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# Coccidia of the grasscutter (*Thryonomys swinderianus*) in southern Côte d'Ivoire

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## Abstract

Coccidiosis is a limiting factor for livestock profitability in Africa. In Côte d'Ivoire, most farmers feed their grasscutters with fodder harvested from forage areas frequented by livestock and wild ruminants. To verify the presence and species of coccidia in grasscutters farms, coprological examinations were carried out on 150 wild and 150 farmed grasscutters from the south of the country. Histological sections were also performed on different portions of the digestive tract to identify the different stages of evolution of coccidia oocysts. Prevalences of coccidia in wild and farmed grasscutters were compared using the Chi-square test. The prevalence of coccidian oocysts in wild grasscutters was 70% compared to 22.6% in farmed grasscutters. Wild grasscutters also showed massive faecal excretion in contrast to farmed grasscutters (3067+/-1077 OPG in wild grasscutters versus 729+/-333 OPG in farmed grasscutters). The histological examinations allowed the identification in the small intestine of two evolution stages of coccidia oocysts: the microgametocyte and the macrogametocyte stage. Coprocultivation showed that all sporulated coccidia oocysts had four sporocysts in their cytoplasm, each containing two sporozoites. These characteristics correspond to that of coccidia of the genus *Eimeria*. Sporulation at ambient temperature occurred from day 6. Therefore six different forms suggesting six species were obtained. This study would contributeto a prophylaxis program based on the observations reported to interrupt the cycle of coccidian infestation in grasscutters farms in Côte d'Ivoire.

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## Introduction

Grasscutter farming is now major speculation in Côte d'Ivoire (Ettian *et al.* 2019) thanks to the awareness and training programs initiated and supported by the school of Fauna and Protected Areas (EFAP) of Bouaflé since 1995, followed in its action by the Project for Commercialization and Regional Initiatives (PACIL) with the National Agency for Rural Development (ANADER) and research and development programs initiated by Nangui Abrogoua University (UNA) and the National Polytechnic Institute Houphouet Boigny (INPHB). However, some factors such as diseases are a barrier to the development of this breeding. Common pathologies in grasscutters include alopecia, staphylococci, enterotoxaemia, helminthosis, dental diseases and coccidiosis (Zouh Bi, 2016).

Coccidiosis can cause heavy losses in breeding and is caused by coccidia which are common parasites of the digestive tract of many animal species. Coccidia is protozoa belonging to the Apicomplexa phylum. They have intracellular development and constitute an important etiology of intestinal disorders and complications. They causes tunting, reduce weight gain, deterioration of the consumption index, and/or diarrhea leading in some cases to death. The works carried out on grasscutters have identified the genus *Eimeria* in Nigeria (Omonona, 2011) in Benin (Mensah and Ekué, 2003) and Côte d'Ivoire (Abé, 2009). However, coccidian has very strong specificity concerning the animal species that they parasitize (Licois, 2010). This study aims to know coccidian different species in grasscutters in Côte d'Ivoire, their prevalence and the sporulation time of their oocysts.

## Material and methods

*Study areas and animals*

Côte d'Ivoire, a country located in the northern hemisphere in the humid and coastal zone of West Africa, is between the tropic of Cancer and the Equator, precisely between 4th and 10th degree of latitude north, and 2nd and 8th degree of longitude west. The study has been carried out from April 2010 to October 2012, on grasscutters selected from eight regions of the south: Abidjan and Yamoussoukro Districts, Agnéby-Tiassa, la Mé, Grands Ponts, Lôh Djiboua, Sud Comoé and Belier regions located in forest zone with high rainfall (Figure 1). These regions have been chosen because of the many existing grasscutters' farms.

The study was conducted on 150 farm grasscutters from fifteen farms. One hundred and fifty (150) wild grasscutters were also submitted for investigations and were from the regions quoted above.

*Parasitic analyzes*

Twenty grams (20g) of rectal contents of each animal were collected for coprological examinations. Coccidia oocysts were investigated and quantified using the modified Mac Master technique according to Gordon and Whitlock (1939) with a saturated solution of sodium chloride (density 1.20). Coccidian genus and species identification were possible thanks to a stool culture made according to the method of Grès *et al.* (2002). Feces were mixed with 2.5% potassium dichromate solution at the rate of one volume of feces for two volumes of potassium dichromate (Aziza and Bothaina, 2001). The preparation was kept in a petri dish at room temperature. Every day, coccidian oocysts were observed in this preparation by the flotation technique until complete sporulation. The length and diameter of each sporulated oocyst were recorded using a micrometer objective. The number of sporocysts, sporozoites, the presence of oocyst residuum and polar granules if possible were also noted. Histological sections were also performed on different portions of the digestive tract to identify the different evolution stages of coccidian oocysts.

*Statistical analysis*

The Microsoft Office Excel 2007 program was used for data entry, calculation of prevalence and parasite intensities. Statistical comparisons of oocysts prevalence and mean numbers were made with the $Khi^2$ test and the Student test, respectively. The difference was significant when p-value was lower than 0.05 ($p < 0.05$).

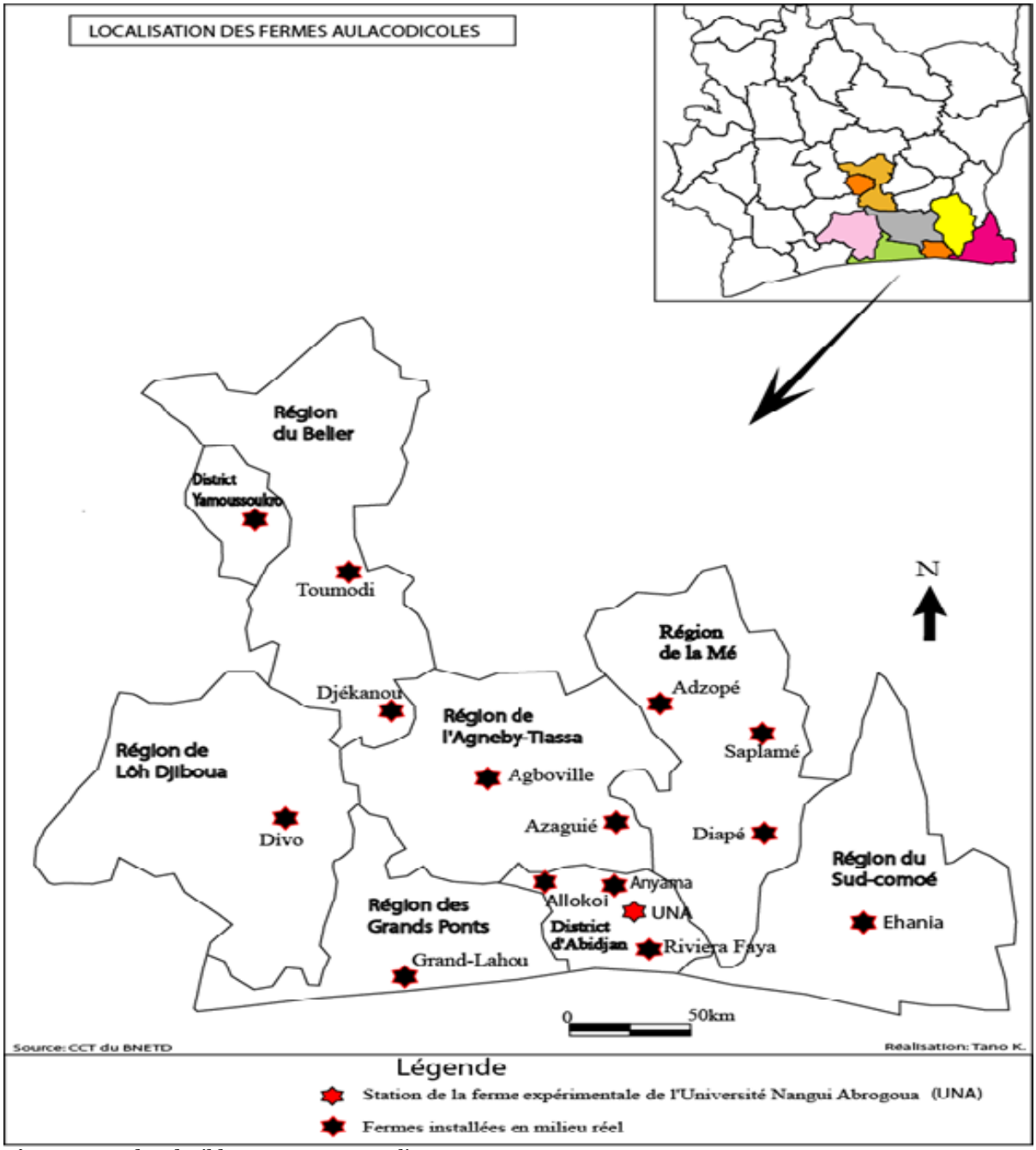


**Fig. 1.** Farmed and wild grass cutters sampling areas.

**Results**

Coccidia oocysts were harvest in both wild and farmed grasscutters in this study.

*Prevalence and average load*

The prevalence of coccidian oocysts in wild grasscutters was 70%. This value was significantly higher than that obtained in farmed grascutters (22.6%) according to the Chi² test ($p<0.05$). Wild grasscutters also experienced massive faecal excretion, unlike farmed ones. The average faecal excretion in wild grasscutters was 3067±1077 oocysts per gram of feces (OPG) with a minimum of 50 and a maximum of 45950. That obtained in farmed grasscutters was 729±333 OPG, with a minimum of 50 and a maximum of 1900.

*Evolution during the year*

The number of coccidian oocysts per gram of feces was very high throughout the experiment duration. In wild grasscutters, values ranged from 450 to 1700 oocysts per gram of feces from January to May. From

June, oocysts number increased to a maximum of 4040 OPG, and then decreased overall with unpredictable peaks to 833 OPG in December (Fig. 2). In farmed grasscutters, excretion remained below 500 OPG from January to May, increased in June to 1242±400 OPG in July, and then decreased continuously until December (245±115 OPG) (Fig. 2).

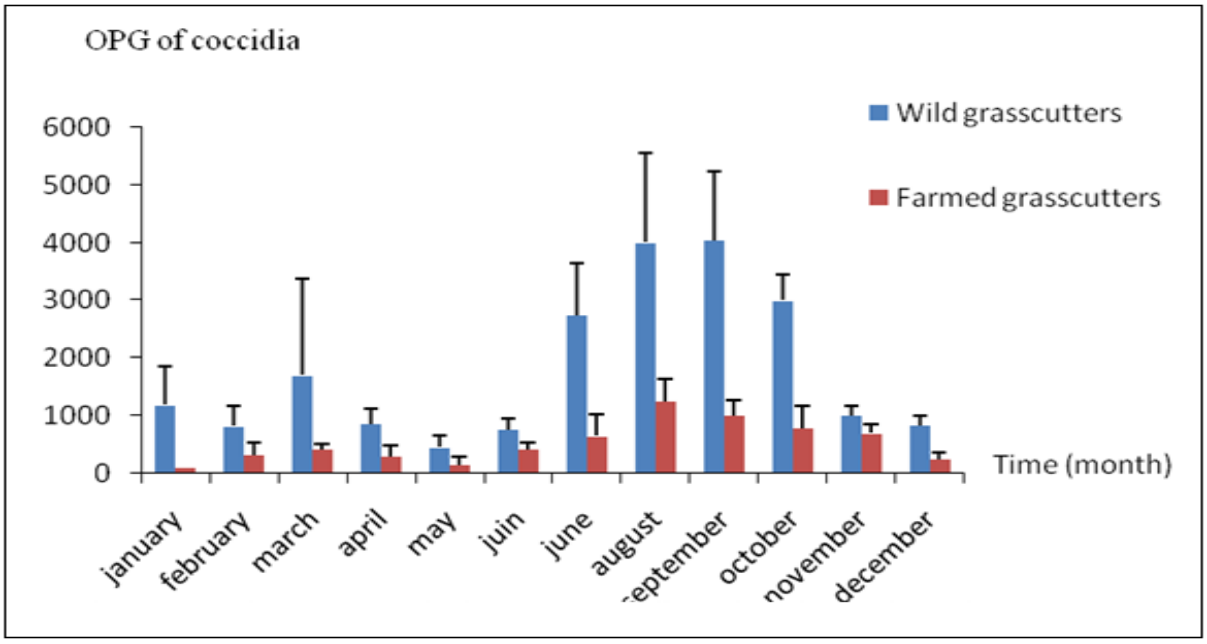


**Fig. 2.** Average load of coccidian ooccysts in grasscutters, during (year) in soth of Côte d'Ivoire.

*Some evolution stages of coccidia oocysts parasitizing the small intestine*

The observation of histological sections allowed the identification, in the small intestine of wild and farmed grasccutters, of two evolution stages of coccidian oocysts: microgametocyte stage (A) and macrogametocyte stage (B) (Fig. 3).


**Fig. 3.** Coccidia oocysts at two different stages in grasscutter' small intestine.

Microgametocytes correspond to oocysts of coccidia at the beginning of evolution and macrogametocytes correspond to those at the end of evolution.

These coccidia are located in the upper part of the intestinal epithelium. They cause moderate atrophy of the intestinal villi. Besides, there is a contingent of inflammatory cells in the *Lamina propia*. Also, there are erosive lesions of the intestinal epithelium.

*Forms of coccidia encountered in grasscutters*

Fecal culture made from grasscutters caecal content showed that all oocysts of coccidia having sporulated had in their cytoplasm four sporocysts, each containing two sporozoites.

Their outer wall is thick, smooth and transparent while the inner one is thin and opaque. These characteristics correspond to that of the genus *Eimeria*. Sporulation at room temperature in 2.5% potassium dichromate solution occurred from day six. The different forms obtained are the following:

*Eimeria* sp1

*Eimeria* sp1 oocyst has a subspheroidal or ovoid shape with a double wall without a distinct micropyle. The mean measurements are 34.56±4.92µm (30 to 45µm) long and 28.00±2.38µm (26.25 to 33.75) wide (the measurement of 50 oocysts) with a ratio of 1.23±0.12. The residual body is visible in some oocysts while others do not. Polar granules are sometimes present. Sporocysts are spherical without apparent Stieda body and have an average diameter of 11.25µm (Fig. 4, 5, 6).

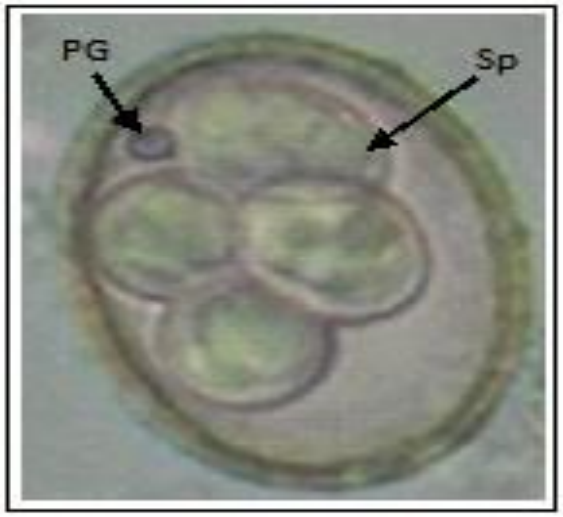


**Fig. 4.** Form with polar granule and without oocyst residuum.

Of all the forms of *Eimeria* identified, this form was the most abundant. It represented 40% of oocysts encountered.

*Eimeria* sp2

*Eimeria* sp2 is the most abundant form after *Eimeria sp*1 (21.40%). The oocyst has a subspheroidal shape with a double wall without a distinct micropyle. Mean measurements are 30±1.92µm (26.75 to 33.75µm) long and 26.25±1.18µm (26.25 to 33.75) wide (25 oocyst measurements) with a ratio of 1.14±0.4. It has no oocyst residuum or polar granule. Sporocysts are subspheoridal without apparent stieda body and have an average diameter of 11.25µm (Fig. 7, 8, 9).

*Eimeria* sp3

The third form *Eimeria* sp3 represents 15.22% of obse rved coccidias. It has an ovoid shape for some and sub-spheroidal shape for others with a double wall without distinct micropyle.


**Fig. 5.** Form without polar granule and oocyst residuum.

The average length is 37.84±3.12µm (33.75 to 41.25µm) and the average width is 28.97±2.42µm (26.25 to 33.75µm) (50 oocysts measu re) witha ratio of 1.27±0.15. There is no oocyst residuum and polar granule in some oocysts while others have it. Sporocysts are ovoid, elongated with 17.43±1.67µm long and 11.04±0.62µm wide. Stieda body is absent in sporocysts (Fig. 10).

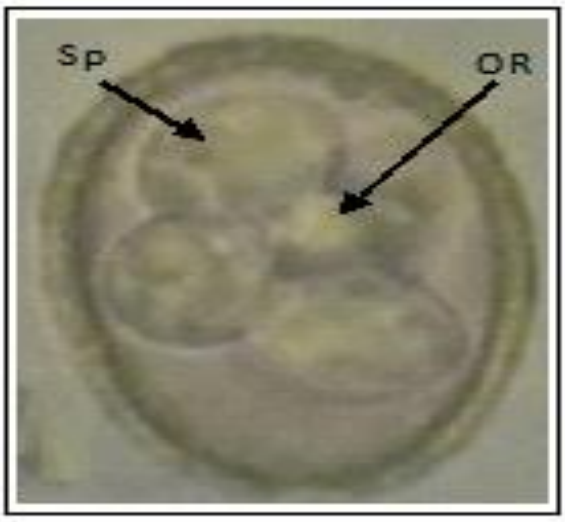


**Fig. 6.** Form with polar granule and with oocyst residuum. PG : Polar granule ; Sp : Sporocyst ; OR : oocyst residuum.

*Eimeria* sp4

The oocyst of these coccidias also has an ovoid shape.

The difference with the others is that it has a double wall with a mycropyle. The oocyst residuum and the polar granule are present. Three sporocysts are ovoid, elongated and the fourth is sub-spherical. There is no Stieda body in these sporocysts. The average oocyst length is 39.31±3.82µm (33.75 to 45µm) and the average width is 30±2.02µm (26.25 to 33.75µm) (30 oocysts measure) with a ratio of 1.31±0.17 (Fig. 11). This form is the least represented (3.39%).

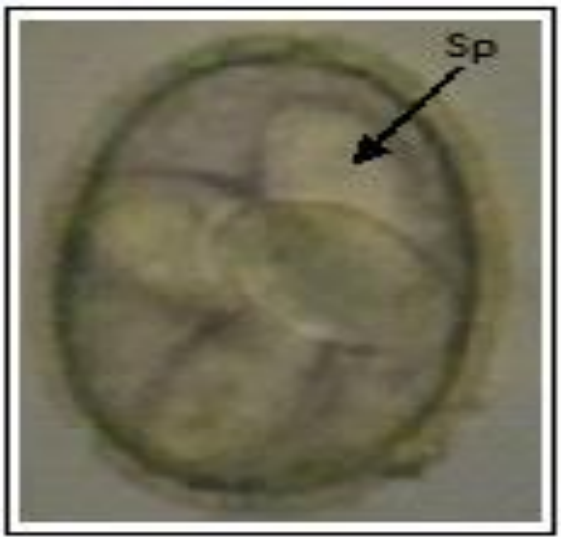


**Fig. 7.** Form without polar granule and oocyst residuum.

*Eimeria* sp5

*Eimeria* sp5 accounted for 5.57% of observed oocysts. The oocyst has an ovoid shape with a double wall without micropyle. There is no oocyst residuum and polar granule.

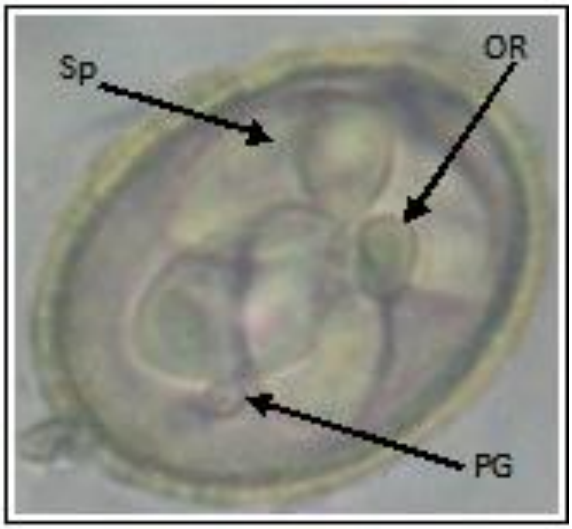


**Fig. 8.** Form with polar granule and oocyst residuum.

Sporocysts are ovoid, elongated and have a Stieda body (Fig 8). Its average length is 41.25±3.75µm (37.75 to 45µm) and its average width is 31.25±2.16µm (30 to 33.75µm) (29 oocysts with a ration of 1.32±0.06. Sporocysts have an average length of 16.87µm (Fig. 12).

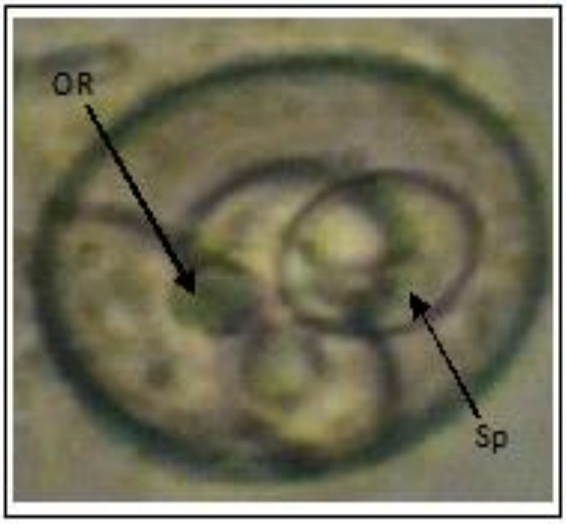


**Fig. 9.** Form without polar granule and with oocyst residuum.

*Eimeria* sp6

The oocyst has an elongated shape with a double wall without micropyle. There is no oocyst residuum but presence of polar granule in the oocyst. Three sporocysts are ovoid, elongated while the fourth is subspheroidal. None of them have stieda body. The oocyst measures 46.87±3.9µm long and 28.12±2.75µm (the measurement of 25 oocysts) wide with a ratio of 1.66 and represents 13.62% of oocysts (Fig. 13).

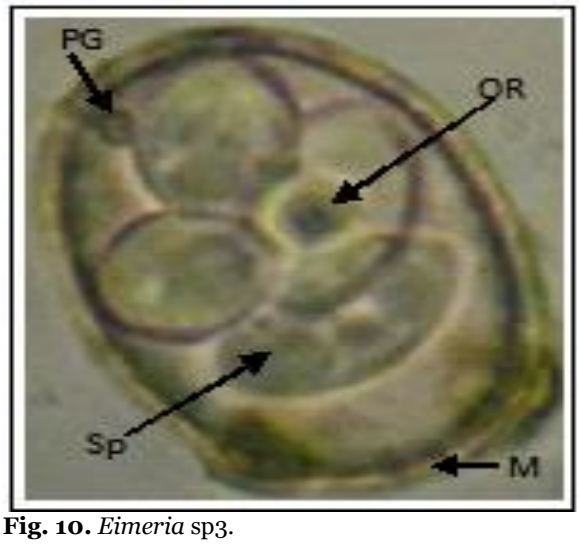


**Fig. 10.** *Eimeria* sp3.

**Discussion**

Coccidian oocysts were collected from wild and farmed grasscutters during this survey. All farms were infected with coccidia. The number per gram of fecal matter was very high throughout the study. Sacramento *et al.* (2010) also noted high levels of coccidia OPG in grasscutters farms in Benin during their work.

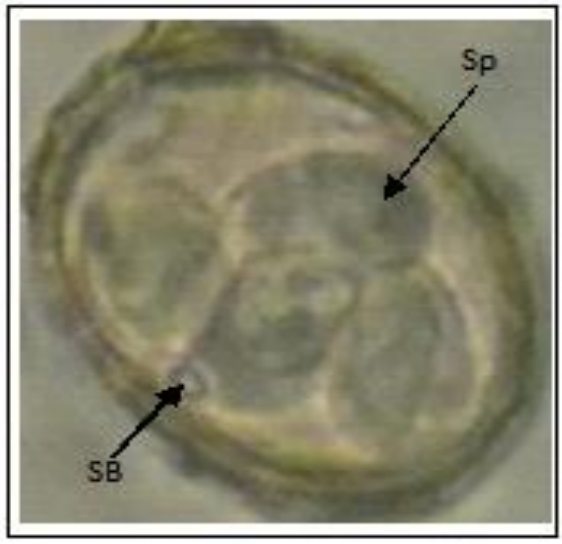


**Fig. 11.** *Eimeria* sp4.

On the other hand, these very high values without apparent symptoms observed in some of the grasscutters farms would lead to suppose the existence of low pathogenic coccidian species in grasscutters. Another hypothesis could be the necessity of higher parasitic loads to make happen clinical impact. Indeed, not all coccidian are pathogenic in rabbits. They can be classified into 4 categories: pathogenic coccidian (*E. Coecicola*), low pathogenic coccidian (*E. perforans*), pathogenic coccidian (*E. media, E. magna, E. piriformis, E. irresidua*) and highly pathogenic coccidian (*E. intestinalis, E. flavescens*) (Burgaud, 2010).

The prevalence and OPG values obtained were significantly higher in wild grasscutters than farmed grasscutters. Also, the prevalence and OPG obtained in farmed grasscutters (22.6% and 729 ± 333 OPG) are lower than those obtained by Adjahoutonon *et al.* in 2007 in Benin (72.73% and 1897 OPG). This could be explained by the hygiene brought to breeding in Côte d'Ivoire, the use of certain medicinal plants and anticoccidians. Indeed, according to Mensah *et al.* (2007), grasscutters breeders use the powder of *Vernonia amygdalina* dried leaves to treat grasscutters' coccidiosis.

The two stages of coccidian evolution (microgametocyte and macrogametocyte) observed in grasscutters' small intestine show that these coccidias normally evolve during the cycle. The digestive tract or, more precisely, grasscutters' intestine would therefore be a favorite site for coccidian. All oocysts examined after coprocultivation had four s porocysts each containing twosporozoites. Theybelon ged to the genus *Eimeria* because according to Levine *et al.* (1980) and Coudert *et al.* (2003), coccidian of the genus *Eimeria* have 4 sporocysts and each sporocyst have 2 sporozoites. This confirms the work of Mpoame (1994) in Cameroun, Mensah and Ekue (2003) in Benin, Yeboah and Simpson (2001) in Ghana, and Ajayi *et al.* (2007), Omonona (2011) and Opara (2012) in Nigeria. These authors have identified only the genus *Eimeria* in grasscutters.

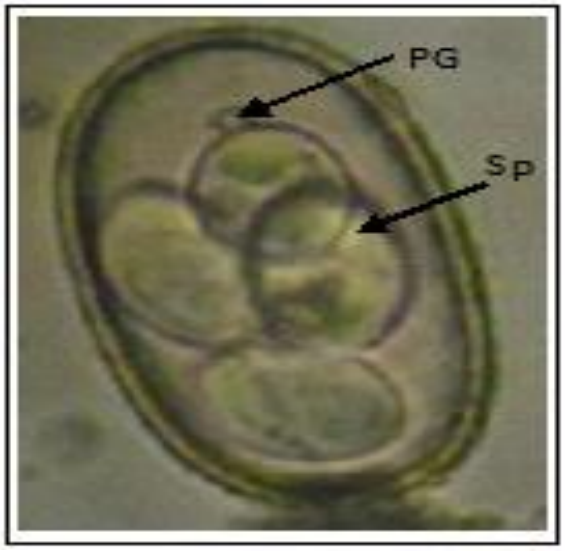


**Fig. 12.** *Eimeria* sp5.

Six forms of *Eimeria* suggesting six different species were identified after sporulation. These forms differ morphologically from species ident ified in rodents from Alaska, USA, and northeastern Siberia, Russia by Duszynski *et al.* (2007) then from the other species present on the identification keys us ed. This would mean that the grasscutter would carry coccidian species peculiar to it. In fact, according to

Licois (2010), coccidian is monoxenes (a single host) and have a very strong specificity for the animal species that they parasitize.

Coccidiosis is prevalent in grasscutters farms, rabbits, chickens, pigs and sheep. Some species have little or no pathogenicity. On the other hand, other species are very pathogenic and play an important role in the decline in production (mortality, growth delay and cost of treatment). They are mainly manifested by diarrhea, under-consumption of water and food, weight loss, dehydration and death (Coudert *et al.*, 2003; Thoto, 2006). Those encountered in grasscutters are not considered zoonoses because human coccidiosis is not caused by coccidian of *Eimeria* genus. Sulfamides are used for curative treatment in farms. Natural anticocidians are also used (*Vernonia amygd alina* dry leaves) (Mensah *et al.* 2007).

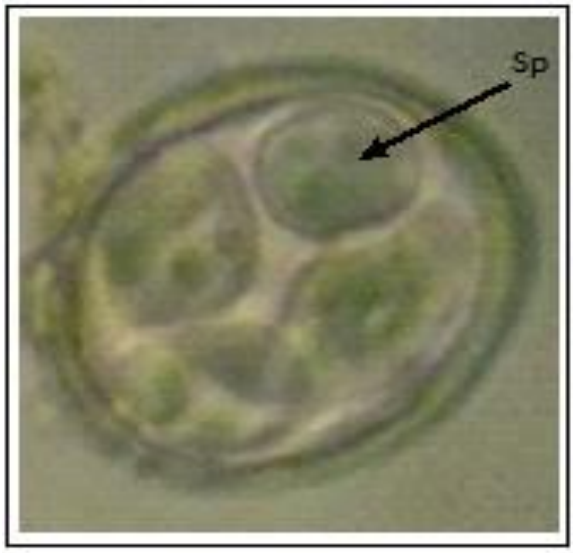


**Fig. 13.** *Eimeria* sp6.

PG : Polar granule ; Sp : Sporocyst ; OR : oocyst residuum; M : micropyle ; SB :Stieda body.

For prophylactic measures, hygiene must be respecte d. The building and livestock equipment must be regularly cleaned and disinfected.

**Conclusion**

Oocysts of coccidia found in grasscutters in Côte d'Ivoire belong to the genus *Eimeria*. The number of oocysts per gram of fecal matter in wild grasscutters is much higher than that of farmed grasscutters. From the morphological point of view, six forms that suggest six different species have been observed. These are different from those observed in lagomorphic rodents such as rabbits. A further study should therefore be carried out to definitively identify the different species of coccidian in grasscutters.

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