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INFLUENCE OF OREGANO ESSENTIAL OIL (OEO) ON PREVALENCE AND OOCYST SHEDDING DYNAMICS OF NATURALLY ACQUIRED *EIMERIA* SPP. INFECTION IN REPLACEMENT DAIRY HEIFERS*

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Abstract

The administration of oregano essential oil (OEO) was tested in a dairy farm (Piacenza, Italy) with a history of sporadic cases of clinical coccidiosis in order to evaluate its influence on infection prevalence and oocyst excretion pattern in calves and replacement dairy heifers. Animals were recruited at 1 month of age and allocated to two groups of 25 animals, experimental (E) and control (C). OEO was added at a concentration of 100 ppm to the feedstuff administered to group E. Prevalence of infection and the number of oocysts per gram of feces (OPG) was evaluated monthly from 30 days of life (d30) till d150, with one extra sampling at d45, for a total of 6 time points. No significant differences were observed regarding the prevalence of infection between the two groups (83% general prevalence, 93% in group E, 72% in group C) although slightly higher prevalence was seen for *Eimeria bovis* and *Eimeria alabamensis* in group C, while the opposite was seen for *Eimeria zuernii*. OEO addition to the diet did not have any effect on the course of coccidial infections in calves and replacement dairy heifers. Other variables, in particular data on growth and production, should likely be tested before ruling out the potential role of OEO, alone or in combination with other control measures, to reduce infection pressure and therefore to improve animal welfare and performance.

Key words: *Eimeria*, coccidiosis, oregano essential oil, dairy cattle, heifers, calves

Bovine coccidiosis mainly affects calves between 1 and 6 months of age, especially in intensive farms. It is caused by several protozoan parasites belonging to the genus *Eimeria*, each characterized by a varying degree of pathogenicity. Severe bloody diarrhea can follow massive infection by species like *Eimeria zuernii* and *E. bovis*, but bovine coccidiosis is usually asymptomatic (Muirhead, 1989; Daugschies and Najdrowski, 2005). To a lesser extent, *Eimeria alabamensis* can also cause clini-

cal disease (Hooshmand-Rad et al., 1994). However, due to damage of the intestinal epithelium, the digestive process can become severely affected, even in the absence of clinical disease, with adverse effects on animal welfare and performance (von Samson-Himmelstjerna et al., 2006). Thus, the economic losses for the cattle industry may be substantial and various attempts have been made to protect calves' intestinal environment, including the use of probiotic (Fitzgerald, 1980; Agazzi et al., 2014). Considering young heifers in particular, impaired growth can delay puberty onset, age at first insemination and subsequently age at first calving (Lormore, 2005). Moreover, poor nutrient availability in the first weeks of life has been demonstrated to compromise mammary gland productive potential at least during the first lactation (van Amburgh, 2004; van Amburgh and Meyer, 2005; Meyer et al., 2006).

Adverse environmental conditions likely play a role in both parasite pressure and host susceptibility to infection. The host immune response plays a key role in keeping coccidial infections under control (Daugschies and Najdrowski, 2005) and can be influenced by stressful environmental conditions, including thermal and humidity extreme ranges. Both sporulation rates and survival of oocysts in the environment are influenced by the same parameters (Taylor et al., 2007) and can affect the risk of infection.

Friedman (2014) has recently reviewed the activity of various essential oils on a diverse range of pathogens, including viruses, bacteria and parasites. Carvacrol and thymol (directly or as biosynthetic precursors, like γ-terpinene and p-cymene) are the major components of many essential oils, including oregano essential oil (OEO; Friedman, 2014). They may constitute up to 93% of the total oil composition and can exhibit considerable antimicrobial and antifungal activity, as well as cytotoxic properties on eukaryotic organisms (Sivropoulou et al., 1996; Adam et al., 1998). Essential oils coming from different plant sources have been shown to be active against several parasites like *Plasmodium* spp. (Dutta et al., 1990), *Toxoplasma gondii* (Ke et al., 1990) and *Trypanosoma* spp. (Santoro et al., 2007). There have been several reports on the effect of OEO and/or related herbal extracts on *Eimeria* spp. in broilers, goats and rabbits, showing increased weight gain and lower oocyst counts compared to infected, untreated controls (Giannenas et al., 2003; Baricco et al., 2005; Oviedo-Rondón et al., 2006; Nosal et al., 2014; Wunderlich et al., 2014). There are no reports, however, on the effects of OEO on *Eimeria* spp. infection in cattle.

The aim of the present study was to evaluate the effect of an OEO-integrated concentrate on the excretion pattern of bovine coccidia (*Eimeria* spp.) in naturally-infected calves and heifers in a large dairy cattle farm in Northern Italy (Province of Piacenza) and to determine if environmental conditions can affect infection dynamics.

Material and methods

Animals
An intensive dairy farm comprised of approximately 300 lactating cattle located in the Province of Piacenza, Italy, was selected, based on a history of sporadic cases

of clinical coccidiosis. A total of 52 female Italian Holstein heifers born in the period between March and July 2011 were recruited at 1 month of age and were divided into 2 equal groups, Control (C, n = 25) and Experimental (E, n = 27), according to the date of birth/age, weaning time and body conditions. The animals were housed in small free stalls on straw bedding and an external paddock with a surface area covered by concrete. The stalls were cleaned weekly by mechanical removal of manure. No other measures against coccidiosis (chemoprophylaxis) were adopted on the farm.

Calves were weaned at 60 days of age. Mixed hay (42.0% Neutral Detergent Fiber – NDF, 14.3% Crude Protein – CP) and starter concentrate (21.6% CP) were administered *ad libitum*. Calves in group E were fed concentrate supplemented with *Origanum heracleoticum L.* EO at the concentration of 100.00 ppm. This gave an estimated daily intake of 2.0 mg per kg bodyweight of pure EO or 200.0 mg per day per animal when animals were consuming 2 kg of concentrate per day.

Fecal analyses

Individual fecal samples were collected directly from the rectum of all calves at 30, 45, 60, 90, 120 and 150 days of age and analyzed for *Eimeria* spp. oocysts. A first screening was performed by flotation in saturated NaCl solution. Positive samples were subsequently analyzed with a modified McMaster technique (detection limit of 50 oocysts per gram – OPG; Ministry of Agriculture, Fisheries and Food, 1986).

Oocysts were identified to the species level for the three pathogenic species *E. zuernii*, *E. bovis* and *E. alabamensis,* according to Ernst and Benz (1986) and Eckert et al. (1995). Oocyst size was the criterion for species determination.

Environmental conditions

Temperature and relative humidity data were collected from the local meteorological station. Thermal Humidity Index (THI) was calculated as

$$(1.18 * Ta \,([1 - Ur \div 100] * [Ta - 14.3]) + 32$$

where:

Ta is the air temperature (ºC);

Ur is the relative air moisture (%), according to Kliber (1964).

The prevalence of animals excreting oocysts, expressed as a percent of the infected animals, was plotted into a timeline graph also including temperature, humidity and THI to show the dynamics of these parameters during the observation period.

No evident alterations of growth and health status were observed during the study in both groups.

Statistical analysis

Differences in infection prevalence were determined using ANOVA (PASW Statistics 18, ver. 18.0.0). The differences between mean OPG of the groups were evaluated using the Kruskal-Wallis test. Statistical significance was set at $P \leq 0.05$ while values of $P \leq 0.1$ were considered as tendency. Differences between prevalence of the groups were evaluated using the Chi-square test.

Results

Prevalence of infection and oocysts excretion intensity

The overall prevalence of infection (% of animals positive at least once) was 83%. Overall prevalence within the groups was 92.59% (group E) and 72.00% (group C) (Table 1).

Table 1. Prevalence of infection (% of animals shedding *Eimeria* oocysts) within groups at the considered intervals (Group E = experimental group; Group C = control group)

Interval	Prevalence[1]	
	Group E	Group C
d30	7.41	16.67
d45	37.04	36.00
d60	22.22	20.00
d90	22.22	20.00
d120	33.33	20.83
d150	15.38	20.00
Average	22.93	22.25

[1]No significant differences were found in prevalence level.

Despite slight numerical differences in excretion intensities at d30, d120 and d150, no significant differences were observed between groups regarding infection prevalence. It should be noted that differences were not consistent since the prevalence was alternately higher and lower in the two groups.

Oocyst shedding was highly variable between groups, the highest values being observed between d30 and d60 in both groups. On average, group C showed higher shedding levels but the numerical variations between groups were not consistent and no trends were observed (Table 2).

Table 2. Intensity of *Eimeria* oocyst shedding (oocyst per gram, OPG) at the considered intervals (Group E = experimental group; Group C = control group)

Interval	Intensity of oocyst shedding (no. of calves)[1]	
	Group E	Group C
d30	11625 (2)	16025 (4)
d45	6890 (10)	4708 (9)
d60	3095 (6)	10365 (5)
d90	479 (6)	485 (5)
d120	480 (9)	320 (5)
d150	106 (4)	706 (4)
Average	735	1147
Median	0	0

[1]No significant differences were found in oocyst shedding.

On average, the prevalence of *E. bovis* was higher in group C (25% vs 10.2%), which showed a tendentially higher prevalence at d120 (P<0.1). The opposite trend was observed for *E. zuernii*, where prevalence was twice as high in group E (14.5%

vs 7.5%), which showed a significantly higher prevalence at d45 (P<0.05). The prevalence of *E. alabamensis* was slightly higher (21.2% vs 16%) in group C where a trend was observed for a higher level of infection at d60 (P<0.1). Non-pathogenic species (i.e. those not belonging to the group of *E. bovis*, *E. zuernii* and *E. alabamensis*) were the most frequently identified (59.25% in group E, 59.63% in group C) and no differences were observed between the two groups (Table 3). Accordingly, all animals remained asymptomatic throughout the study period.

Table 3. Prevalence of pathogenic (*E. bovis*, *E. zuernii* and *E. alabamensis*) and other (nonpathogenic) *Eimeria* species at the considered intervals (Group E = experimental group; Group C = control group).

Species	Group	Interval (d) – Prevalence (%) – Positive to the Species/Infected (n)						
		d30	d45	d60	d90	d120	d150	mean
n positive	E (n=25)	2	10	6	6	9	4	
n positive	C (n=27)	4	9	5	5	5	4	
E. bovis								
Group E	E	0 (0)	0 (0)	1 (16.7)	2 (33.3)	1 (11.1) x	0 (0)	10.2
Group C	C	0 (0)	0 (0)	0 (0)	2 (40)	3 (60) x	2 (50)	25
E. zuernii								
Group E	E	0 (0)	4 (40) xx	0 (0)	0 (0)	2 (22.2)	1 (25)	14.5
Group C	C	1 (25)	0 (0) xx	1 (20)	0 (0)	0 (0)	0 (0)	7.5
E. alabamensis								
Group E	E	1 (50)	1 (10)	0 (0) x	0 (0)	1 (11.1)	1 (25)	16
Group C	C	1 (25)	2 (22.2)	2 (40) x	1 (20)	1 (20)	0 (0)	21.2
Nonpathogenic *Eimeria* spp.								
Group E	E	1 (50)	5 (50)	5 (83.3)	4 (66.7)	5 (55.5)	2 (50)	59.25
Group C	C	2 (50)	7 (77.8)	3 (60)	2 (40)	4 (80)	2 (50)	59.63

x = P<0.1; xx = P<0.05.

Environmental conditions and infection dynamics

The temperature, humidity and THI dynamics were consistent with the ones observed by Segnalini et al. (2011) in the Mediterranean basin area. During the observation period, minimum and maximum temperature were –3 and 36°C, with averages of 18.5°C. The maximum and minimum relative humidity values were 30 and 99%, with averages of 67.1%. Thermal humidity index was calculated to be 63.0 on average, with a minimum of 35.7 and a maximum of 80.3, the latter being reached during the third week of August 2011.

Results of plotting of environmental conditions and excretion pattern are shown in Figure 1. Two peaks of oocyst excretion were observed, one towards the beginning of July and one towards the end of October. Their occurrence was preceded by two humidity peaks taking place respectively at the beginning of June and at the beginning of October.
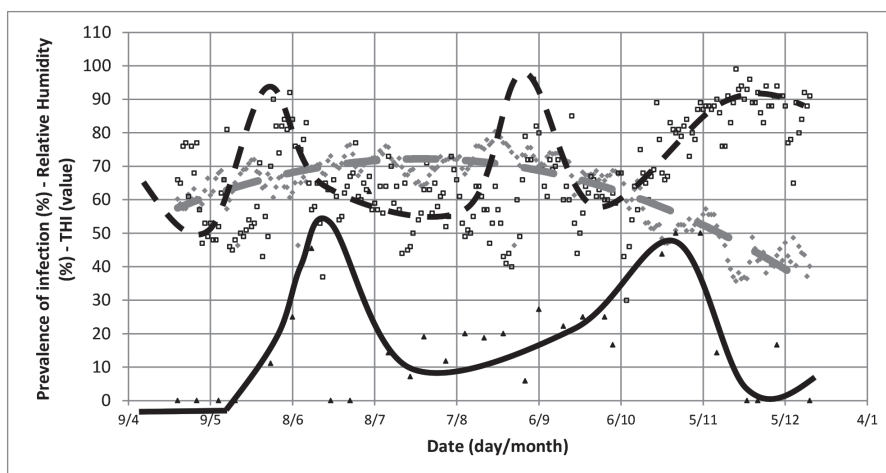
Figure 1. Prevalence of infection (black triangles, continous black line), Relative Humidity (white squares, black dotted line) and Thermal Humidity Index (THI; grey squares, grey dotted line) during the observation period. Lines are showing the approximate trends

Discussion

In a recent review, Friedman (2014) has reported that oregano oil and its major constituent carvacrol exhibit exceptionally high antimicrobial properties and antioxidant activity in many tissues, including the intestine. It has been suggested that addition of OEO can lead to better productive performance in several food-producing animals. There are only a few studies of the effect of essential oils on coccidial infections in animals. Giannenas et al. (2003) reported that supplementation with dietary OEO given two weeks after infection with *E. tenella* in broilers resulted in higher weight gains and feed conversion ratios compared to the infected control group, but in lower values compared to the group receiving lasalocid. Broilers treated with OEO also showed less severe symptoms (bloody diarrhea), higher survival rate, lower lesion scores and OPG values compared to non-treated, infected birds. However, the anti-coccidial effect of OEO against *E. tenella* was consistently lower than that exhibited by lasalocid. Supplementation with ground oregano was also able to reduce the adverse effects of *E. tenella* infection, as shown by increased body weights and the significantly improved feed conversion ratio values compared to the challenged control group (Giannenas et al., 2004). Finally, Oviedo-Rondón et al. (2006) showed that treatment of *Eimeria* spp.-infected broilers with two specific EO blends (containing thymol, eugenol, curcumin, piperin) prevented major shifts in the intestinal microbial communities as compared to non-treated controls, leading to less severe clinical signs. The results of these studies suggested that in broilers, essential oils, including OEO, could be an alternative to drugs and/or vaccination for protection against *E. tenella* infection. To the authors' knowledge, the only study

on the effect of essential oils on coccidial infection in ruminants is that of Baricco et al. (2005), who evaluated the effects of OEO in goats infected with *Eimeria* spp. Treated goats (2.5 mg of OEO per kg b.w./day for 30 days) showed lower OPGs and improved fecal scores compared to untreated controls.

In the present study, the addition of OEO had no significant influence on infection prevalence and oocyst shedding of *Eimeria* spp. in naturally infected calves. Results showed a progressive decrease of oocyst shedding during the first months of age in both groups, confirming the self-limiting nature of the infection.

Growth performance was not evaluated in the study. However, all animals remained asymptomatic and the vast majority of animals had a predominance of non-pathogenic species of *Eimeria*. The absence of clinical signs is probably related to the low OPG values when compared to other studies (Bangoura et al., 2011). It would be worthwhile to evaluate the effects of OEO on clinical signs and growth performance in calves experimentally infected with *E. bovis* and *E. zuernii*. It would also be interesting to evaluate the *in vitro* activity of essential oils on pathogenic species of *Eimeria* in cattle, including studies of phenolic compounds (carvacrol, thymol) on *Eimeria* oocysts, as already performed in avian *Eimeria* (Remmal et al., 2011).

It has been reported that cattle undergo heat stress during the summer months (Pennington and Van Devender, 2004). In the present study, an increase in infection prevalence was observed during two specific periods: in the month of June, when the increase of prevalence followed an increase in humidity and was possibly associated to heat stress that could have affected host immunity (Carrol et al., 2012) and during the second half of October, when the defense capacity of the host could have been reduced as a consequence of the rapid decrease in environmental temperature, associated with an increase in humidity (Sejian and Srivastava, 2010).

The use of OEO at the dose tested here in weaning and growing replacement heifers did not show a significant anti-coccidial activity. With the current lack of research for new compounds and the increase in awareness regarding drug use in animal husbandry, more efforts should be employed to confirm or to rule out the role of such compounds on coccidial infection control, growth parameters, feed efficiency and ultimately on animal welfare.

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