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Contamination of Slaughterhouse Meat by Cryptosporidia in the Tonkpi Region, Western Cote D'ivoire

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Abstract **Original Research Article**

A study was carried out on meat from the main abattoirs with the general aim of avoiding health risks in meat from abattoirs in the Tonkpi Region, in the west of Côte d'Ivoire. Post-mortem inspection was used to carry out health and veterinary checks on cattle carcasses from the abattoirs. The methodology used was simple random sampling, in which individuals with the same probability of being selected were drawn at random. A total of 390 samples were taken from the intestines that had been in contact with the carcasses during transport to the markets, and these samples were sent to the laboratory and prepared using histopathological methods so that they could be observed under a light microscope. The samples were stained with May-Grünwald Giemsa (MGG) to characterise the lesions and identify the tissue or cell locations of the aetiological agent. The laboratory results revealed the presence of *Cryptospridium parvum* oocysts in the intestines, which contaminated bovine carcasses intended for human consumption. High prevalence rates (41.54%) of cryptosporidiosis were observed in meat from abattoirs, notably 15.64% in Man, 14.10% in Danané and 11.79% in Biankouma. This disease is one of the emerging zoonoses and is mainly found in immunocompromised subjects. The comparison of prevalences between the different abattoirs in the Tonkpi Region was not significant ($p>0.05$). Generally speaking, meat produced in abattoirs and sold on markets in the region revealed a potential public health risk.

Keywords: Meat, prevalence, cryptosporidiosis, abattoirs, consumers.

1. INTRODUCTION

Food of animal origin can be a source of consumer exposure to a variety of agents that can cause disease or injury (OIE, 2012). Contamination of food by biological, chemical or physical agents can occur at any stage of production, transport, storage, distribution and preparation for consumption (FAO/WHO, 2006). This is why the country's veterinary officers play an essential role in the prevention and control of food-borne zoonoses, even in the absence of clinical signs, by inspecting and certifying the sanitary quality of the meat produced (Diarrassouba, 2011; Mckenzie and Hathaway, 2002). Unfortunately, the separation of animal dressing operations into healthy and contaminated sectors is not often respected in the main abattoirs in the Tonkpi Region. Respect for the forward movement and non-crossing of circuits in a slaughterhouse is far from being achieved. Butchers eviscerate the carcasses after dressing, and place the viscera, including the intestines, on the carcasses before sending them to market. It has been observed that the contents of the intestines spill onto the carcasses during transport. These carcasses are often transported on the backs of men to private

2. MATERIALS AND METHODS

2.1- Study site

The study area is the Tonkpi Region, which has a population of 992,564 and is predominantly rural and agricultural (Figure 1). It comprises five departments (Biankouma, Danané, Man, Sipilou and Zouan-Hounien), of which Man is the regional capital. The equatorial climate is characterised by two dry seasons (a long dry season from November to March and a short dry season from July to the end of August) and two rainy seasons (a long rainy season from March to June and a short rainy season from September to October). The

relief is relatively monotonous with the presence of scattered hills, numerous low-lying areas with sature ferralitic soils and hydromorphic soils (INS, 2016).

2.2- Sampling

The sample size to be taken was determined from Thrusfield's (2005) formula for simple random sampling in which:

- δ = standard deviation (function of risk α which is the precision error of the estimate);
- p = maximum expected prevalence rate for the study;
- i = tolerated margin of sampling error and
- c = correction coefficient.

Assuming a maximum prevalence p of 50%, a risk α of 5% (δ =1.96), a margin of sampling error i of 5% and a correction factor c of 1, the sample size n is 385 subjects. In fact, 390 intestinal samples were taken during this study.

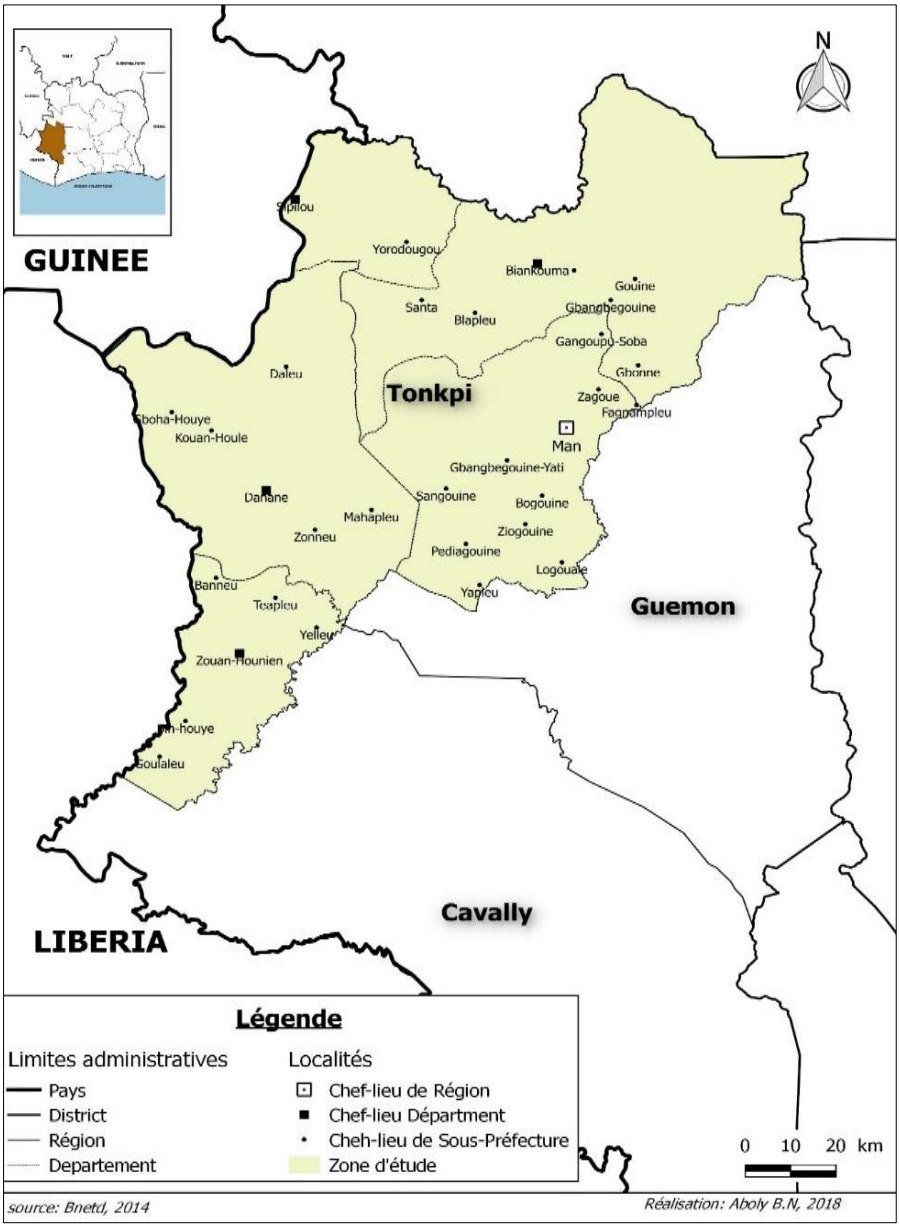

Figure 1: Map of the Tonkpi Region

2.3- Sampling methods for laboratory analyses

Samples were taken from the intestines of cattle in contact with the carcasses during transport to the slaughterhouse. To detect parasite contamination, the oocysts were isolated directly from the food sample (intestines) and then observed under a microscope. The sample obtained was prepared using histological methods so that it could be observed under the microscope (Kristin *et al.,* 2012). It is immersed in a container designed to allow reagents to penetrate the tissue. It is immersed in successive baths with increasing concentrations of ethanol, leading to complete

dehydration. The sample is first immersed in toluene or xylene (with care, as these are toxic products). Then it is finally immersed in a preservative (often melted paraffin). This fixes the sample, but makes it different from the original fabric. The final image must therefore be 'interpreted' by a specialist. The tissue prepared in this way is then removed from the preparation cassette and placed in a mould into which paraffin is poured, which freezes into a block as it cools.

By cutting very thin slices (2 to 7 micrometres thick) from this block using a microtome, we obtain slices of biological tissue thinner than the average thickness of a cell. These slices are then placed on a glass slide and, if necessary, examined.

2.4- Specific staining of samples

To characterise the lesions and highlight the tissue or cell locations of the aetiological agent, the samples were specially stained with May-Grünwald Giemsa (MGG). The slides were read and interpreted using a LEICA DM 1000 optical microscope.

2.5- Data collection and analysis.

The data collected were entered into the Microsoft Excel 2013 database system. The data were analysed using R Version 3.4.0 and Stata 14.2 statistical software. Prevalence was calculated by dividing the proportion of meat infested with Cryptosporidium parvum by the total number of carcasses examined and multiplying by 100%. The determinants of cryptosporidiosis were studied using percentage values and the Chi-square test, the significance threshold of which was set at 5% ($p < 0.05$).

Prevalences were compared on the basis of 95% confidence intervals (CI). Where the intervals overlapped, there was no statistical difference. However, when they do not overlap, there is a statistical difference between the proportions compared. It should be noted that the confidence interval is a mathematical indicator used to quantify the area of uncertainty in a survey or poll based on a population sample. It frames a real value that we are trying to estimate using measurements taken.

$$IC_{95\%} = \left[p - Z\alpha \frac{s}{\sqrt{n}} ; p + Z\alpha \frac{s}{\sqrt{n}} \right]$$

3. RESULTS AND DISCUSSION

3.1. RESULTS

3.1.1. Risk factors for meat contamination at the abattoir

The results of the study showed that dressing requires particular attention because of the handling of the meat and its exposure to environmental conditions. The current practice of dressing meat on the floor in abattoirs and slaughter areas, and the transport of carcasses mixed with viscera on tricycles, exposes the meat to very high risks of contamination. These risks are mainly contamination by hands, knives and faeces, due to the practice of evisceration on the floor (Figures 2 and 3).

Figure 2: Cattle skinned on the ground

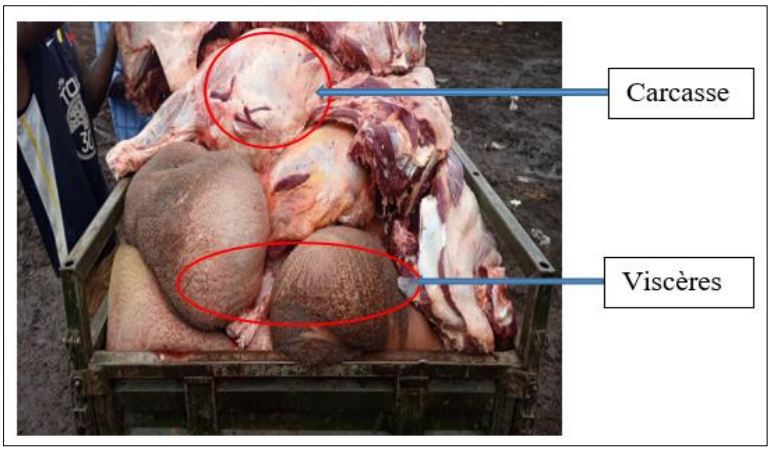

Figure 3: Carcass transported in a tricycle with the viscera

3.1.2- Laboratory results of intestinal samples per abattoir

Samples were taken from the intestines of animals aged between less than 1 and more than 2 years of age of both sexes (Table I). A total of 390 samples were taken, of which 162 were positive (41.54%). Most of the beef cattle over 2 years old, of both sexes, were slaughtered in abattoirs with a high number of head of cows (216 cattle). It was also in this age bracket that the number of parasitized animals was highest in the region's abattoirs (55%).

Table 1: Collection of intestinal samples by abattoir and sex of cattle

Slaughterhouses	Animal sex	Animal Age	Number of Samples	Positive Laboratoiry Results
Biankouma	Male	<1 year	0	0
		1 à 2 years	5	1
		> 2 years	12	3
	Female	<1 year	0	0
		1 à 2 years	17	9
		> 2 years	61	33
	Subtotal 1		**95**	**46**
Danané	Male	<1 years	3	0
		1 à 2 years	34	12
		> 2 years	7	3
	Female	<1 year	0	0
		1 à 2 years	16	8
		> 2 years	65	32
	Subtotal 2		**125**	**55**
Man	Male	<1 year	5	1
		1 à 2 years	29	11
		> 2 years	13	7
	Female	<1 year	0	0
		1 à 2 years	37	13
		> 2 years	86	29
	Subtotal 3		**170**	**61**
Total (1+2+3)			**390**	**162**

3.1.3- Cryptosporidiosis prevalence by abattoir

The prevalence of cryptosporidiosis in meat from the main abattoirs was high from one abattoir to another. Out of a total of 390 samples taken, positive laboratory results were estimated at 41.54% (95% CI [36.60%; 46.60%]). The Man abattoir recorded the highest prevalence rate (15.64%). This corresponds to the presence of oocysts in the intestines of cattle that had been in contact with the meat. Analysis of the comparison of prevalences between the different abattoirs in the Tonkpi Region was not significant ($p>0.05$).

4- Identification of pathogens

The samples were stained with May-Grünwald Giemsa (MGG). When read using a LEICA DM 1000 optical microscope with a ×100 objective, the results revealed the presence of cryptosporidium oocysts in the intestines. The species identified was *Cryptosporidium*

parvum, present in the intestines of cattle whose contents had contaminated the carcass.

Table 2: Prevalence of cryptosporidiosis by abattoir

Abattoirs	Number of samples	Laboratory results		Prevalence (95% CI)
		Negative	Positive	
Biankouma	95	49	46	11,79 (8,77-15,42)
Danané	125	70	55	14,10 (10,80- 17,96)
Man	170	109	61	15,64 (12,18- 19,63)
Total	**390**	**228**	**162**	**41,54 (36,60- 46,60)**
Khi-deux				0,57143
p-value				0,7515

CI: Confidence interval

3.2.DISCUSSION

Meat is the product of muscle processing after the death of the animal. It is traditionally considered to be the vehicle of many food-borne diseases in humans due to hygiene defects (Dennaï *et al.,* 2001; Fosse *et al.,* 2006). Slaughterhouses are establishments in which animals intended for human consumption are slaughtered and prepared (Dieye, 2011). They are the preferred site for meat health risks, due to the concentration of slaughtering, and are the most representative for postmortem health inspection of cattle (Mckenzie and Hathaway, 2006). The main sources of contamination are constant contact between operators' hands and exposed carcass surfaces, contact between skinned carcasses and unnecessary handling of carcasses by apprentice butchers (Aboly, 2022).

When it comes to transport, the means of transport are not at all appropriate, and the carcass can also deteriorate due to poor transport or storage conditions. This is the case for the contents of cattle intestines, which come into contact with carcasses as they are transported from slaughterhouses to butchers' shops. A total of 390 intestinal samples were taken and sent to the laboratory for analysis. Some samples tested positive (41.54 (36.60- 46.60)), and the pathogen species identified was *Cryptosporidium parvum*.

Statistical analysis of the results from one abattoir to another was not significant. It should be emphasized that cryptosporidiosis is a cosmopolitan parasitosis, observed in sporadic or epidemic form (drinking water reservoir, swimming pools, direct human-to-human, crèche, infected animals...), with highly variable incidence and prevalence (ANOFEL, 2016). This disease is one of the emerging zoonoses and opportunistic diseases of AIDS (AFFSA, 2002). According to Khelef (2007), human contamination occurs through ingestion of oocysts, which lead to more serious disorders. This could be explained by the fact that contamination can be direct between an infested host and a healthy host in cases of healthcare-associated infestations, where an endogenous cycle (self-infestation) is possible and favored by immunodepression. The respective proportions of the different sources or modes of contamination (human-to-human, dietary, environmental) are not known. Contamination may be indirect, through ingestion of water or food contaminated with oocysts. It can also result from aquatic activities such as swimming (Aboly, 2022). Numerous epidemics are regularly observed in industrialized countries, due to fecal contamination of drinking water distribution networks. In these contexts, the use of chlorine on Cryptosporidium is virtually ineffective (ANOFEL, 2016). Usually, the disease heals spontaneously in the immunocompetent patient, but can be persistent and severe in the case of acquired immunodeficiency syndrome (AIDS). Medical and paramedical staff, livestock farmers and veterinarians are particularly at risk. Travel to countries with low levels of hygiene can be considered a risk factor for contracting cryptosporidiosis (ANSES, 2010).

The vast majority of cases of human cryptosporidiosis (>90%) are due to *Cryptosporidium parvum* (main animal reservoir: ruminants) or *Cryptosporidium. hominis*. The other species are mainly found in immunocompromised subjects (AFFSA, 2002).

4. CONCLUSION

Meat from abattoirs in the Tonkpi Region is subject to contamination during transport to the point of sale. Cryptosporidiosis prevalence (41.54%) was observed on carcasses from abattoirs that had been in contact with the intestines, including 15.64% in Man, 14.10% in Danané and 11.79% in Biankouma. Cows over 2 years of age slaughtered in abattoirs accounted for 55% of infected animals. Analysis of the comparison of prevalences from one abattoir to another revealed a non-significant difference (p> 0.05). Meat produced in slaughterhouses or slaughter areas and sold on the market thus represented a real risk for consumers. These risks are mainly contamination via hands, knives, butchers' protective aprons and the practice of evisceration on the floor. It should be noted that cryptosporidiosis is one of the emerging zoonoses and is mainly found in immunocompromised subjects. As for the future, more exhaustive studies are needed to better define all the epidemiological and etiological aspects (virological, bacteriological, parasitological). Awareness-raising

campaigns are essential to encourage butchers and slaughterhouse workers to adopt good hygiene practices.

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STATEMENT OF AUTHORS CONTRIBUTIONS

The study and writing of the manuscript were carried out in a collegial manner. Nevertheless, according to the following steps, ABN and TA participated in the design and planning of the study. TA and ABN performed the laboratory analyses. ABN, TA and CF collected the data and drafted the first version of the manuscript. YA, MB and ABN performed statistical analyses and data interpretation. YA, MB and CF critically revised the manuscript.

Conflicts of Interest

The study was carried out without any conflicts of interest.

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