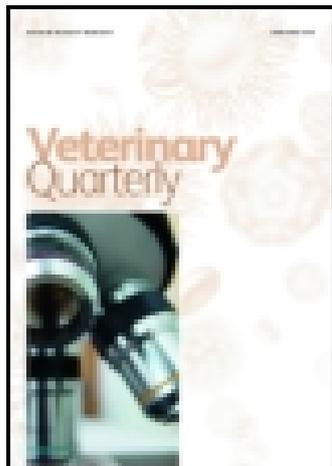


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### Prevalence, pathology and risk factors for coccidiosis in domestic rabbits (*Oryctolagus cuniculus*) in selected regions in Kenya

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## ORIGINAL ARTICLE

### Prevalence, pathology and risk factors for coccidiosis in domestic rabbits (*Oryctolagus cuniculus*) in selected regions in Kenya

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**Background:** The potential for rabbit production in Kenya is high. However, high morbidity and mortality of domestic rabbits were reported.

**Objective:** The aim of the study was to determine the pathology, prevalence and the predisposing factors to coccidiosis in domestic rabbits in selected regions in Kenya.

**Animals and methods:** A total of 61 farms keeping rabbits in six different counties were visited in the survey. A total of 2680 live rabbits were examined and 61 rabbits and 302 fecal samples were randomly collected from the farms and examined for coccidian oocysts by ante-mortem and post-mortem methods. The predisposing factors to coccidiosis were assessed through questionnaires and direct observation. Chi square ( $\chi^2$ ) statistics was used with  $P$  values < 0.05 considered statistically significant.

**Results:** Of the 302 fecal samples, 85% ( $P < 0.001$ ) contained coccidian oocysts and 2% harbored nematode eggs (*Passalurus ambiguus*). The overall prevalence of *Eimeria* spp. infestation was 85.1% in the study area and 90.2% in the individual rabbits, while prevalence of intestinal coccidiosis and hepatic coccidiosis was 29.5% and 11.5%, respectively. Higher counts of coccidian oocysts per gram of feces were recovered in weaners than in growers and adults rabbits ( $P < 0.001$ ), rabbits that were kept in high density group housing ( $P < 0.05$ ) and housing with more than two tiers.

**Conclusion:** This study identified group housing of rabbits of different ages and inadequate control of concurrent infections as the major risk factors associated with coccidiosis in domestic rabbits in Kenya.

**Keywords:** rabbit; coccidiosis; *Eimeria stiedae*; *Passalurus ambiguus*; Kenya

#### 1. Introduction

The potential for rabbit production in Kenya is high. However, diseases of rabbit is a major challenge to majority of the rabbit farms in Kenya (Hungu et al. 2013; Serem et al. 2013). Majority of farmers are able to recognize symptoms of illness in rabbits, whereas few sought treatment probably due to limited technical information on rabbit diseases since emphasis is laid on other food animals (Borter & Mwanza 2011).

Studies on the diseases of domestic rabbits in Kenya are rare, scant and are based on retrospective evaluation of either cases presented at the small animal clinic (Aleri et al. 2012) or for post-mortem examination (Ngatia et al. 1988) at the University of Nairobi, Kenya. However, these reports are mainly from rabbit farms situated close to the university facility. Hence, there is a necessity to conduct a study on disease situation in domestic rabbit farms in the country.

Coccidiosis is caused by different species of the protozoan *Eimeria* parasites. The condition in domestic rabbits occurs either in the intestines (intestinal coccidiosis) or liver (hepatic coccidiosis) and mainly in young rabbits (Coudert et al. 1995; Pakandl et al. 2008; Papeschi et al. 2013) and rabbits housed in poor environmental sanitation and poor

hygienic practices (González-Redondo et al. 2008). Depending on the clinical symptoms including weight loss, diarrhea and subsequent mortality, intestinal coccidian species can be classified into three types. These are non-pathogenic to slightly pathogenic coccidia (*Eimeria media*, *Eimeria exigua*, *Eimeria perforans*, *Eimeria coecicola*), moderately pathogenic (*Eimeria irresidua*, *Eimeria magna*, *Eimeria piriformis*) and very pathogenic coccidia (*Eimeria intestinalis*, *Eimeria flavescens*). Hepatic coccidiosis is caused by *Eimeria stiedae* (Coudert et al. 1995).

Coccidiosis is associated with major economic losses in rabbit farming including morbidity and mortality, growth retardation and condemnation of affected livers in cases of hepatic coccidiosis (Lebas et al. 1986; Coudert et al. 1995; Darzi et al. 2003).

In Kenya, 83% morbidity and 69% mortality of domestic rabbits on the farms were reported (Hungu et al. 2013). However, no systematic research has been performed to determine the prevalence of coccidiosis in domestic rabbits as a possible cause of these losses. The goal of this study was to determine the prevalence and pathology of coccidiosis in domestic rabbits and the associated risk factors in rabbit farms in selected regions in Kenya.

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## 2. Materials and methods

### 2.1. Study area

The study was carried out in sixty-one randomly selected rabbit farms within six counties where domestic rabbit keeping is common practice in Kenya. These areas included: Nairobi County and its surrounding areas of Karen, Ngong', Dagoretti, Ongata Rongai, Kiambu County (Thika town, Kabete and Kikuyu), Nyeri County (Nyeri town, Othaya, Mukurweini and Karatina), Meru County (Central Imenti, South Imenti), Nakuru County (Nakuru town and Gilgil) and Taita-Taveta County (Wundanyi and Taita) (Ministry of Livestock Development [MOLD] 2010; Borter & Mwanza 2011; Serem et al. 2013). The actual sampling sites are illustrated in Figure 1.

### 2.2. Study design and population

Households with rabbits were randomly selected with assistance of the Department of Livestock Production in the Ministry of Livestock Development, which already had data on areas where rabbit production in Kenya has been established. A cross-sectional survey was carried out in the selected districts from January 2012 to May 2013.

Using simple random sampling method, 80% of all the registered rabbit farms from each location were randomly selected from the list of rabbit keepers as obtained from the livestock production offices in each area. However, due to the variation in number of registered rabbit keepers in each county, the number of rabbits kept per farm and husbandry practices, larger numbers of rabbits (2680 out of 3350) were examined. This is due to the fact that larger

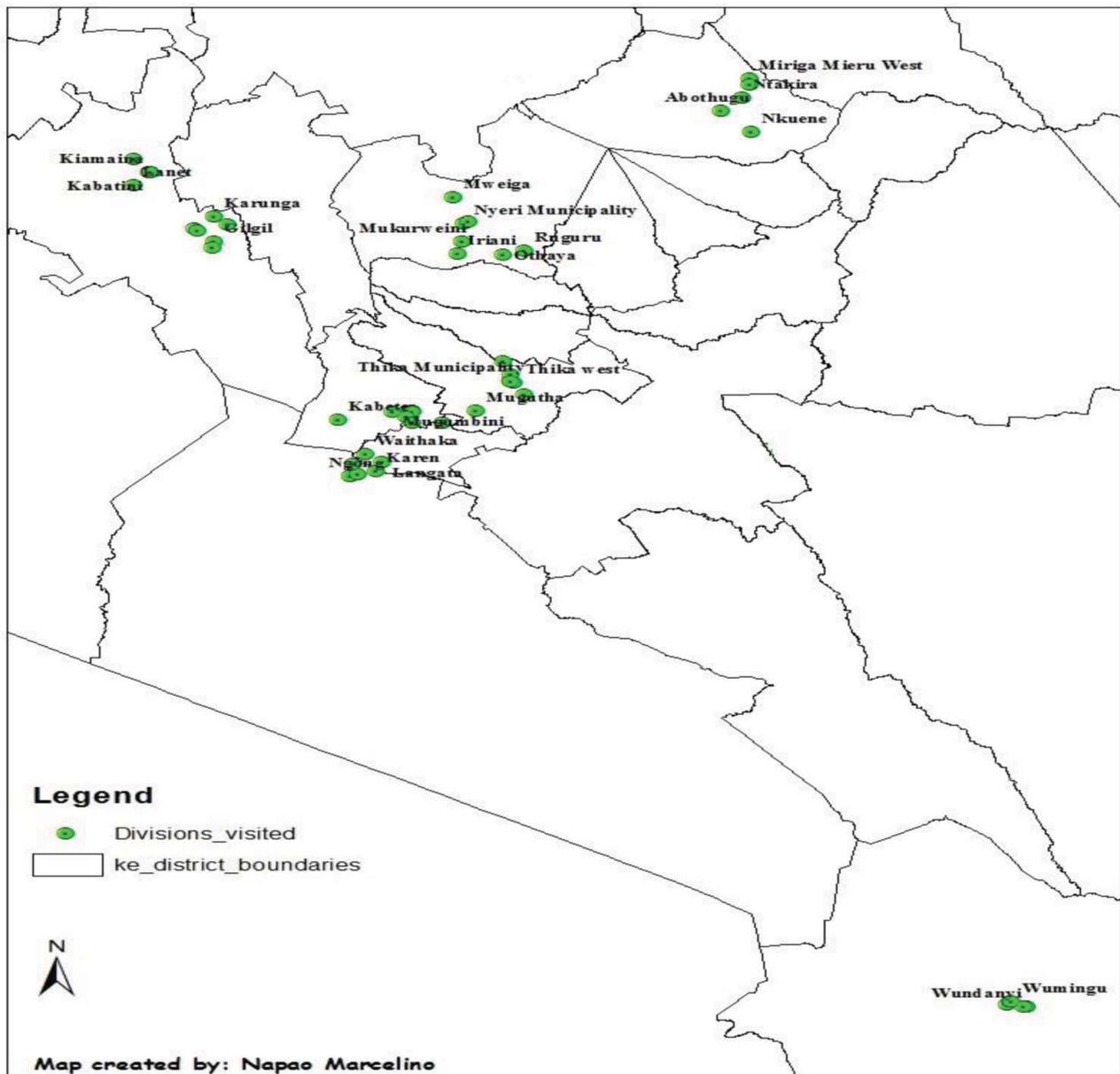


Figure 1. Map of Kenya showing administrative divisions where samples on rabbit diseases were collected between January 2012 and May 2013.

samples more accurately represent the characteristics of the populations from which they are derived (Marcoulides 1993). In each farm visited, a questionnaire on rabbit husbandry practices was filled with either the rabbit attendant or the owner.

The study was approved by the Institutional Animal Care and Use Committee of the University of Nairobi, Kenya.

### 2.3. Prevalence of coccidiosis

Infection of rabbits with coccidiosis was assessed by examination of rabbits. Clinical coccidiosis was recorded in rabbits, where typical lesions of coccidiosis including diarrhea, matted perineum, loss of condition and mortality were observed. However, the rabbits which only showed intestinal or hepatic lesions at necropsy without clinical signs were recorded as subclinical coccidiosis (Jithendran & Bhat 1996). Five fecal samples comprising 25 grams of fresh feces were obtained from the litter and under the cages of each farm. Where rabbits were housed in groups, samples were collected from different areas of the cage(s) (Cerioli et al. 2008). The samples were stored in plastic fecal pots and refrigerated at 4 °C until examination by flotation technique in order to determine number of coccidian oocysts per gram of feces (OPG) using Mc Master Technique as described previously (MAFF 1986).

The numbers of eggs and coccidian oocysts within each grid of chamber were counted under a compound microscope at 10x magnification. The total numbers of nematode eggs or coccidian oocysts were multiplied by 50 to give either the eggs per gram of feces (EPG) or OPG. Morphological and color differences were used to distinguish various eggs (Soulsby 2005). The average EPG and OPG were calculated for each farm.

The non-sporulated oocysts obtained from each farm were pooled and suspended in 2.5% (W/V) aqueous potassium dichromate, placed in Petri dishes in air to sporulate at room temperature ( $25 \pm 2$  °C). The samples were examined daily under light microscope using the oil immersion lens and recorded when all the sporozoites within the sporocysts were fully formed. The species were identified using sporulation time and morphological features (curvature, presence or absence of oocyst residuum and micropyle) (Coudert et al. 1995).

### 2.4. Pathology of coccidiosis

One live rabbit was randomly sampled from each farm for further laboratory examination at the Department of

Veterinary Pathology, Microbiology and Parasitology of the University of Nairobi. The rabbits were humanely euthanized for post-mortem examination by intraperitoneal injection of sodium pentobarbitone (Euthasol<sup>®</sup>, Virbac AH, Texas, USA) at 100 mg/kg body weight. Necropsies were performed using a comprehensive technique at the Department of Veterinary Pathology, Microbiology and Parasitology of the University of Nairobi. Twenty-five grams of intestinal fecal content were collected from each rabbit for examinations as described above. The liver and intestinal sections showing gross lesions were collected, preserved in 10% buffered formalin and processed for histopathology examination as described by Kiernan (1981).

### 2.5. The risk factors for coccidiosis in Kenya

A survey was conducted through questionnaires and verified through direct observations. Structured questionnaires were used to assess the farm husbandry practices, namely feeding and feeding equipments, housing and housing sanitation and the disease symptoms previously encountered in the farm and control measures.

### 2.6. Statistical analysis

The statistical analytical system SAS V9 (SAS Institute Inc. 2002) was used for data analysis. Chi square ( $\chi^2$ ) statistics was used to show association between the husbandry practices and occurrence of coccidiosis and *P* values of less than or equal to 0.05 was considered statistically significant.

## 3. Results

A total of 61 farms were surveyed, 2680 out of 3350 rabbits in total on these farms were examined and 302 fecal samples collected from the farms. In addition, 61 rabbits (43 females and 18 males) were euthanized for necropsy and 61 intestinal contents analyzed for coccidian oocysts. The rabbits included 25 New Zealand white, 22 Californian whites, 13 crosses of these two and one Dutch breed. Nyeri County had the highest average number of rabbits kept per farm while Taita-Taveta County had the lowest (Table 1).

Diarrhea was reported in 50 (86.2%) farms and frequently observed during clinical examination (11.5%). Diarrhea was indicated by matted perineum, the presence

Table 1. Average number of rabbits kept per farm in the six counties in Kenya in which the survey was conducted between the periods January 2012 to May 2013.

County	Farms visited	Average number of rabbits/farm $\pm$ SD	Fecal samples collected	Rabbits for necropsy
Kiambu	17	59.2 $\pm$ 50.4	84	17
Meru	6	48.0 $\pm$ 41.6	30	6
Nakuru	12	34.8 $\pm$ 26.4	60	12
Nairobi	13	59.9 $\pm$ 43.8	65	13
Nyeri	7	61.9 $\pm$ 49.8	35	7
Taita-Taveta	6	24.2 $\pm$ 13.5	28	6
Total	61	2680	302	61

Table 2. Clinical findings from the 61 rabbit farms in the six counties in Kenya between the periods January 2012 and May 2013.

Clinical signs	Frequency of farms (%)	
	Observed previously	Observed during clinical examination
Soiled perineum	86.2	11.5
Unthriftiness	13.8	9.8
Found dead	77.6	8.2
Abdominal distention	72.4	4.9
Depressed	12.0	9.8
Nervous signs	–	3.3

of watery, mucoid and abnormally soft feces in 1/61 (1.6%), 2/61 (3.3%) and 5/61 (8.2%) of farms, respectively. Other clinical signs included sudden onset of limb in coordination (nervous signs) and bloating (abdominal distension) (Table 2).

A total 257 (85.1%) fecal samples contained coccidian oocysts, while 6 (2.0%) revealed pin worm (*Passalurus ambiguus*) eggs of between 6000–35,000 EPG. Of the 45 (14.9%) fecal samples that tested negative for coccidian oocysts, 30 were collected from 6 (9.8%) farms. Relatively high coccidian OPG (>6000) were recovered in fecal samples collected from farms in Nyeri (57.1%) and Nairobi (46.1%) County, while majority of farms in Kiambu (76.8%) and Taita-Taveta (66.6%) County had low coccidian OPG counts. There was no significant variation in the average coccidian OPG within the different counties ( $P = 0.4163$ ). The distribution of average coccidian OPG of the farms in each county were as shown in Table 3.

Of 61 fecal samples collected from intestines and caeca of euthanized rabbits, 90.2% were positive for coccidian oocysts. High numbers of coccidian OPG ( $>8.0 \times 10^3$ ) were recovered in weaners aged between four and five weeks more frequently than in growers and adult rabbits ( $P < 0.001$ ) (Table 4).

At necropsy, enteritis was encountered in 29.5% rabbits confirmed with clinical (13) and sub-clinical (5) intestinal coccidiosis. The intestinal mucosa of these rabbits had the following gross lesions; general congestion

27.9%, petechial hemorrhages 8.2%, bloody content 13.1%, yellowish slightly mucoid content 13.1% and watery content 4.9% (Figure 2). Fecal samples collected from these rabbits predominantly revealed mixed infections with the following *Eimeria* species; *E. perforans* 6/18 (33.3%), *E. magna* 5/18 (27.8%), *E. piriformis* 6/18 (33.3%), *E. intestinalis* 5/18 (27.8%), *E. flavescens* 5/18 (27.8%) and *E. coecicola* 3/18 (16.7%). Histology revealed lymphocytic infiltration and presence of coccidian oocysts and coccidian schizonts in the lamina propria of intestinal epithelium (Figure 3).

Hepatic coccidiosis was diagnosed in seven (11.5%) rabbits. One rabbit (10 weeks of age) showed diarrhea, two rabbits were reported with history of poor growth rate (unthriftiness). However, two rabbits were apparently healthy. Livers of these rabbits grossly showed raised, multifocal whitish to yellowish nodules of about 0.5–1 millimeter in diameter. Histology revealed multifocal areas of coagulative liver necrosis, bile duct proliferation, hyperplasia of the epithelial cells of the bile duct and presence of *Eimeria stiedae* oocysts and gametocytes within the bile ducts (Figure 4). Necropsy also revealed concurrent disease conditions in the rabbits with intestinal coccidiosis including; mucoid enteropathy 3 (4.9%), helminthosis due to *Passalurus ambiguus* 2 (3.3%), gastric ulcers 1 (1.6%) and intussusceptions 1 (1.6%), while 9 (14.8%) of the rabbits were emaciated.

Tiered cages were observed in 28 (45.9%) farms. These were significantly higher in Nairobi 9 (69.2%), Meru 4 (65.6%) and Nakuru 6 (50%), but lower in Taita-Taveta (0%) and Nyeri 1 (14.3%) ( $P < 0.01$ ). In 31 (50.8%) farms, rabbits were housed in groups according to their age and sex, while in 15 (24.6%) farms rabbits were housed in groups irrespective of age and sex.

Poor to very poor cage sanitations as characterized by dirty floors, soiled water and feed/feeding equipment, poor urine drainage and cage odor with pungent ammonia smell were observed in 14 (23%) farms. However, 17 (27.9%) farms had fair housing sanitation characterized by presence of fresh fecal pellets on cage floors, feed/feeding equipment on the floor, proper ventilation and properly maintained house. The majority of the farms (49.2%) had good to very good housing sanitation. These were farms with clean hutch floors free from feces, feed and water/feeding equipment were raised above the floor

Table 3. Average farm fecal coccidian count for the six counties in Kenya in which the survey was conducted between the periods January 2012 to May 2013.

County	Coccidian OPG $\times 10^3$								Total
	0	0.1–2.0	2.001–4	4.001–6	6.001–8	8.001–10	10.001–60	>60.0	
Kiambu	4	7	1	1	–	1	–	3	17
Meru	–	4	–	–	–	–	2	–	6
Nakuru	–	6	–	3	–	–	1	2	12
Nairobi	2	4	1	–	–	2	2	2	13
Nyeri	–	2	1	–	–	1	2	1	7
Taita-Taveta	–	3	1	–	1	–	1	–	6
Total	6	26	4	4	1	4	8	8	61

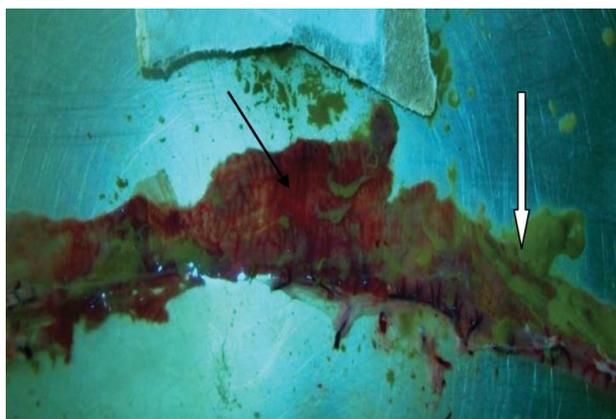


Figure 2. An opened segment of the intestines from a rabbit showing hemorrhages and congestion on the intestinal mucosa (arrow) and yellowish mucoid intestinal content (bold arrow) in a case of hemorrhagic enteritis due to intestinal coccidiosis.

and the farms also had well ventilated and properly maintained houses.

However, there was no statistical significant difference in cage sanitation in the counties. The study revealed significant association between the occurrence of high coccidian load ( $>8.0 \times 10^3$  OPG) and age of rabbits ( $P < 0.001$ ), grouped housing ( $P = 0.0293$ ) and concurrent infections ( $P = 0.0425$ ), but not number of cage tiers ( $P = 0.0572$ ) or cage sanitation ( $P = 0.6312$ ).

#### 4. Discussion

Fecal examination showed ubiquitous infection of domestic rabbits with coccidian parasites. The overall prevalence of *Eimeria* spp. infestation was 85.1% in the study area and 90.2% in the individual rabbits, while prevalence of intestinal coccidiosis and hepatic coccidiosis was 29.5% and 11.5%, respectively. This prevalence was higher than reported by Aleri et al. (2012) in Kenya and Jithendran and Bhat (1996) in India. These findings suggest that both clinical and subclinical coccidiosis occur in domestic rabbits in Kenya and are major causes of

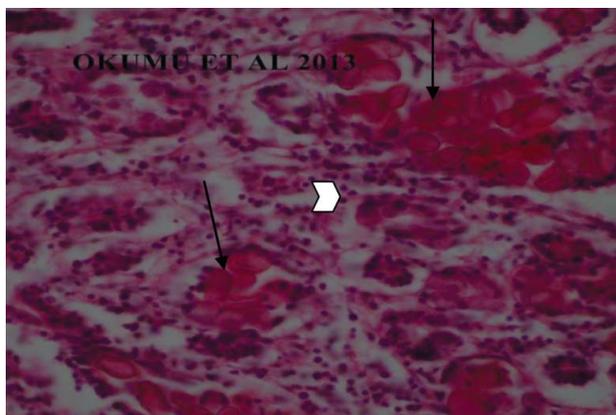


Figure 3. Histological section of a rabbit intestine showing coccidian oocysts in the intestinal epithelium (arrow) and lymphocytic infiltration in the lamina of the villi (arrow head) in a case of intestinal coccidiosis (X 400 H/E).

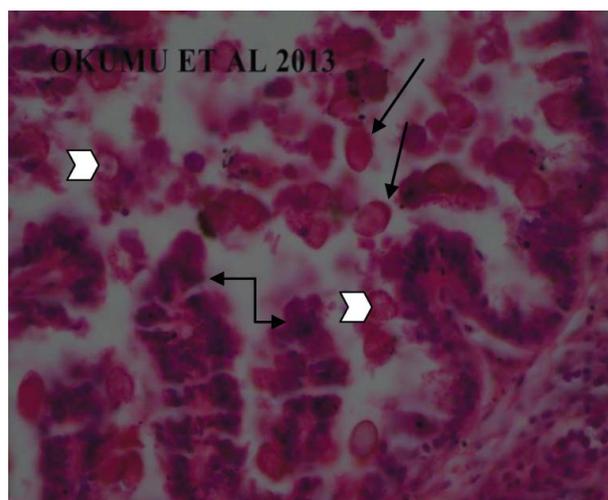


Figure 4. Liver section of a rabbit showing coccidian oocysts (arrow), gametocytes (arrow head) and proliferation of bile duct epithelium (double arrow) in a case of hepatic coccidiosis (X 400 H/E).

diarrhea and death (Rashwan & Marai 2000; Rosell et al. 2010). However, mixed infection with more than one *Eimeria* species is common (Jithendran & Bhat 1996). In two rabbits aged 7 and 10 weeks which clinically presented with limb incoordination (nervous signs) grossly showed hemorrhagic enteritis, intussusceptions at the ilea-cecal junction and uncountable number of coccidian OPG (too many to count). Intussusceptions could be associated with intestinal hyperperistalsis induced by heavy coccidian infection (Weisbroth & Scher 1975). Emaciation (14.8%) and unthriftiness in rabbits could partly be attributed to the insidious nature of coccidiosis in rabbits and to the concurrent infections (Rosell & De La Fuente 2008) encountered in this study.

The 9.8% of farms that were negative for coccidian OPG had treated the rabbits with sulphonamides. However, use of toltrazuril (Peeters & Geeroms 1986) and diclazuril (Vanparijs et al. 1989) are recommended as they can be used for both prevention and treatment of coccidiosis compared to sulphonamides, which are used mainly for treatment (Pakandl 2009). Despite good cage sanitation, fecal coccidian oocyst loads from majority of the farms were unsatisfactory ( $>1000$  OPG). This was in contrary to the findings by González-Redondo et al. (2008) and Pakandl et al. (2008) that good farm hygiene is sufficient to maintain low coccidian levels on a farm.

The frequent recovery of high loads of coccidia ( $>8.0 \times 10^3$  OPG) in weaning rabbits ( $P < 0.001$ ) and in rabbits housed in groups ( $P = 0.0293$ ) could be attributed to several factors. First, naïve rabbits are more susceptible to infection from adult carriers especially after weaning (Pakandl et al. 2008; Papeschi et al. 2013) and since most rabbit keepers in Kenya (50.8%) mainly housed their rabbits in groups, housing and husbandry practices are likely risk factors. Second, weaning stress has been reported to lower immunity of rabbits to infection (Papeschi et al. 2013). In this regard, ingestion of coccidian contaminated solid feed during weaning period may raise the intensity of infection for the weaners.

Table 4. Distribution of coccidian OPG in feces collected at post-mortem from intestines and ceca in different age groups of the 61 rabbits sampled from the six counties in Kenya between the periods January 2012 and May 2013.

Age group (weeks)	Coccidian oocysts per gram of feces (OPG) $\times 10^3$								Total
	0	0.1–2.0	2.001–4	4.001–6	6.001–8	8.001–10	10.001–60	>60.0	
Weaners (1–5)	0	1	1	2	0	2	5	3	14
Growers (6–24)	3	11	2	1	1	0	1	3	23
Adults (>24)	3	14	1	1	0	2	2	2	24
Total	6	26	4	4	1	4	8	8	61

High coccidian OPG were frequently recovered from farms where rabbits were housed in tiered cages probably because of difficulty encountered in cleaning the tiered cages. However, this was not statistically significant ( $P = 0.0572$ ). In this regard, farms where tiered cages are used need to be more thorough in maintaining hygiene in the rabbit houses. The findings of this study suggest that group housing of rabbits of different ages and inadequate control of concurrent infections are the likely risk factors associated with coccidiosis in domestic rabbits in Kenya. The study therefore recommends further investigation on the epidemiology and management of coccidiosis in domestic rabbits in Kenya.

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