

Etiology of a diarrhoea epidemic among employees of a poultry farm in the city of N'zérékoré, Republic of Guinea

Abstract

Objective: Acute diarrheal diseases are a primary and recurrent health concern among poultry workers. This study is designed to determine the causative agent of an epidemic which broke out among the 510 employees of a poultry farm in the City of N'zérékoré.

Methodology: Systematic survey was implored according to a list drawn up by the personal chef of the farm. To detect germs commonly implicated in food-borne illnesses, a biological analysis of the stools with the Polymerase Chain Reaction was performed, followed by a direct examination of the stools and poultry feed. Statistical processing was achieved with the BiostatGV software.

Results: 104 cases (20,4%) of *Salmonella typhi* DNA positivity were detected in these stool samples, including 50 cases (9.8%) positive in men versus 54 cases (10.59%) positive in women. The most affected are: (18-29) years old 6.5%, followed by that of (30-41) years old with 5.7%. 73 cases (14,3%) of DNA positivity of *Campylobacter spp* detected including 44 positive cases in men i.e., 8.6% against 29 positive cases in women, i.e., 5.69%. 32 positive cases (6,3%) of *Tapeworm spp* eggs detected, including 12 positive cases in men, i.e., 2.4% against 20 positive cases in women, i.e., 3,92%.

Conclusion: Among the 510 farm employees, *Salmonella typhi*, *Campylobacter spp*, and *Tapeworm spp* were responsible for the acute diarrhea epidemic declared on 01 / July / 2022.

Keywords: diarrhea, epidemic, employees, *Salmonella*, *Tapeworm*

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Sangare Mory,¹ Sangaré Lanciné,² Namory Keita,³ Sidibé Youssouf⁴

¹University of N'zérékoré / International Research Centre on Tropical Infections in Guinea (CIRITG), Guinea

²Institut Supérieur Agronomique et vétérinaire de Faranah, Department of agriculture, Guinea

³Université de Kindia, Guinea

⁴Molecular Biology Laboratory of Nzérékoré Regional Hospital, Guinea

Correspondence: Mory Sangaree, University of N'zérékoré / International Research Centre on Tropical Infections in Guinea (CIRITG), Guinea, Tel +224 622 38 04 95; Email morisangar@yahoo.fr

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Introduction

Poultry farm workers are highly susceptible to the causative agents of diarrheal infections, including Enterobacteriaceae and intestinal parasites. Following the declaration of an epidemic of febrile diarrhea, sometimes accompanied by abdominal pain, abdominal distension, nausea, and vomiting, the International Center for Research on Tropical Infections in Guinea conducted a parasitological and bacteriological investigation on July 1, 2022, among 510 employees of this poultry farm in the city of N'zérékoré. These employees were identified by the head of personnel of the farm and submitted to a medical consultation. It is reported that in 2009, Europe had 5,550 cases of CFTI (Collective Foodborne Toxi-infection), which affected 48,964 people, of which 4,356 had to be hospitalized and 46 died.^{1,2} The two main bacteria that cause CFTIs are *Salmonella* and *Campylobacter*. Concerning the first one, in Europe, the number of cases of salmonellosis in humans has been constantly decreasing for the last 5 years. Between 2008 and 2009, the infection rate fell by 17.4%. This decrease is the result of a specific control plan implemented in poultry farms in order to guarantee a higher sanitary quality of eggs.¹ As for *Campylobacter*, the number of cases has remained stable. *Campylobacter* (from the Greek, curve) is a genus of gram-negative, micro aerophilic, oxidase-positive, non-spore-forming bacteria that cause food poisoning. They are present in the intestines of many animals, especially farm animals. They are considered as a source of zoonosis in livestock, and as the main bacterial cause of human gastroenteritis in the world, with an increasing incidence in developed countries, which could be due to the concentration of livestock.² The mortality rate of these bacteria is 0.8% and 0.2% respectively.¹⁻³ On the other hand, in sub-Saharan Africa, among the food-borne infections that still threaten the populations of poor areas, typhoid fever is high on the list.¹ Typhoid

fever is a bacterial infection caused by *Salmonella enterica serotypes typhi* (Eberth's bacillus) and *Paratyphi A, B* or *C*.⁴ The reservoir of germs for this disease is strictly human¹: contamination occurs through the stools of sick or healthy carriers.¹⁻⁴ Transmission is most often orofaecal by ingestion of water or food contaminated with germs.⁵ It can also occur through direct contact with infected stool or by eating food handled by a germ carrier.⁶ Parasitosis can be caused by improper handling of feed, including the feeding of poorly stored fish from unsafe waters to poultry. Tapeworm infection is caused by ingestion of feed contaminated with tapeworm eggs or larvae. Once swallowed, tapeworm eggs can form cysts in tissues and organs (invasive infection).^{6,7} Currently, there is a worsening of risk factors related to increasing poverty, global climate change associated with the degradation of sanitary services on poultry farms (lack of clean water and personal hygiene of farmers), and the massive displacement of populations who sometimes live in unsanitary conditions.^{5,8} Thus, diarrhoeal diseases are rampant in most poor countries and remain frequent in countries with low hygiene. Levels.^{5,9,10} There are no clear national statistics concerning collective toxi-infection in Guinea, data are scattered and it could be one of the receptacles of child malnutrition. Given this alarming context, the importance of this poultry farm and the number of people involved, it was important to write this article whose objective was to determine the causal agent of the epidemic that broke out among its 510 employees.

Methods

Location and type of studies

The city of N'zérékoré was the site of the studies. It has a mainly agropastoral population and had 224,791 inhabitants in 2016. The study was an experimental survey conducted on July 1, 2022 in the Microbiology and Nutrition sections of the International Research

Center on Tropical Infections in Guinea (CIRIT-G) in Nzérékoré. It involved 510 employees of a poultry farm in the urban commune of N'zérékoré.

Ethical aspect

All patients involved in the study were informed of the objective of the survey and agreed following informed consent to participate in the study.

Training and follow-up of investigators.

Preparations: After developing the data collection sheet, establishing a schedule in collaboration with the chief of staff and the owner of the poultry farm, and arranging the necessary logistics and financial resources, interviewers were trained. Most of the interviewers were already involved in the health care of the population. All the interviewers have a high school or university degree. During a one-day training session, the objectives of the survey and sampling techniques were explained. The training session was concluded by a pre-survey.

Teams of doctors and laboratory scientist: Two doctors and a laboratory investigator were reinforced by training on the concept of fever syndromes, temperature taking, stool sampling, polymerase chain reaction (PCR), and the filling of forms.

Conduct of the survey, data collection; consumables and non-consumables of the laboratory: The laboratory technician collected the faeces and excreta from the chickens and forwarded them 30 minutes later to the laboratory for real-time Polymerase Chain Reaction (PCR). Doctors provided the medical consultation. For each case of fever, the full name, date of birth, age, sex and weight were recorded.

The analysis included: 510 persons 18 to 65 years old with systematic survey using a list made by the Chief of Staff. The sampling tubes, containing distilled water, the cooler at +4°C, the RT thermocycler, the microscope, the slide and slide, the centrifuge, the reagents for the real time PCR were used as survey materials.

Table 1 Simple distribution of patients from 18 to 65 year by age group and sex

Age/year	Male		Woman		Total		Ratio Male/Female
	number.	%	number.	%	number.	%	
18 - 29	80	16	70	14	150	29	1,1
30 - 41	60	12	62	12	122	24	1,0
42 - 53	62	12	65	13	127	25	1,0
54 - 65	56	11	55	11	111	22	1,0
Total	258	51	252	49	510	100	1,0

Men are 51% against 49% for women. The boy/girl ratio is 1.0. The age groups (18-29) of 29% and (42-53) of 25% concern the major part of the patients.

Identification of the causes of the epidemic in the poultry farm by the PCR method With different primers used, the results are recorded in the following tables:

Table 2 Positive cases of *Salmonella typhi* by polymerase chain reaction by age group

Age/ year	Male			Woman			Total		
	Number	positive case	%	Number	positive case	%	Number	positive case	%
18-29	80	15	2,9	70	18	3,53	150	33	6,5
30-41	60	10	2,0	62	19	3,73	122	29	5,7
42-53	62	17	3,3	65	6	1,18	127	23	4,5
54-65	56	8	1,6	55	11	2,16	111	19	3,7
Total	258	50	9,8	252	54	10,59	510	104	20,4

104 cases (20.4%) of *Salmonella typhi* DNA positivity were detected in these stool samples, including 50 positive cases in men (9.8%) and 54 positive cases in women (10.59%). (PCR in real time). The most affected age groups are: (18-29) years 6.5%, followed by (30-41) years with 5.7%.

Statistical treatment with Biostat TGV online to calculate the p (95% value and confidence interval).

Real-time PCR technique: At the mobile laboratory of the Russian Federation (Rospotrebnadzor) in Guinea, the analysis was carried out as follows: The first step was to homogenize the samples. Samples were diluted 1/10th (25 g of stool in 225 ml of sterile distilled water). After cell lysis, DNA was extracted using different kits to search for the corresponding bacteria.^{9,10} The main pathogen sought in the stool samples was *Salmonella typhi*, *Campylobacter spp* and others, causative agent. The test for bacteria using the PCR method is based on the polymerase chain reaction technique.¹⁰ This method multiplies a specific DNA sequence of a bacterium: The first step consists in extracting the DNA of the bacterium present in the sample thanks to solvents which break the envelope of the cells. This DNA is then filtered and purified. In a second step, the DNA is placed in a thermocycler where it is amplified over forty cycles. The DNA sequences duplicated in one cycle serve as the basis for duplication in subsequent cycles. This allows an exponential multiplication of the number of significant DNA fractions. During this duplication phase a fluorescent chemical marker is attached to the duplicated bacterial sequences to measure semi-quantitatively (+++, ++, +/-, -) by spectrometric reading, the presence of the bacteria in the initial sample.¹⁰ One week after the survey, the data was entered and analyzed with BiostaTGV and Excel software to determine the p (p-Value) at 95% confidence interval (CI).

Stool parasitology

Poultry excreta and feed were diluted with physiological water in a thin smear between slide and coverslip and observed directly under the microscope, with 10X and 40X objectives. Willis concentration was also performed on each sample, only the deposit, in thin smear between slide and coverslip was observed; the supernatant was discarded.

Results

Tables (1–5)

Table 3 Positive cases of *Campylobacter spp* by Polymerase Chain Reaction(PCR) by age group

Age/ year	Male			Woman			Total		
	Number	positive case	%	Number	positive case	%	Number	positive case	%
18-29	80	11	2,2	70	9	1,76	150	20	3,9
30-41	60	9	1,8	62	8	1,57	122	17	3,3
42-53	62	18	3,5	65	7	1,37	127	25	4,9
54-65	56	6	1,2	55	5	0,98	111	11	2,2
Total	258	44	8,6	252	29	5,69	510	73	14,3

73 cases (14.3) of *Campylobacter spp* DNA positivity were detected in these stool samples of which 44 positive cases in males or 8.6% against 29 positive cases in females or 5.69%. (PCR in real time).The most affected age groups are: (42-53) years 4.9%, followed by (18-29) years with 3.9%.

Table 4 Positive cases of *Tapeworm spp.* by age group (direct slide and slideexamination of patients' stools)

Age/ year	Male			Woman			Total		
	Number	positive case	%	Number	positive case	%	Number	positive case	%
18-29	80	2	0,4	70	5	0,98	150	7	1,4
30-41	60	3	0,6	62	6	1,18	122	9	1,8
42-53	62	3	0,6	65	4	0,78	127	7	1,4
54-65	56	4	0,8	55	5	0,98	111	9	1,8
Total	258	12	2,4	252	20	3,92	510	32	6,3

32 cases (6.3%) of positive eggs of *Taenia ssp* were detected in these stool samples of which 12 positive cases in men or 2.4% against 20 positive cases in women or 3.92%. (Direct examination in fresh air).The most affected age groups are: (30-41) years 1.8%, followed by (54-65) years with 1.8%.

Table 5 Positive cases of *Campylobacter spp*, *Salmonella typhi ssp* (real-time PCR) and *Taenia spp* by direct examination of feces and feeds from farm chickens

Positive cases						
Number	<i>Salmonella typhi</i>	%	<i>Campylobacter Spp</i>	%	<i>Tapeworm Spp</i>	%
510	54	10,6	54	10,6	30	5,9

Of the 510 samples of excreta and feed from farm chickens, 54 cases of *S. typhi*, 54 cases of *Campylobacter spp.* and 30 cases of *Taenia spp.* were identified.

Discussion of the results

510 stool samples from patients aged 18 to 65 years and 510 faeces and food samples were examined. Males were 51% against 49% for females. The male to female ratio was 1.0. The age groups (18-29) of 29.% and (42-53) of 25.% concern the major part of the patients. Statistically insignificant difference between men and women $p = 0.5$ at 95% [-258.5974; 291.5974]. Following real time PCR examinations, 104 cases (20.4%) of *Salmonella typhi* DNA positivity were detected in these stool samples, of which 50 positive cases in males, i.e.,9.8%, against 54 positive cases in females, i.e.,10.59%. The most affected age groups were: (18-29) years 6.5%, followed by (30-41) years with 5.7%. $P = 0.7$ at 95% [-110.9909; 120.0909]. In 2005, in Senegal, N. Lefebvre et al. presented higher percentages were out of 70 patients, 65 had a positive blood culture i.e., 92.85%.⁵ Another study conducted on 65 children in Cameroon by P. Doumb et al.¹¹ in 2007 showed 77% of *Salmonella typhi* cases.⁷ Although the difference between the two studies was not statistically significant, $p = 0.8$ at 95% [-865.4; 829.2], the difference may be located in the method used in this study having a high specificity and sensitivity, the real time PCR [10]. 73 cases (14.3%) of *Campylobacter spp* DNA positivity were detected in these stool samples of which 44 positive cases in males or 8.6% against 29 positive cases in females or 5.69%. The most affected age groups are: (42-53) years 4.9%, followed by (18-29) years with 3.9%. These results, as a whole, are consistent with a recent epidemiological report from en.wikipedia.org/wiki/Campylobacter indicating that 50% to 80% of human campylobacter cases could be attributed to chicken.^{3,7} Following direct fresh stool examination, 32 cases (6.3%) of positive *Taenia ssp* eggs were detected of which 12 positive cases in males or

2.4% against 20 positive cases in females or 3.92%. The most affected age groups are: (30-41) years 1.8%, followed by (54-65) years with 1.8%. *Taenia spp.* infection is caused by the ingestion of food (fish) contaminated with tapeworm eggs or larvae, especially dried fish, which is found poorly preserved and is an essential component of poultry feed. Once swallowed, *tapeworm* eggs can cause diarrhea or form cysts in tissues and organs (invasive infection).⁷ To establish a link between infected employees and the health status of the farm, 510 samples of chicken feces and feed were analyzed by PCR and direct examination in thin smear between slide and slide. Fifty-four cases (10.6%) of *S. typhi*, 54 cases (10.6%) of *Campylobacter spp.* and 30 cases (5.9%) of *tapeworm spp* were identified. The statistically insignificant difference between the infection rates of the workers and those found in the poultry feces and feeds indicates a high exposure of the workers to the latter, $p = 0.5$ at 95% [-38.4357; 52.9357].

Conclusion

The epidemic of diarrhea declared on 01/06/2022 in a poultry farm in the city of N'zérékoré, among 510 employees is due to *Salmonella typhi*, *Campylobacter spp.*, and *Taenia spp.* Indeed after analysis of 510 stool samples of the patients with real-time PCR, and after direct observation of 510 samples of excreta and food from the poultry farm, it was identified: 20.4% *S.typhi*, 14.3% *campylobacter spp.*, 6.3% *tapeworm spp.* These employees are highly exposed to contaminated feces and food in the proportions of 10.6% *S.typhi*, 10.6% *Campylobacter spp* and 5.9% *Tapeworm spp.* These results were provided and the patients were managed. The farm was sanitized.

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None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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