundii*, including 06 in Hadjer-Lamis, 03 in N'Djamena and 01 in Chari-Baguirmi;

✓ 07 positive samples for *Escherichia coli*, including 06 from Chari-Baguirmi and 01 from Hadjer-Lamis;

✓ 01 samples positive for *Pseudomonas* at Hadjer-Lamis).

However, the remaining 28 samples (46.66%) were not contaminated.

The result in **Figure 6** enabled us to identify the microorganisms in the poultry droppings:

- Of the 60 samples of droppings taken in the study area, we detected:

✓ 17 samples positive for *Salmonella paratyphi*, including 6 in Hadjer-Lamis, 9 in Chari-Baguirmi and 02 in N'Djamena;

✓ 07 samples positive for *Citrobacter freundii*, including 04 in Hadjer-Lamis, 02 in N'Djamena and 01 in Chari-Baguirmi;

✓ 01 sample positive for *Escherichia coli* in Chari-Baguirmi.

However, the remaining 35 samples (58.33%) were not contaminated.

The result in **Figure 7** enabled us to identify the microorganisms in the samples taken from the poultry habitat surfaces:

- Of the 120 samples analysed from scrapings of poultry habitats in the study area, we identified:

✓ 26 samples positive for *Salmonella paratyphi*, including 11 from Hadjer-Lamis, 9 from Chari-Baguirmi and 06 from N'Djamena;

✓ 08 samples positive for *Citrobacter freundii*, including 05 from Hadjer-Lamis, 01 from N Djamenaand 02 from Chari-Baguirmi;

✓ 05 positive samples for *Escherichia coli*, including 03 from Hadjer-Lamis, 01 from N'Djamena and 01 from Chari-Baguirmi;

✓ 02 samples positive for *Pseudomonas* in N'Djamena.

However, the remaining 79 samples (65.83%) were not contaminated.

## 4. Discussion

Our field visit suggests that modern poultry farming in Chad is making slow progress due to lack of resources, pathologies and lack of professionalism. This assertion corroborates that of Bastianelli, who researched intensive poultry production in Burkina Faso.

The results of our work have shown that traditional livestock farming strengthens resilience and generates significant income for rural communities. This assertion is similar to that of Barkot, who conducted research in 2007 on the structure and importance of the commercial and traditional poultry sectors in Morocco.

The characteristics of poultry farming in our study area are similar to those studied by Elgroud and colleagues, who carried out research in 2009 on 30 farms in the Wilaya of Constantine in Algeria [18].

Our studies have enabled us to isolate the microorganisms responsible for farm contamination. Our results corroborate those of Mollenhorst and colleagues who conducted research on salmonella in the Netherlands in 2005 [19] and those of Namata H. *et al.* in 2008 [20]. The risk of contamination of poultry farms by *Salmonella paratyphi* therefore increases with the size of the farm and the sampling period.

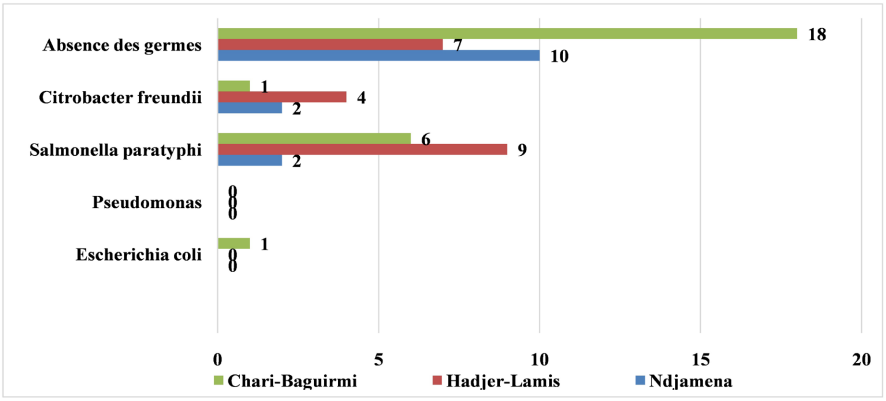


**Figure 6.** Microorganisms identified in poultry droppings from the study area.

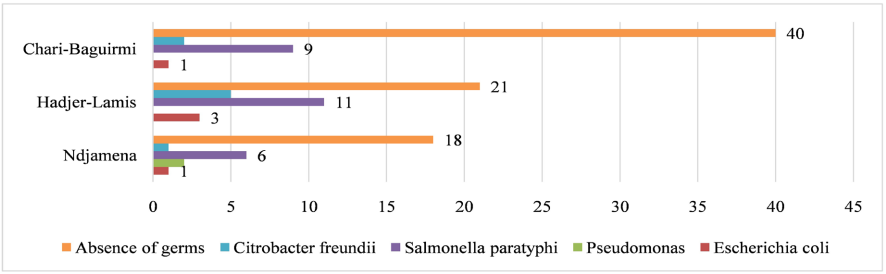


**Figure 7.** Microorganisms identified in samples taken from poultry habitats in the study area.

Poultry water in the study area contains microorganisms because the hygiene, washing and disinfection of drinking troughs are defective. However, our results differ from those of Koffi, who carried out research in Côte d' Ivoire in 2015 on water from drinking troughs and found only *salmonella paratyphi* (4.2%) because most farmers observed hygiene [21].

Our study detected *Salmonella Paratyphi* in the droppings because the poultry had not been vaccinated or biosecured, and the farmers were not familiar with good poultry farming practices. Our results are therefore similar to those of Abdallah Chaiba and colleagues, who also carried out research in October 2007 on droppings in Meknès (Morocco) and found 24% of farms positive for *Salmonella Paratyphi* [22].

## 5. Conclusions

The aim of the study, carried out in N'Djamena and the Hadjer-Lamis and Chari-Baguirmi border areas, is to identify the micro-organisms responsible for contaminating poultry farms.

Research was carried out on 300 samples, including 70 from N'Djamena, 100 from Hadjer-Lamis and 130 from Chari-Baguirmi. Samples were taken from feed, water, droppings and poultry housing surfaces.

IRED analyses revealed 121 samples positive for microorganisms (*Salmonella paratyphi*, *Citrobacter freundii*, *Escherichia coli* and *Pseudomonas)* (40.33%),

but the remaining 179 samples (59.67%) were free from contamination.

Nevertheless, we recommend that poultry farmers respect hygiene, follow the vaccination schedule, implement biosecurity measures, carry out a sanitary vacuum, change the litter at the end of the deadline, and clean and disinfect the buildings. Particular attention must also be paid to the quality of the water, the feed and the environment in which the chickens are kept, which are considered to be potential sources of contamination for farms and the entire production chain.

It is by adopting this strategy that we can guarantee poultry a stable health status.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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