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Seroprevalence of Toxoplasmosis in Pigs (*Sus Scrofa Domesticus*) in the Department of Korhogo

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Abstract | **Original Research Article**

The aim of this study was to contribute to the fight against toxoplasmosis in pigs in Côte d'Ivoire. Pig samples were analysed using the ELISA technique for the detection of antibodies (IgG) against *T. gondii*. A total of 180 pigs, including 86 females and 94 males, were screened in the study. The results of the analysis showed a seroprevalence of 18.09% for males and 15.12% for females. By age, 23.08% of pigs aged under 5 months were seropositive, compared with % of pigs aged between 5 and 11 months and % of pigs aged over 11 months. As for the localities involved in the study, all were infested with Toxoplasma. The data from this preliminary study could be used by animal health professionals to reinforce existing preventive measures aimed at protecting consumer health.

Keywords: Seroprevalence, Toxoplasmosis, Pigs, Korhogo.

INTRODUCTION

Infectious and parasitic diseases are the second leading cause of death in humans worldwide (who, 2004) and around two-thirds of these are zoonoses (cleavelande *et al.*, 2001); diseases that can be transmitted to humans by animals. According to an INVS report (2003), parasites are involved in 46% of cases of food-borne infection. Toxoplasmosis is an ubiquitous, cosmopolitan parasite caused by the protozoan *Toxoplasma gondii*. It is one of the most common parasitic zoonoses (Radu *et al.*, 2013). The parasite is adapted to infect all warm-blooded animals, including humans (Vitomir, 2016). Human contamination occurs either through ingestion of cysts contained in meat, particularly pork or mutton, raw vegetables or drinking water, especially in poor countries where hygiene conditions are precarious, or through the placenta, causing congenital toxoplasmosis (Adoubryn, 2004). In humans, these parasites can cause severe forms of toxoplasmosis (Radu *et al.*, 2013; Montoya *et al.*, 2004; Pappas *et al.*, 2009; Brenier *et al.*, 2003). In the case of congenital transmission in non-immune pregnant women, it can lead to abortion, as well as serious malformations of the unborn child [10]. (French Ministry of Agriculture and Fisheries, 2006). However, in animals, this disease is one of the main causes of abortion and stillbirths, and causes significant economic losses, particularly in small domestic ruminant and pig farms (Dia, 1992; Dubey, 2009). In Africa, the prevalence of toxoplasmosis is estimated at 40-70% in pregnant women and 20-55% in domestic animals (Adoubryn *et al.*, 2004). In Côte d'Ivoire, where pig production is estimated at 56,128 head [13]. (Anonyme, 2006), the prevalence of toxoplasmosis in pregnant women has been estimated at 60% (Adoubryn *et al.*, 2004). This represents a major public health risk, as well as a source of economic losses for the livestock industry. In most villages in the north of Côte d'Ivoire, pigs are widely reared in the wild. In this region of the country, few studies have been conducted on the seroprevalence of toxoplasmosis in pigs reared on traditional farms, which means that there is a lack of reliable data on which to base a better control strategy. The general objective of this study is to contribute to an update of existing data on swine toxoplasmosis in the Korhogo department in order to better contribute to its control. More specifically, the aim will be to determine the prevalence of toxoplasmosis

and to identify the factors that expose pigs to toxoplasmosis.

MATERIALS AND METHODS

Study Site

Korhogo department is located between 5°16 and 6°16 west longitude, and 8°32 and 10°20 north latitude (N'guetta, 2012). It is bordered to the north by the department of M'bengué, to the north-east by the department of Sinématiali, to the north-west by the Bagoué region, to the south-west by the department of Mankono and to the south-east by the Hambol region. It has sixteen (16) sub-prefectures: Korhogo, Karakoro, Komborodougou, Koumborokoura, Koni, Sohouo, Nionfoin, Ngannon, Lataha, Sirasso, Tioro, Napié, Nafoun, Kiémou, Dassoungbo, Kanoroba (figure 1).

Spread over an area of 12,500 km², or 3.9% of the national territory, the population of the Department is 536,851 (RGPH, 2014). The population is made up of indigenous Senufo, non-natives from various regions of Côte d'Ivoire and non-natives from ECOWAS countries, particularly Burkina Faso and Mali.

The climate in northern Côte d'Ivoire is dry tropical, also known as Sudano-Guinean. The climate is marked by two main seasons. The rainy season from May to October and a dry season from November to April. The dry season is accompanied by the harmattan, which begins in December and ends in February, with hot spells.

Hydrographically, the waters are drained by the Bandama catchment area. The network is relatively dense. It is a tributary of Bandama. Annual rainfall in the north varies between 1,200 and 1,400 mm (Anonymous, 2013).

In terms of geology, Korhogo's soils are ferralitic and typical of granite. There are two (2) main types of soil in the region:

- Moderately desaturated ferralitic soils;
- Soils on basic rocks and armouring zones.

In terms of soil typology, it should be noted that the soils are mainly formed on granite (acid rock).

The relief of Korhogo department is characterised by a vast series of plateaux, topped in places by a few isolated elevations, made up of granite domes and hills with a predominance of plateaux whose altitudes vary from 300 to 500 m. There are isolated mountains and granite mountain ranges. The whole area is made up of buttes and lateritic breastworks.

The majority of the population of Korhogo department derives its income from the main agricultural crops, both perennial (cotton, cashew nuts and mangoes) and food crops (rice, maize, millet, groundnuts, chillies, etc.).

Cotton is the leading cash crop, with annual production estimated at 180,000 tonnes in the Korhogo department. Food crops ensure the subsistence of local populations and are also an important source of wealth for the population. With a total production of 13,488 tonnes of maize with an average turnover of 2,036,688,000 CFA francs and 4,944 tonnes of chilli peppers with a cash value of 2,259,408,000 CFA francs were produced in Korhogo according to the Office d'aide à la Commercialisation des Produits Vivriers in 2012. As the natural environment is very favourable, livestock farming plays a key role in the local economy. The industrial sector is poorly represented by agro-industries.

The hydrographic network of the Korhogo department is drained by the Bandama watershed. The main tributaries are the Solomougou, the N'Zi, the Bedanon, the Lopkoho and the Koko-badeni. Most of these watercourses are temporary and often dry up during the dry season. A large number of dams have also been built in the Korhogo department. There are 122 pastoral dams (Ouattara, 2009). These dams are used to irrigate rice paddies and market garden crops located downstream in the baffons, they also provide watering points for animals and a source of drinking water for the local population.

Choice of Site

To carry out our work, we randomly selected four (4) sub-prefectures (Korhogo, Lataha, Tioro and Napié) from the list of sub-prefectures in the department of Korhogo. In each sub-prefecture, three (3) villages were drawn at random. We visited 16 villages.

Methods

Sampling

Sampling consisted of defining the size of the study sample and selecting the site and target population.

Sample size

The size of the survey population had been determined according to the following formula [17]. (Atobla *et al*., 2015):

$$n = \frac{P * (1 - P)}{(\frac{E}{1{,}96})^2}$$

In this formula:

n = minimum survey population size
P = the prevalence of pork consumption
E = margin of error

Given the lack of data on the prevalence of swine toxoplasmosis in Côte d'Ivoire, we used a prevalence (P) of 50%. The margin of error (E) was 5% and the confidence interval 95%. The sample size (n) was 180 subjects.

Figure 1: Map of Korhogo Department.
Source: www.Tourismeci.com

Blood Sampling

After restraining the animals, we punctured an auricular vein and collected the blood in 5 ml VENOJET tubes. The dry 5 ml VENOJET tubes used to collect the blood were placed on a rack, which was then placed in the cooler for better preservation.

Serum Collection

The tubes containing the blood were kept at room temperature for half a day to allow the blood to settle.

The serum was then collected and purified by centrifugation at 3000 rpm for 10 minutes. The final serum was collected in micro-tubes and stored at -120°C until use.

Laboratory Analysis Method (ELISA Technique)

The entire blood sample is taken in order to extract the serum using regular methods. The serum must be clear and free of hemolysis. Before carrying out the analysis, the platelet to be analyzed must be prepared. To do this, we start by preparing the washing solution. In the kit, we take the concentrated wash buffer 10 times and bring it to room temperature before use. If it contains salt crystals, shake to dissolve them, and then we used distilled water to dilute 10 times. The resulting wash solution can be stored for one week at 4°C. Next, the serum sample is placed on the serum dilution plate at a rate of 1/100. Be sure to record the position of each serum on the plate.

For the ELISA test, a plate previously coated with the anti-toxoplasma antigen has been mobilised to receive 100µl of the contents of the dilution plate, ensuring that each well in the antigen plate corresponds to a single well in the dilution plate. Add 100µl of negative and positive control to the wells provided for the reservoirs, shake gently before covering. Incubate at 37°C for 30 minutes. When the time has elapsed, pour the liquid out of the wells, add 250µl of diluted wash solution to each well, pour and repeat 4 to 6 times, then dry well using absorbent paper. Add 50µl of enzyme conjugate to each well and incubate at 37°C for 30 minutes. When the time has elapsed, pour the liquid out of the wells, add 250µl of the diluted wash solution to each well, pour and repeat 4-6 times, then dry well using absorbent paper again.

Add 100µl of substrate (TMB) to each well, homogenize and leave for 10 minutes at 37°C in the dark.

Statistical Analysis

Data were processed using Excel. This software was used to produce graphs and tables and to calculate prevalences. The prevalence (P) of toxoplasmosis was calculated using the following formula:

$$\text{Prevalence en \%} = \frac{\text{Number of cases of the disease} * 100}{Total\ number\ of\ sick\ individuals}$$

RESULTS

Characteristics of the Populations Studied

Of 180 pigs sampled in Korhogo department, 86 (47.77%) were females compared with 94 males (52.23%). The animals sampled during this study were mostly pigs aged between 5 and 11 months. They accounted for 45% of the animals compared with 28.89% of pigs aged less than 5 months and 26.11% of pigs aged over 11 months.

At sub-prefecture level, 62 (34.44%) pigs were sampled in Korhogo, 44 (24.44%) in Lataha, 38 (21.11%) in Tioro and 36 (20.01%) in Napié respectively (Table I).

Table I: Breakdown by locality, sex and age

		Sub-prefecture				Department
Risk factors		Korhogo	Lataha	Tioro	Napié	
Sex	Female	28	26	15	17	86
	Male	34	18	23	19	94
Age	<5 months	20	13	10	9	52
	5 à 11 months	18	25	18	20	81
	+11 months	24	6	10	7	47
Total		62	44	38	36	180

Global Prevalence

Out of 180 tested pigs, 30 were positive for anti-*T. gondii* IgG antibodies infected with toxoplasmosis, giving an overall prevalence of 16.67%.

Overall Prevalence in Pigs Sampled By Sex

The prevalence of toxoplasmosis in pigs sampled according to sex was higher in males (18.09%) than in females (15.12%) (Figure 2).

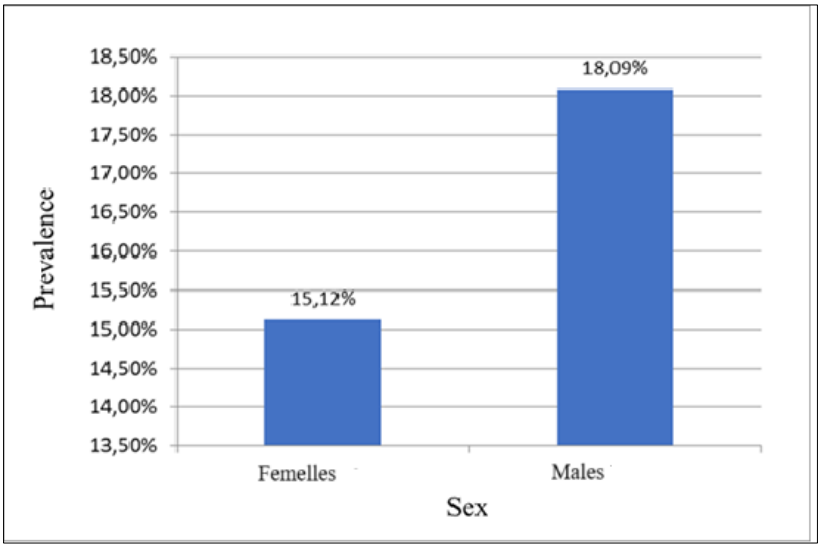

Figure 2: Prevalence of toxoplasmosis according to sex

Prevalence in Pigs Sampled According to Age

In the Korhogo department, the prevalence of toxoplasmosis in pigs varied according to the age of the animals sampled (Figure 3).

Of all the age groups defined, the highest prevalence (23.08%) was found in animals aged less than 5 months. This was followed in decreasing order of importance by animals aged over 11 months (14.89%) and animals aged between 5 and 11 months (13.58%). Thus, the seroprevalence of toxoplasmosis appears to decrease between the first 2 age groups before increasing between the last 2 age groups (figure 3).

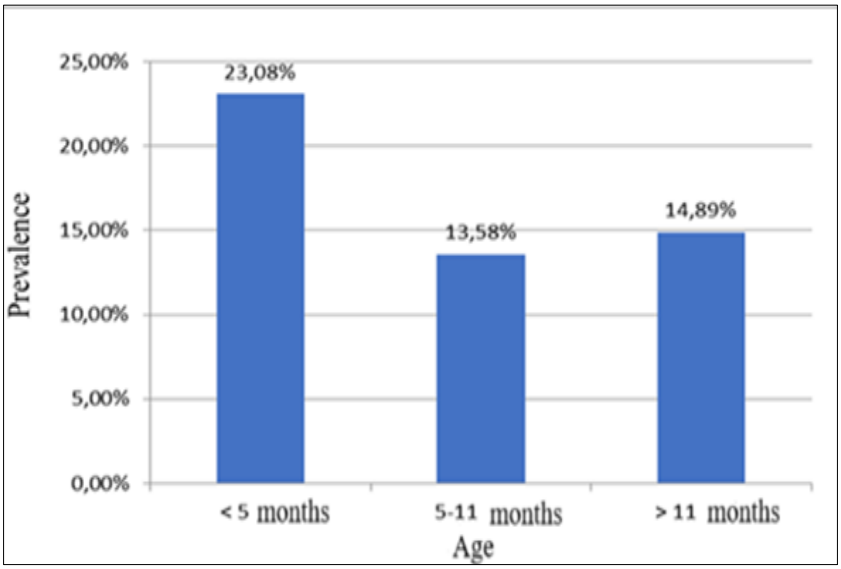

Figure 3: Prevalence of toxoplasmosis according to age

Prevalence in Pigs by Locality

The prevalence of toxoplasmosis in pigs by locality is shown in Figure 4 and varies considerably from one sub-prefecture to another. The highest rates were found in the sub-prefectures of Tioro and Korhogo. They were 21.05% and 20.97% respectively. On the other hand, the lowest prevalence was observed in the sub-prefecture of Lataha, with an infestation rate of 9.09%. In Napié, the average prevalence of the disease was 13.89% (Figure 4).

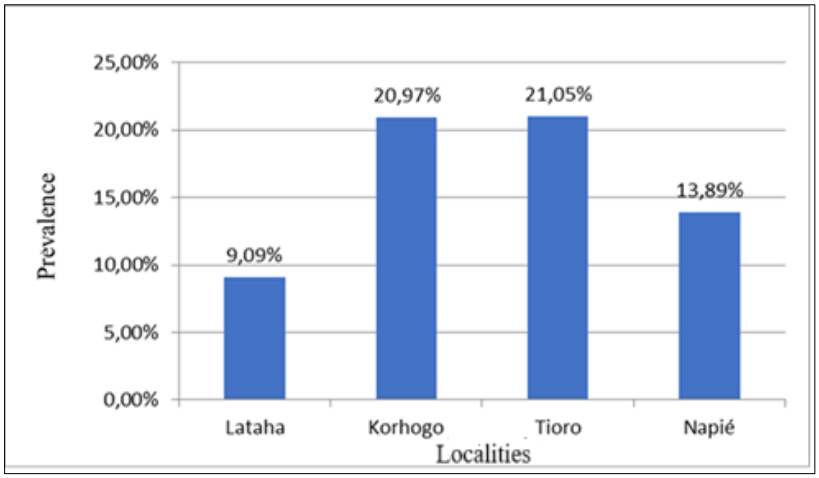

Figure 4: Prevalence of toxoplasmosis in the sub-prefectures studied

Toxoplasmosis Infection Rate in the Sub-Prefectures Studied

Analysis of the laboratory data according to the villages investigated revealed that all 11 localities sampled were contaminated with *T. gondii*. The seroprevalence of toxoplasmosis varied in these villages. It ranged from 5.26% to 36.36%. In the villages of the Tioro sub-prefecture, the seroprevalence varied between 11.11% and 36.36%. On the other hand, in the sub-prefecture of Lataha, seroprevalence varied from 6.67% to 12.5%. In Korhogo, the seroprevalence of toxoplasmosis in the villages sampled ranged from 5.56% to 13.04%, compared with 5.26% to 23.53% in the Napié sub-prefecture.

Table II: Contamination rates and prevalence in villages sampled in each sub-prefecture

Localities	Number of sampled villages	Number of infected villages	Percentage of infected villages	Extremes values for villages prevalences
Lataha	3	3	100,00%	6,67%-12,5%
Korhogo	3	3	100,00%	5,56%-13,04%
Tioro	3	3	100,00%	11,11%-36,36%
Napié	2	2	100,00%	5,26%-23,53%
Total	11	11	100,00%	5,26%-36,36%

Factors in the Exposure of Pigs to Toxoplasmosis by Age Group

Prevalence of Toxoplasmosis in Young Pigs Under 5 Months of Age

As shown in Figure 5, young male pigs were more infested with toxoplasmosis than young females. In males, the prevalence was 24.14% compared with 21.74% in females (Figure 5). The prevalence of toxoplasmosis varied greatly between the different sub-prefectures. It ranged from 10% to 50% (Table III).

The lowest prevalence in this age group was noted in the Korhogo sub-prefecture (10%) compared to 50% in the Tioro sub-prefecture. In the sub-prefectures of Napié and Lataha, 33.33% and 15.38% of animals in this age group were infested with the parasite (Table III).

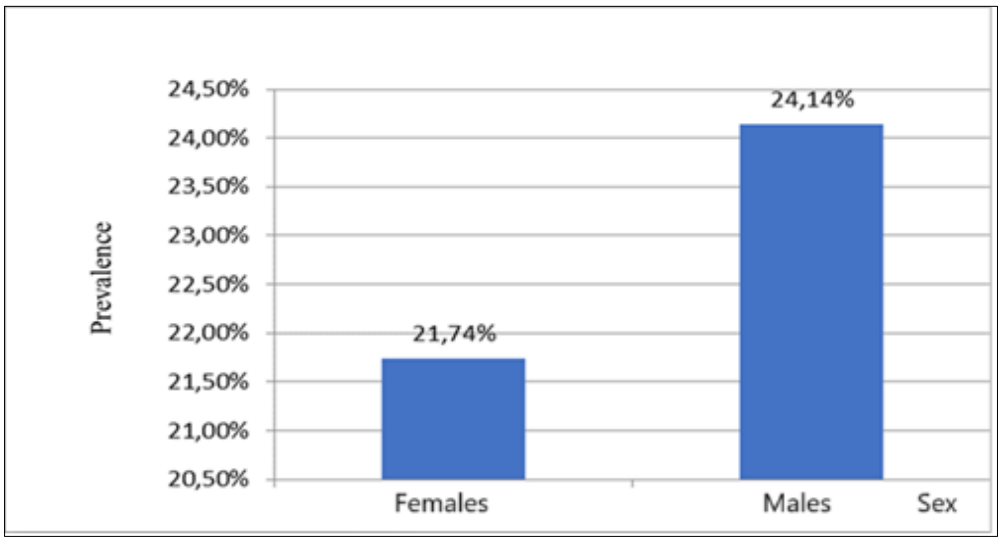

Figure 5: Prevalence in young pigs aged less than 5 months, by sex

Table III: Prevalence of toxoplasmosis in young pigs by locality

Localities	Workforce	Positifs	Prevalence
Lataha	13	2	15,38
Korhogo	20	2	10
Tioro	10	5	50
Napié	9	3	33,33
Total	52	12	23,08

Seroprevalence of Toxoplasmosis in Fattening Pigs

In pigs aged between 5 and 11 months, seroprevalence was uneven. It was higher in males (15.00%) than in females (12.00%) (Figure 6). Similarly, in the sub-prefectures investigated, the seroprevalence of toxoplasmosis in animals in this age group varied (Table IV). Depending on the sub-prefecture, the prevalence of toxoplasmosis ranged from 4% to 38.89%. The highest seroprevalence of toxoplasmosis in animals in this age group was recorded in Korhogo. It was 38.89% in this zone, compared with 11.11% in Tioro. The lowest seroprevalence rates were recorded in Lataha and Napié. In these localities, they were 4.00% and 5.00% respectively (Table IV).

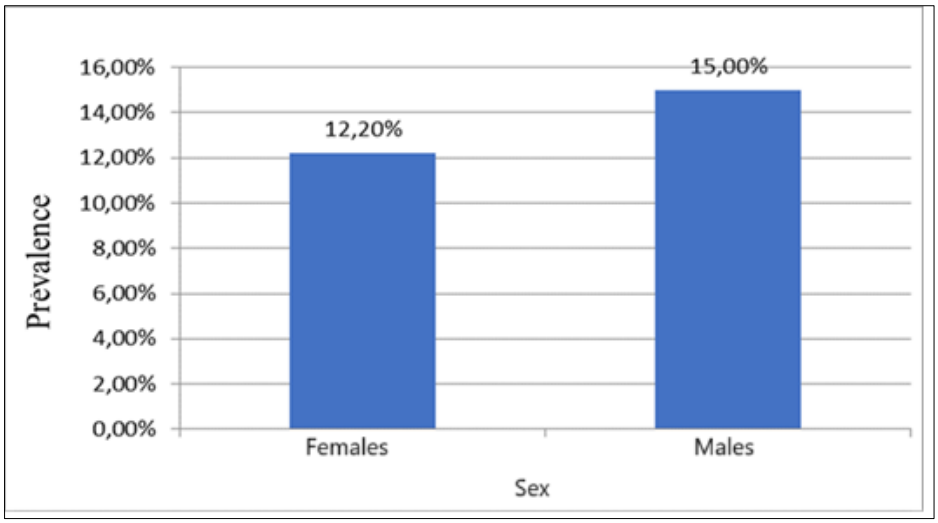

Figure 6: Seroprevalence of toxoplasmosis in fattening pigs by sex

Table IV: Seroprevalence of toxoplasmosis in fattening pigs by locality

Localities	Workface	Positifs	Prevalence
Lataha	25	1	4,00%
Korhogo	18	7	38,89%
Tioro	18	2	11,11%
Napié	20	1	5,00%
Total	81	11	13,58%

Prevalence of Toxoplasmosis in Adult Pigs

Seroprevalence of toxoplasmosis in adult male pigs was higher (16%) than in adult female pigs (13.64%) (Figure 7). In the sub-prefectures selected for this study, the prevalence of toxoplasmosis varied from 10% to 16.67% (Table V). Of 47 adult pigs sampled during the study, only seven were positive for toxoplasmosis, giving an overall prevalence of 14.89%. However, in the sub-prefectures of Lataha and Korhogo, this prevalence was 16.67% compared to 14.29% and 10% respectively in Napié and Tioro (table V).

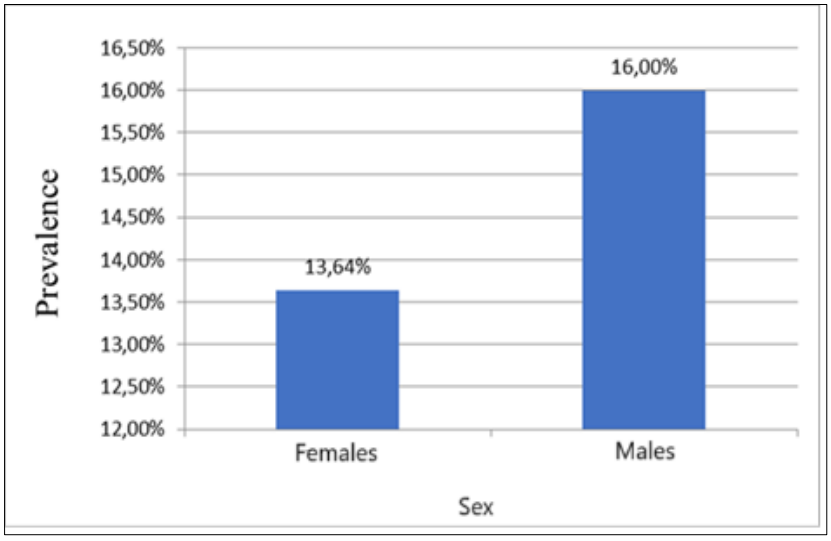

Figure 7: Seroprevalence in adult pigs according to sex.

Table V: Seroprevalence in adult pigs by locality

Localities	Workforce	Positifs	Prevalence
Lataha	6	1	16,67%
Korhogo	24	4	16,67%
Tioro	10	1	10,00%
Napié	7	1	14,29%
Total	47	7	14,89%

Factors in the Exposure of Pigs to Toxoplasmosis, By Sex

Seroprevalence of Toxoplasmosis in Females by Age Group

Of all the age groups defined, animals under 5 months of age showed the highest prevalence (21.74%). This was followed in order of decreasing importance by that of subjects aged over 11 months (13.64%) and that of animals aged between 5 and 11 months (12.20%). Thus, the seroprevalence of toxoplasmosis appears to decrease between the first two age groups before increasing between the last 2 age groups (Figure 8).

Of 86 females sampled, 13 were infested with the parasite *Toxoplasma gondii*, with prevalence varying from one locality to another. Tioro had a high prevalence of 26.64%, while Lataha and Napié had a low prevalence of 11.54% and 11.76% respectively (Table VI).

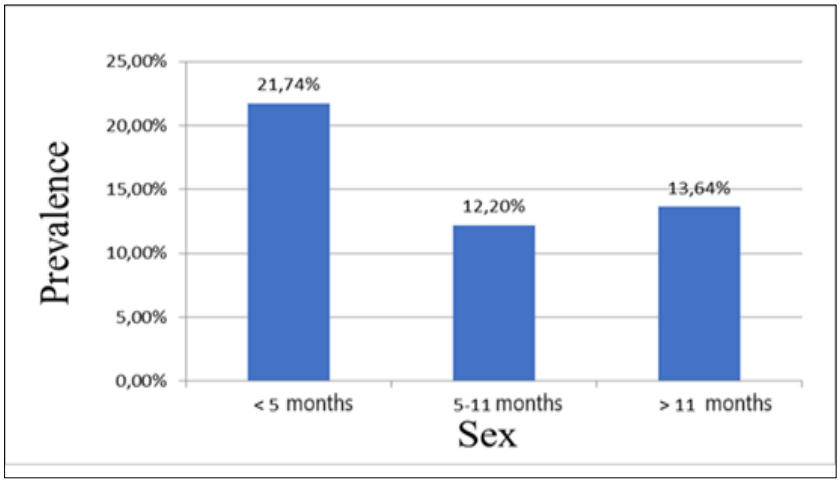

Figure 8: Prevalence of toxoplasmosis in females as a function of age

Table VI: Prevalence in females according to locality

Localities	Workforce	Positifs	Prevalence
Lataha	26	3	11,54%
Korhogo	28	4	14,29%
Tioro	15	4	26,67%
Napié	17	2	11,76%
Total	86	13	15,12%

Prevalence of Toxoplasmosis in Males by Age Group

Males under 5 months of age had a high prevalence of 24.14% compared with males aged 5-11 months, which had a lower prevalence of 15.00%, followed by males over 11 months, which also had a low prevalence but slightly higher than males aged 5-11 months, with a prevalence of 16.00% (Figure 9).

Of the 94 male pigs sampled, 17 were positive for toxoplasmosis. However, this prevalence varied from one locality to another. The Korhogo sub-prefecture had the highest prevalence, with an infestation rate of 26.47%, compared with 17.39% and 15.79% for the Tioro and Napié sub-prefectures respectively. The lowest prevalence for animals in this age group was recorded in the sub-prefecture of Lataha with a rate of 5.56% (Table VII).

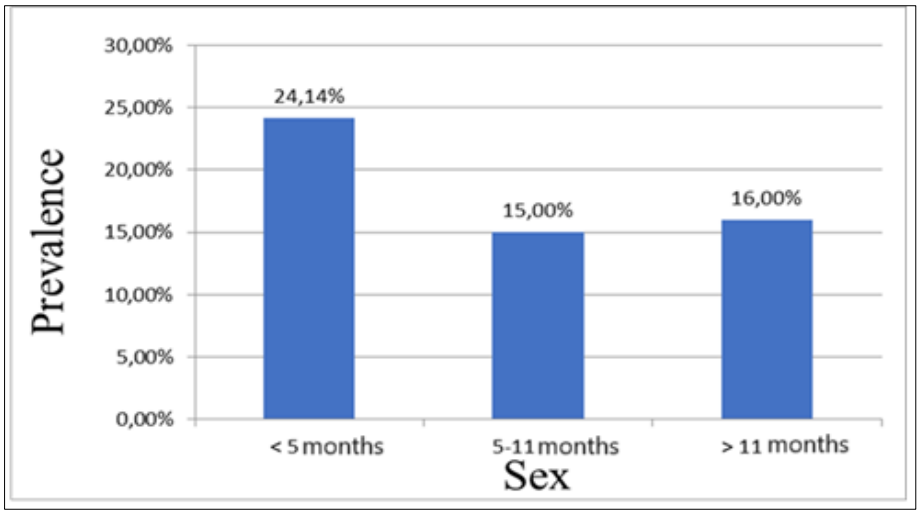

Figure 9: Prevalence of toxoplasmosis in males by age

Table VII: Prevalence of toxoplasmosis by locality.

Localities	Workforce	Positifs	Prevalence
Lataha	18	1	5,56%
Korhogo	34	9	26,47%
Tioro	23	4	17,39%
Napié	19	3	15,79%
Total	94	17	18,09%

DISCUSSION

During this study, 180 pigs were sampled in 4 sub-prefectures of the Korhogo department. The results of the ELISA test showed that the overall prevalence of toxoplasmosis in this department was 16.67%. This result is similar to that obtained in Spain by García-Bocanegra and his collaborators[18] but differs from those of many authors. Indeed, the seroprevalence of toxoplasmosis determined in the Korhogo department is lower than that of obtained in Ibadan in Nigeria (29.14%) (Onyiche and Ademola, 2015), in Hubei in central China (24.5%) (*Tao et al.*, 2011), in Germany (69.1%) [21]. (Damriyasa *et al.*, 2004) and in Italy (42.3%) (Villari *et al.*, 2009). However, the anti-*T. gondii* antibody positivity rate of pigs reared in the Korhogo department remains higher than that obtained by Powell and his collaborators [23], in the United Kingdom and Obijiaku and his collaborators in Makurdi, Nigeria.

Analysis of the results obtained revealed that the seroprevalence of toxoplasmosis in the Korhogo department was higher in males (18.09%) than in females (15.12%). These results are in agreement with the work of Obijiaku and his collaborators [24], at the Makurdi abattoir in Nigeria, which determined a prevalence of 5.4% in males compared with 4% in females. However, our results contradict those obtained by Onyiche and Ademola (2015) in Ibadan, also in Nigeria. During their epidemiological survey, these authors noted a prevalence of anti-*Toxoplasma gondii* antibodies of 37.5% in females compared with 17.46% in males.

With regard to the age risk factor, data analysis revealed that the seroprevalence of toxoplasmosis in pigs aged under 5 months was 23.08%. This was higher than pigs aged between 5 and 11 months (13.58%) and in animals aged over 11 months (14.89%). These results contrast with those of Tao and his collaborators [20], and Onyiche and Ademola [19], who worked on the risk factors associated with T. gondii seropositivity in pigs in Hubei, China and Ibadan, Nigeria. According to these authors, the seroprevalence of *T. gondii* infection in adult pigs was higher than in piglets due to the increasing exposure of pigs to *T. gondii*, confirming the work of Silva and his collaborators [25].

The change in the prevalence of toxoplasmosis observed in our study could be explained by three hypotheses. The very high prevalence of anti-*Toxoplasma gondii* antibodies in animals aged less than 5 months compared with other age groups could be due to the fact that some animals in this group still had maternal antibodies at the time of sampling. In addition to this fringe of the sub-population of young animals, other subjects, healthy at the time of their weaning, very quickly became infested because they were put out to pasture at an early stage. Under these conditions, these young animals come into contact with sources of contamination in the environment very early on.

The drop in prevalence observed between the group of animals aged less than 5 months and those aged between 5 and 11 months could be explained by the fact that beyond the age of 5 months, all young animals have lost their maternal antibodies. As a result, seropositive animals in this age group were probably contaminated from outside. On the other hand, the increase in the prevalence of toxoplasmosis between the group of fattening pigs (5 to 11 months) and the animals aged over 11 months could be due to the length of contact between the animals and the sources of contamination in the outside environment. In addition, of the 11 villages sampled, 11 were contaminated, i.e. 100%. This result confirms the fact that *Toxoplasma gondii* is a cosmopolitan parasite, as highlighted by Kamani and his collaborators.

CONCLUSION

This work was carried out in the department of Korhogo (Côte d'Ivoire) from February to May 2019. It showed that the overall prevalence of this parasitic disease was 16.67% in the pigs sampled. The presence of toxoplasmosis was higher in males than in females. Age-wise, pigs less than 5 months old were more affected.

The high prevalence and presence of the parasite in all the villages sampled confirms that this parasite is endemic in the Korhogo department, where all the conditions for its spread are present. This should justify stepping up screening when pigs are slaughtered and alerting the veterinary authorities to the risk of toxoplasma contamination. New lines of research need to be explored by researchers into the epidemiology of protozoa (*T. gondii* and *N. caninum*) in extensive livestock farms in northern Côte d'Ivoire. This work should make it possible to assess the level of contamination of abnormal foodstuffs and the risk they represent for local populations.

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