Anticoccidial Activity of Amprosul (Agrar®) on Sporulation of *Eimeria maxima* Oocysts

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ABSTRACT Sporulation is necessary if the life cycle of an *Eimeria* oocyst is to continue. Therefore, the study on anticoccidial activity of Amprosul (Agrar®) on sporulation of *Eimeria maxima* oocysts was investigated. The sporulation rates of oocysts of *Eimeria maxima* isolated from broiler chickens infected but not medicated and those infected and medicated with 0.5g and 1g of Amprosul (Agrar ®)/l of water were studied at room temperature. Normal oocysts, N (isolated from infected, unmedicated birds) sporulated faster and in greater numbers than principal oocysts P_1 and P_2 isolated from infected, medicated chickens (with 0.5g and 1g of Amprosul/l of water respectively). Oocysts isolated from chickens that received the higher dose of the drug sporulated slower and in lower numbers than those isolated from chickens treated with the lower dose. Average sporulation rate in relation to the treatments at 36, 48 and 72 hours respectively showed a very high significant difference ($P < 0.001$) while no significant variation ($P > 0.05$) was observed across treatments at the 96th hour. In conclusion, this study shows that Amprosul (Agrar®) drug can inhibit the sporulation of chicken *Eimeria* oocysts which will help in the termination of coccidiosis breaks in poultry houses resulting to huge economic gain.

KEYWORDS: Chickens, *Eimeria maxima*, Oocysts, Sporulation, Amprosul (Agrar ®)
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Short Communication

INTRODUCTION

Coccidiosis is caused by various species of *Eimeria*, an Apicomplexa protozoan parasite. It is one of the common diseases in poultry, which is responsible for major economic losses worldwide (Razmi & Kalideri, 2000; Nematollahi *et al.*, 2009; Gharekhani *et al.*, 2014). The disease occurs only after ingestion of sporulated oocysts in susceptible hosts. Both clinically infected and recovered birds shed oocysts in their droppings, which contaminate feed, dust, water, litter, and soil. Oocysts may be transmitted by mechanical carriers (e.g., equipment, clothing, insects, farm workers, and other animals (Hadipour *et al.*, 2011).

Sporulation is necessary if the life cycle of an *Eimeria* oocyst is to continue. An unsporulated oocyst is not infective to the host even when taken in large number (Felici *et al.*, 2021). Becker (1959) studied the sporulation time of 7 species of chicken *Eimeria* oocysts under different temperatures. At room temperature he reported the sporulation time to be 48 hours for *E. tenella, E. necatrix, E. maxima* and *E. mitis*, 24 – 48 hours for *E. brunetti* and *E. hagani* and 25 hours for *E. acervulina*. At 29 °C it was 18 hours for *E. mivati* and 48 hours for *E. praecox* (Soulsby, 1986). Edgar (1955) reported that almost all the oocysts of each species of chicken coccidia reached the infective stage (i.e. sporulated) by 18 hours at 28 °C - 29 °C + 1 °C except *E. maxima* which was first found to be infective at 30 hours thereby making 28 °C + 1 °C or 29 °C + 1 °C most suitable temperatures for sporulation.

Although the efficacies of many anticoccidials have been reported, coccidiosis breaks have been reported in laying flocks after preventive medication had been discontinued (McDougald & Reid, 1971; Vinay *et al.*, 2013). Poultry International (1992) reported the presence/diagnosis of subclinical coccidiosis which resulted in major losses in broiler production even though a whole battery of anticoccidial drugs was available. In Nigeria, there are several anticoccidial drugs in the market yet coccidiosis still ravages the poultry industry (Lawal *et al.*, 2016). According to Yakubu & Ajayi (1995), these drugs are being used indiscriminately for the control of coccidiosis in livestock without any regard to the consequences of them under dose or overdose usage. Depending on how they are used, the consequences are either advantageous or otherwise (Ajayi, 1976; Ajayi & Todd, 1977). These reports suggest the desirability to examine the effect of anticoccidials on sporulation of *Eimeria* oocysts.

MATERIALS AND METHODS

Sample Collection and Processing

Oocysts of *Eimeria maxima* were obtained from the small intestine of necropsied birds reported for coccidiosis outbreaks at the ECWA Veterinary Clinic, Bukuru, Plateau State, Nigeria between February and April 1996. The oocysts after being sporulated in 2.5% aqueous potassium dichromate solution in petri dishes were propagated by passaging them in two 4-weeks old coccidia-free chickens using the methods of Joyner & Davies (1960), Hodgson (1970) and Amer *et al.* (2010). The faecal droppings of the chickens were collected and the recovered oocysts sporulated and stored in specimen bottles in the refrigerator at 3 - 4 °C (El-Morsy *et al.*, 2016). An oocyst was regarded as sporulated when it has fully formed 4 sporocysts each containing 2 sporozoites (Laverty *et al.*, 2021).

Twelve 3-weeks old coccidia-free Euribrid hybro-broilers (brooded from day-old by the researcher) were divided into 3 groups (4 birds in each group). All the birds were infected with 10,000 sporulated oocysts of *Eimeria maxima* and challenged with 20,000 sporulated oocyts of same (Brito *et al.*, 2014). The first group were not medicated, the second group were medicated with 0.5g of Amprosul/l of water (which is the normal dose of drug) and the third group was medicated with 1g of Amprosul/l of water (which is the double dose).

The anticoccidial Amprosul (Agrar ®) contained the following active ingredients per gram:

i. Sulphaquinoxaline - 166mg
ii. Amprolium - 166mg, and
iii. Vitamin K_3 - 20mg

Freshly discharged oocysts obtained from the faeces of birds in group 1 (i.e infected, challenged and unmedicated) were regarded as Normal oocysts (N) while those recovered from Amprosul-treated birds receiving normal dose (group 2) were regarded as Principal oocysts one (P_1) and oocysts from double dose (group 3) were regarded as Principal oocysts two (P_2). Two collections of faeces were made on the same day for each of the groups mentioned above. The oocysts were recovered and kept in petri dishes (2 for each group) containing 2.5% aqueous potassium dichromate solution and covered with foil paper. Sporulation was done at room temperature (27 – 30 °C). A drop from each petri dish was observed every 24 hours and when sporulation started it was observed every 12 hours. The number of oocysts that sporulated out of 100 and the time in hours were recorded.

Statistical Analysis

Data obtained was analyzed using R Console software (Version 4.0.2). Pearson's Chi-square test was used to compare sporulation rates in relation to treatments per time. Level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The sporulation rates of the normal (N) and principal (P_1 and P_2) oocysts at room temperature are shown in Table 1. At the end of 24 hours, none of the oocyst sporulated in relation to the treatments. This agrees with Laverty *et al.* (2021) who opined that sporulation is said to occur only when up to four (4) oocysts become sporocysts with each containing 2 sporozoites.

Normal oocysts sporulated faster from the 36th to 96th hour and had the highest number of sporocysts over the principal oocysts P_1 (i.e. oocysts treated with normal dose of Amprosul (0.5g/l of water)) which sporulated slowly but much faster than P_2. Therefore, there was a very high significant difference in average sporulation rate in relation to the treatment groups at 36, 48 and 72 hours respectively (36 hours: $\chi^2 = 70.967$, df = 2, $P = 3.888 \times 10^{-16}$; 48 hours: $\chi^2 = 61.205$, df = 2, $P = 5.122 \times 10^{-14}$; 72 hours: $\chi^2 = 24.222$, df = 2, $P = 5.498 \times 10^{-6}$) but showed significant difference at the 96th hour ($\chi^2 = 1.9297$, df = 2, P = 0.381).

The highest number of sporulated oocysts for both principal oocysts P_1 and P_2 was recorded at 96 hours while for the normal oocysts it was between 36 and 48 hours. The oocysts recovered from birds medicated with double dose of Amprosul (1g/l water) P_2 did not sporulate well and on time nor in large numbers (i.e. sporulation was slow and in small number).

Table 1. Average Sporulation Rates of Normal Oocysts (N) and Principal Oocysts (P_1 and P_2) at Room Temperature

Type of oocysts	Source of oocysts	Time (hours) and number of sporulated oocysts out of 100				
		24	36	48	72	96
Normal (N)	Infected, Challenged, Non-medicated birds	0	68	92	92	92
Principal 1 (P_1)	Infected, Challenged, Medicated birds (0.5g/l of Amprosul)	0	15	36	60	89
Principal 2 (P_2)	Infected, Challenged, Medicated birds (1g/l of Amprosul)	0	8	18	37	75

The result of this study showed that both levels of the drug Amprosul had effect on the sporulation rates of *E. maxima* oocysts. The drug probably had a certain effect on the formation of oocysts or on the gametes, causing some destruction of zygote nucleus. Kogan (1960) reported a similar situation in oocysts of *E. necatrix* isolated from chicks that had been treated with sulphathiazole or phthalysulphathiazole; the oocysts sporulated very poorly. The result also conforms to the result obtained by Brackett and Bliznick (1949) who reported partial inhibition of sporulation of *E. tenella* oocysts by sulphaquinoxaline and nitrophenide. Joyner & Norton (1977) reported that chicks infected with the Weybridge strains of *E. maxima* and *E. acervulina* were not protected by the normal levels of amprolium, but the sporulation of the oocysts was inhibited. Sporulation of *E. brunetti* was reduced by the higher dosage of amprolium levels. Dinitolmide also reduced sporulation of *E. maxima* and *E. acervulina*. Much higher level was required obtain the effect with *E. brunetti*. Monensin at 120ppm affected neither oocysts numbers nor sporulation in any of the species. Ruff *et al.* (1993) also reported that sporulation of oocysts from chickens medicated with

amprolium was reduced compared with that of oocysts from unmedicated chickens. Sporulation was reduced by levels of 0.250% amprolium for *E. acervulina* and by levels of 0.0060% for *E. maxima* and the susceptible *E. tenella*. Amprolium medication had no effect on the sporulation of an amprolium-resistant *E. tenella*. They further reported that those oocysts from amprolium medicated chickens that did sporulate when fed to unmedicated chickens were as infective as oocysts recovered from unmedicated chickens.

CONCLUSION

Although the study did not work on the infectivity of the sporulated oocysts recovered from medicated birds, any drug (such as Amprosul (Agrar®)) that can inhibit the sporulation of chicken *Eimeria* oocysts will indeed be very useful in termination of coccidiosis breaks in poultry houses.

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