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## Examination of a Novel Practical Poultry Management Method to Enhance the Effect of Live *Eimeria* Vaccination for Conventionally Housed Replacement Layer Pullets

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**Abstract:** Coccidiosis, an enteric disease caused by *Eimeria* species, continues to be a substantial economic burden on the global poultry industry. With increased concerns regarding prophylactic antimicrobials to control coccidiosis alternate methods of preventative control have been developed, yet innovation to strengthen these methods has been limited. Live *Eimeria* vaccination stimulates immunity from the first small dose of vaccinal oocysts and is enhanced through low dose fecal-oral transmission ("cycling") of these oocysts. This immune response manifests as no pathogenic effects with minimal parasite reproduction. The success of a live coccidiosis vaccine is inextricably linked to poultry management techniques that successfully balance oocyst cycling with modest numbers of infective oocysts. The breadth of cage floor coverage using a durable fibre tray (lasting ~5 weeks) to improve oocyst cycling in live vaccinated caged pullets was investigated. Pullets were inoculated via oral gavage with mixed vaccinal *Eimeria* species and reared under simulated commercial conditions with 0, 20, 40 or 60% tray coverage from hatch to 42 days of age, then subsequently challenged with homologous *Eimeria* species. Mean total oocyst shed (output) between birds housed with or without coverage differed significantly after challenge infection. Pullets on 40% coverage had significantly lower mean oocyst output than the other treatment groups (>99% compared to no coverage). No difference in mean total oocyst output was found between the 20 and 60% treatment groups. Lesion scores mirrored oocyst shed results. Increased access to oocysts using 40% cage floor coverage with fibre trays over 5 weeks allowed for improved vaccine success in live coccidiosis vaccinated caged pullets.

**Key words:** Cage environment, coccidiosis, oocyst cycling, poultry environment, poultry production, management

### INTRODUCTION

Parasitic diseases, such as coccidiosis, differ from viral and bacterial infections due to several factors including a complicated life cycle, methods of transmission and means of control (McDougald, 2003). Coccidiosis, a disease complex produced by host-specific *Eimeria* species, has profound negative economic impact on global poultry production due to the cost of coccidial control (i.e. medication and vaccination), predisposition to secondary disease, morbidity (i.e., decreased performance due to impaired growth rate, poor feed conversion or temporary reduction in egg production) and, even though less common in current poultry production, some mortality (Foster, 1949; Williams, 1999; Dalloul, 2006; Kitandu and Juranová, 2006; Lee *et al.*, 2009; Sharman *et al.*, 2010). The direct impact on the

global layer industry has yet to be adequately realized but has been noted to be important in modern rearing (Soares *et al.*, 2004). While nine species of *Eimeria* infecting *Gallus gallus* var. *domesticus* have been described (Reid and Long, 1979), only seven species (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella*) are commonly associated with coccidial infections in long-lived commercial chickens such as layer birds (Williams, 1999). These distinct species can infect the host simultaneously or sequentially, hence the need for coccidiosis to be regarded as a disease complex in commercial production (Williams, 1999). Environmentally resistant oocysts transmitted via the fecal-oral route ("cycling") are the only means of transmission among birds in a flock (McDougald, 2003).

Poultry are capable of developing long-lasting species/strain-specific immunity to *Eimeria* species with minimal cross-immunity (Long and Millard, 1979; Allen and Fetterer, 2002). In the face of a disease challenge, manifestation of the immune response can be best represented by a spectrum of disease reduction. At one end of the spectrum is partial immunity, when challenged birds may demonstrate reduced infection severity compared with naïve birds but still shed oocysts (Chapman, 1978, 1999). The opposite end of the spectrum is complete immunity in which birds are able to fully block endogenous parasite development and no excretion of oocysts occurs. Complete immunity can be achieved after a single exposure with *E. maxima* (Schnitzler and Shirley, 1999; Williams, 1999). For coccidiosis generated by any species other than *E. maxima* or by simultaneous infections with multiple species, protective immunity, defined functionally by the absence of pathogenic effects with limited parasite reproduction and minimal oocyst output, is a realistic target to combat coccidiosis (Joyner and Norton, 1976). Daily inoculations of low levels of oocysts over a minimum of two to four weeks have been shown to establish protective immunity (Joyner and Norton, 1976; Nakai *et al.*, 1992; Parry *et al.*, 1992; Stiff and Bafundo, 1993; Chapman *et al.*, 2005a). Live *Eimeria* vaccines initiate generation of protective immunity by providing the first low level dose of oocysts (Reid, 1990; Chapman *et al.*, 2002).

Contrary to the belief that rearing birds in wire cages throughout production eliminated coccidiosis in these birds (Bell, 2002), coccidiosis in combination with necrotic enteritis has been recorded as a recent problem in conventionally reared pullets and layers (Gingrich, 2007). Layer and broiler breeder birds at approximately 18 to 24 weeks of age have been involved in coccidiosis outbreaks shortly after movement to the egg production barn (McDougald *et al.*, 1990; Soares *et al.*, 2004). This phenomenon is known in the poultry industry as “new house syndrome” (McDougald *et al.*, 1990; Chapman, 2000; Soares *et al.*, 2004). A reason for this condition developing in a flock is a lack of protective immunity that should have been generated at an earlier age (Chapman, 2000).

Replacement layer pullets are frequently reared in a tiered cage system with wire mesh floors that have 1 cm or larger spacing to allow feces to fall into a manure disposal system (Price, 2012). In this situation, pullets have minimal access to feces so little oocyst cycling may occur (Price, 2012). Some commercial pullet producers had used heavy paper from day of hatch to cover a portion of the cage floor to help with oocyst cycling in live vaccinated pullets; unfortunately, these birds still showed signs of coccidiosis once moved to the layer barn. It was postulated that the paper degraded too quickly (approximately 10-14 days) to permit adequate

oocyst cycling for the live vaccinated birds. Previous workers have suggested durable trays to be used for cage floor coverage to enhance oocyst cycling but such trays have not been tested in laboratory controlled settings (Soares *et al.*, 2004; Morgan *et al.*, 2009). A preliminary study demonstrated that live *Eimeria* vaccinated caged pullets reared on a moulded fibre tray that covered 10% of the cage floor and lasted approximately 5 weeks had improved protective immunity compared to similar birds reared on paper (KRP, unpublished observations; Price, 2012).

The objective of the present study was to determine the effect, if any, of different percentages of cage floor coverage by moulded fibre trays on the cycling of vaccinal oocysts in cage-reared pullets. Subsequent protection against homologous coccidial challenge was then assessed at 42 days of age to determine any differences in vaccine success within the modified cage environments.

## MATERIALS AND METHODS

**Vaccination:** Coccivac-D<sup>®</sup> (Merck Animal Health, Summit NJ) was used as the live coccidiosis vaccine in this trial. On day of hatch, each chick was inoculated via oral gavage with 250 oocysts of mixed *Eimeria* species as found in Coccivac-D<sup>®</sup> (contains proprietary numbers of each *E. tenella*, *E. mivati*, *E. acervulina*, *E. maxima*, *E. brunetti*, *E. hagani*, *E. necatrix* and *E. praecox*) diluted in 1 ml distilled water. Vaccine was delivered via oral gavage directly into the crop of birds receiving vaccine to minimize dose variability.

**Experimental design treatment stage:** A total of 280 commercial White Lohmann-LSL Lite day of hatch chicks (Archer's Poultry Farm Ltd., Brighton, Ontario, Canada) were vaccinated as per standard protocol in the hatchery, then inoculated as described above, wing tagged and randomly assigned into Hellmann<sup>®</sup> Poultry GmbH and Co. (London, Ontario, Canada) rearing cages (disinfected to be coccidia-free) at a bird density of 200 cm<sup>2</sup> per bird (approximately 35 birds per cage unit) from 0 to 42 days of age, as per Canadian Agri-Food Research Council (Anonymous, 2003) and institutional Animal Care Committee recommendations within the Central Animal Facility's Animal Isolation Unit (University of Guelph) in accordance with Canadian Council on Animal Care guidelines (Tennessen *et al.*, 2009). These cages and bird densities were used to simulate typical Canadian commercial replacement layer pullet brood conditions (Anonymous, 2003). The cage environment had 0, 20, 40 or 60% cage floor coverage (treatment groups) provided by the moulded fibre trays (Huhtamaki, Franeker, The Netherlands) and each treatment group was replicated for a total of eight isolated cages (Fig. 1). Cages were set up to prevent cross-contamination with oocysts among groups during

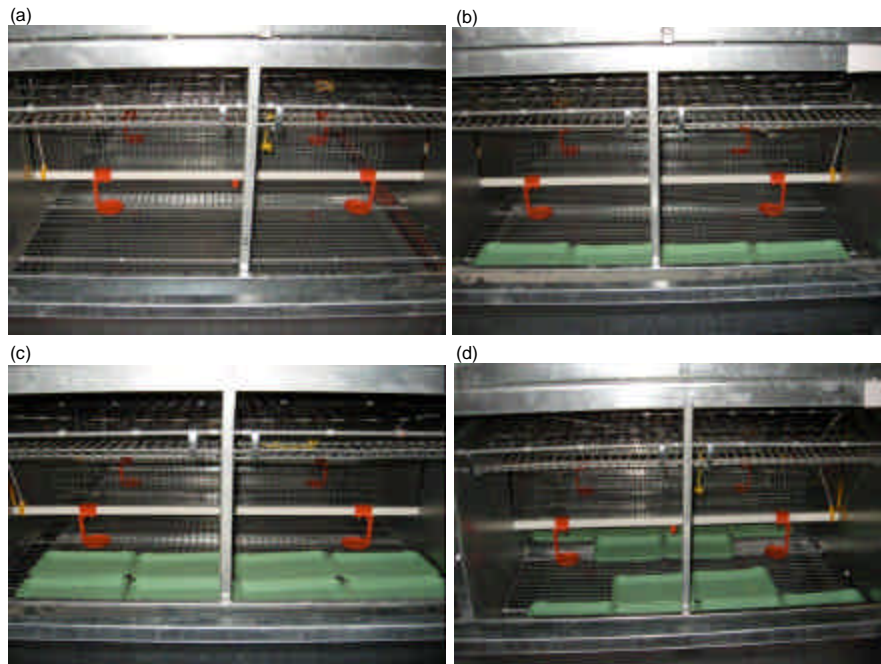


Fig. 1(a-d): The separate treatment cages with 0% (A), 20% (B), 40% (C) and 60% (D) wire coverage. The fibre trays were organized to avoid the nipple drinkers to prevent early degradation; however, pullets moved the trays around during the treatment stage of the experiment

the treatment phase. Positions of cages in the research treatment room were assigned randomly. Light intensity, temperature and relative humidity levels were monitored to replicate standard commercial production conditions as outlined by the Lohmann-LSL management guide (Anonymous, 2005) and the Canadian Agri-Food Research Council (Anonymous, 2003). Birds were provided a standard replacement layer pullet diet and water *ad libitum*. Brown paper (Fisher Scientific, Canada) was placed on the cage floor beneath the tray coverage for the first 13 days of life to prevent chicks' feet from falling through the mesh cage floor. This paper was changed daily to remove any feces that would have otherwise dropped through the cage floor (feces landing on the fibre trays was not disturbed). All feces on the removed papers (6, 9 and 12 days post-inoculation [DPI]) or dropping through the wire flooring (commencing 15 DPI) was collected for a 24 hour period every 3 days from 6 to 41 DPI to assess cycling of the parasite (see *Oocyst Output*, below). Mortalities that occurred (a total of 19) were not associated with any treatment. At 42 days of age all birds were weighed individually and assessed for animal welfare as described in *Animal Welfare Assessment*, below (Welfare Quality<sup>®</sup> Consortium, 2009).

**Experimental design challenge infection:** At the conclusion of the 6 week period, all pullets were given a homologous challenge dose of 13,000 oocysts of mixed *Eimeria* species as found in Coccivac-D<sup>®</sup> via oral gavage

to assess the manifestation of the immune response of birds within each treatment group. The number of parasites given in the challenge dose was established by a pre-challenge titration experiment on age and strain matched immunologically naïve birds that maximized host oocyst excretion without generating confounding severe disease (data not shown). From 1 to 5 days post challenge infection, pullets were placed into clean cages. At 6 days post challenge infection all pullets were weighed and 133 randomly selected vaccinated and challenged pullets were killed humanely by cervical dislocation (Charbonneau *et al.*, 2010) for lesion score analysis of the intestinal tract (see Johnson and Reid, 1970). The remaining vaccinated and challenged pullets (128) were kept to enumerate oocyst output. The latter pullets were randomly distributed into cages within randomly allocated rooms at 1 bird per cage.

**Oocyst output:** Total fecal output from each bird was collected from metal trays below each cage for 24 hours each day from 6 to 11 days post challenge infection to determine the total number of oocysts shed during this period. Samples were stored at 4°C. Total oocyst output from blinded samples was determined by the McMaster counting chamber technique using saturated NaCl as the flotation medium (Long *et al.*, 1976). Each sample was counted twice, blindly and the two counts were averaged to provide a single mean total oocyst count for each sample.

**Animal welfare assessment:** Foot pad dermatitis, hock burn and plumage cleanliness were chosen to be evaluated using the Welfare Quality<sup>®</sup> Assessment Protocols for Poultry (Welfare Quality<sup>®</sup> Consortium, 2009). These scores are normally assessed for birds reared on the floor; however, by placing trays covering a portion of the cage floor these pullets may share similar welfare issues. Additionally, foot pad dermatitis associated with cages ("wire floor foot pad dermatitis" or bumble foot) was considered because this may, in part, be generated by hygiene problems. All birds were assessed, blindly, using recording and analysis guidelines from the Welfare Quality<sup>®</sup> Assessment Protocols for Poultry (Welfare Quality<sup>®</sup> Consortium, 2009).

**Statistical analyses:** All statistical analyses were performed using the analytical software SAS (SAS 9.2, Cary, NC). The PROC MIXED general linear mixed model generated standard error values, upper and lower 95% confidence intervals and p-values. The PROC GLIMMIX model was used for categorical data (lesion scores and animal welfare) in addition to the PROC MIXED model. Comprehensive residual analysis was used to examine Analysis of Variance (ANOVA) assumptions. The residual analysis was conducted as it may suggest the use of transformations, the presence of outliers, unequal variance or other patterns suggesting problems with the ANOVA assumptions. For all experimental tests  $p < 0.05$  was deemed significant.

**Weight data:** The least square means of the weights prior to challenge were calculated to determine whether there were any significant differences between treatment groups at the end of the treatment period using a Tukey-Kramer test. The difference between least square means of body weights after challenge and the body weights prior to challenge (treatment by time interaction), accounting for differences in body weights prior to challenge, were assessed for any significant changes.

**Challenge: Total fecal oocyst output data:** Residual analysis indicated that the data had to be natural log transformed to account for large variances. The average of the two counts for each sample was used as the dependent variable. Cage and room (random) effect analysis was conducted to determine whether the oocyst output from a treatment group over each day could be totalled and averaged. Least square means were calculated to determine which group had the lowest average total oocyst output. Differences of least square means were calculated and compared using Duncan's test to establish any significant differences between treatment groups.

**Animal welfare and lesion score data:** Both an ANOVA and a GLIMMIX analysis were performed on these

categorical data. For the ANOVA model, the assumption was made that the difference in severity between a score of 0 and 1 was similar to the difference in severity between a score of 1 and 2 and so on. Under this assumption, score means may be analyzed for the ANOVA analysis. Lesion scores range from 0 to 4, while the animal welfare qualities assessed had different score ranges: plumage cleanliness scores range from 0 to 3; foot pad dermatitis scores range from 0 to 4; hock burn scores range from 0 to 4 and wire floor foot pad dermatitis scores range from 0 to 2 (Johnson and Reid, 1970; Welfare Quality<sup>®</sup> Consortium, 2009). Tests of random and fixed effects were performed. The differences of least square means were calculated to determine any significant differences between lesion scores among separate intestinal regions (upper intestine, middle intestine, lower intestine and cecum). The GLIMMIX model directly analyzes categorical data without any assumptions regarding the nature of the scoring data. The data was determined to have a Poisson distribution and a Tukey-Kramer test was used to determine whether there were any statistically significant differences between treatment groups. Some categorical data was not suitable for analysis using the GLIMMIX model because of score distribution. In these cases no GLIMMIX analysis was performed.

## RESULTS

**Treatment:** Chicks exhibited normal activity upon the fibre trays and defecated freely on them (Fig. 2). The mean total oocyst output per bird for each cage floor coverage treatment over days post inoculation is shown in Table 1.

Table 2 summarizes the animal welfare scores (plumage cleanliness, foot pad dermatitis, hock burn and wire floor foot pad dermatitis) for each treatment group for the ANOVA analysis. Animal welfare scores in



Fig. 2: Pullets on the moulded fibre trays, with temporary paper below, at one week of age. Pullets actively used and defecated on the fibre trays

Table 1: The mean total oocyst output numbers per bird for each treatment group over days post initial inoculation to provide the temporal pattern of oocyst shedding

	6 DPI	9 DPI	12 DPI	15 DPI	18 DPI	21 DPI	24 DPI	27 DPI	30 DPI	33 DPI	36 DPI
0%	3,739,489	1,437,174	3,966,752	2,661,864	5,441,239	314,337	54,207	TFTC <sup>a</sup>	TFTC	TFTC	TFTC
20%	2,548,617	660,955	3,609,343	3,422,355	2,004,504	351,016	102,437	TFTC	TFTC	TFTC	TFTC
40%	5,954,388	2,238,996	10,253,872	5,816,825	2,442,300	565,810	401,476	51,330	22,861	TFTC	TFTC
60%	3,037,401	1,768,450	7,484,236	3,709,522	2,977,144	603,371	220,023	76,784	8,180	TFTC	TFTC

<sup>a</sup>TFTC (too few to count) was used when no oocysts can be detected from a NaCl flotation

Table 2: The least square mean<sup>a</sup> scores ( $\pm$  standard error) for the plumage cleanliness, foot pad dermatitis, hock burn and wire floor foot pad dermatitis animal welfare parameters for birds housed on wire cage floor with different portions of the cage floor covered with resilient fibre trays

	Plumage cleanliness (score 0-3)	Foot Pad Dermatitis (score 0-4)	Hock Burn (score 0-4)	Wire Floor Foot Pad Dermatitis (score 0-2)
0% coverage	0.1 $\pm$ 0.07 <sup>a</sup>	0 <sup>+</sup> $\pm$ 0.07 <sup>a</sup>	0 <sup>+</sup> $\pm$ 0.07 <sup>a</sup>	0.2 $\pm$ 0.07 <sup>a</sup>
20% coverage	0.2 $\pm$ 0.07 <sup>b</sup>	0 <sup>+</sup> $\pm$ 0.07 <sup>a</sup>	0 <sup>+</sup> $\pm$ 0.07 <sup>a</sup>	0.1 $\pm$ 0.07 <sup>a, b</sup>
40% coverage	0.6 $\pm$ 0.07 <sup>c</sup>	0.3 $\pm$ 0.07 <sup>b</sup>	0 <sup>+</sup> $\pm$ 0.07 <sup>a</sup>	0 <sup>+</sup> $\pm$ 0.07 <sup>b</sup>
60% coverage	0.7 $\pm$ 0.07 <sup>c</sup>	0 <sup>+</sup> $\pm$ 0.07 <sup>a</sup>	0 <sup>+</sup> $\pm$ 0.07 <sup>a</sup>	0 <sup>+</sup> $\pm$ 0.07 <sup>b</sup>

0<sup>+</sup> indicates a non-zero average lesion score that rounds to 0 at 1 significant decimal place, Groups displaying the same letters within a column only do not differ significantly ( $p>0.05$ ). <sup>1</sup> For the purpose of ANOVA analysis, the difference in severity between a score of 0 and 1 was considered similar to the difference between a score of 1 and 2 and so on (SAS 9.2)

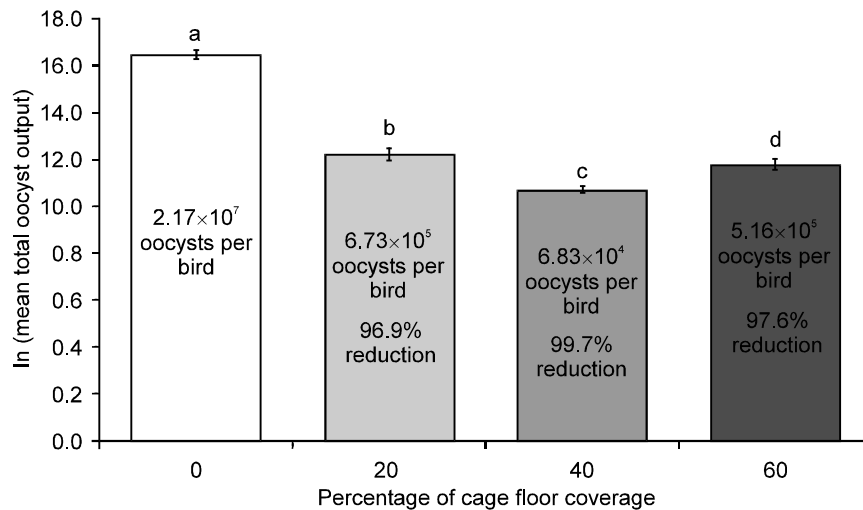


Fig. 3: The natural log transformed mean total oocyst output numbers, with standard error bars, after challenge for each treatment group. Percent reduction of mean total oocyst output numbers compared to the 0% treatment group is given within the graph bars. Groups displaying the same letters do not differ significantly ( $p>0.05$ )

all categories and all treatment groups were modest. No significant differences were found between treatment groups for the hock burn scores. Birds housed with 40% cage floor coverage had significantly higher ( $p<0.05$ ) foot pad dermatitis score ( $0.3 \pm 0.07$ ) than birds housed with 0% ( $0.0 \pm 0.07$ ), 20% ( $0.0 \pm 0.07$ ), or 60% ( $0.0 \pm 0.07$ ) cage floor coverage. Birds reared with no cage floor coverage experienced significantly higher ( $p<0.05$ ) wire floor foot pad dermatitis least square mean scores than birds reared with 40% or 60% cage floor coverage ( $0.3 \pm 0.07$  compared to  $0.0 \pm 0.07$  for both the 40% and 60% treatment groups, respectively). Birds reared with any cage floor coverage had significantly worse ( $p<0.05$ ) plumage cleanliness scores than birds reared with no floor coverage.

Mean body weights at the end of the treatment phase (immediately prior to challenge) at 42 days of age (Table

3) showed significant differences between some treatment groups. Birds in the 0% treatment group had the highest mean body weight pre-challenge ( $530.6g \pm 5.17$ ) but this mean weight was not significantly different from the weight of birds in the 20% treatment group ( $519.2g \pm 5.21$ ). Pullets from the 40% and 60% treatment groups had significantly lower weights ( $506.4g \pm 4.90$  and  $502.4g \pm 5.05$ , respectively) than birds from the 0% treatment group.

**Challenge: Total oocyst output, lesion score and weight data:** Total oocyst output was assessed from 6 to 11 days following the challenge infection initiated at 42 days of age (Fig. 3). The mean total oocyst output from birds reared with no cage floor coverage during treatment after challenge with 13,000 mixed *Eimeria* species oocysts was  $2.17 \times 10^7$  oocysts per bird. Birds

Table 4: The least square mean<sup>1,2</sup> lesion scores ( $\pm$  standard error) for the upper, middle, lower and cecal intestinal region for birds housed on wire cage floor with different portions of the cage floor covered with resilient fibre trays

	Upper Intestine	Middle Intestine <sup>3</sup>	Lower Intestine <sup>3</sup>	Cecum
0% coverage	1.3 $\pm$ 0.11 <sub>A</sub> <sup>a</sup>	0.1 $\pm$ 0.11 <sup>a</sup>	0.1 $\pm$ 0.11 <sup>a</sup>	0.9 $\pm$ 0.11 <sub>A</sub> <sup>a</sup>
20% coverage	0.6 $\pm$ 0.11 <sub>A</sub> <sup>b</sup>	0 $\pm$ 0.11 <sup>a</sup>	0.1 $\pm$ 0.11 <sup>a</sup>	0.2 $\pm$ 0.11 <sub>B</sub> <sup>b</sup>
40% coverage	0.2 $\pm$ 0.10 <sub>B</sub> <sup>c</sup>	0 $\pm$ 0.10 <sup>a</sup>	0 $\pm$ 0.10 <sup>a</sup>	0.1 $\pm$ 0.10 <sub>B</sub> <sup>b</sup>
60% coverage	1.1 $\pm$ 0.10 <sub>A</sub> <sup>a</sup>	0.1 $\pm$ 0.10 <sup>a</sup>	0 $\pm$ 0.10 <sup>a</sup>	0.1 $\pm$ 0.10 <sub>B</sub> <sup>b</sup>

Lesion scores for each region can range between 0 and 4, 0<sup>\*</sup> indicates a non-zero average lesion score that rounds to 0 at 1 significant decimal place, Groups displaying the same letters within a column only do not differ significantly ( $p>0.05$ ). <sup>1</sup>For the purpose of ANOVA analysis, the difference in severity between a score of 0 and 1 was considered similar to the difference between a score of 1 and 2 and so on (SAS 9.2). <sup>2</sup> Lower case superscript letters represent ANOVA analysis and upper case subscript letters represent GLIMMIX analysis (SAS 9.2). <sup>3</sup>For the middle and lower intestinal region GLIMMIX analyses did not converge

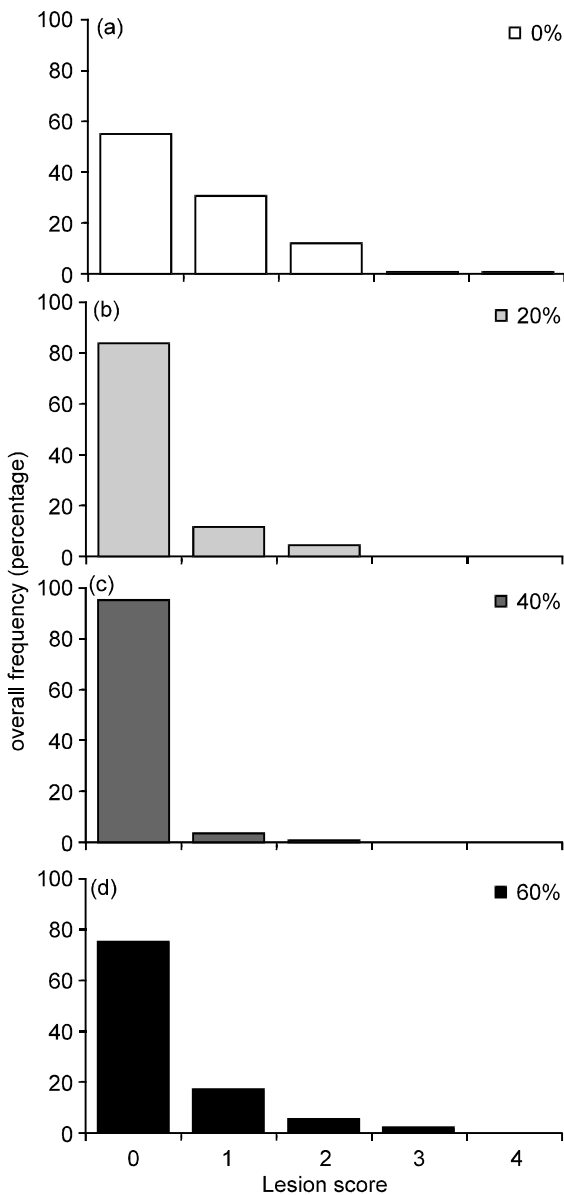


Fig. 4(a-d): The overall lesion score frequency percentage for each treatment group at six days post challenge infection around six weeks of age. Lesion scores are measured on a scale from 0 to 4

reared with trays covering a portion of the cage flooring shed fewer oocysts after challenge: for birds reared with 20% cage floor coverage, mean total oocyst output per bird was reduced 96.9% to  $6.73 \times 10^5$ ; for birds reared with 40% cage floor coverage, mean total oocyst output per bird was reduced 99.7% to  $6.83 \times 10^4$  and, for birds reared with 60% cage floor coverage, mean total oocyst output per bird was reduced 97.6% to  $5.13 \times 10^5$ . Birds reared during treatment with 40% cage floor coverage had a significantly lower ( $p<0.05$ ) natural log transformed mean total oocyst output ( $\ln(10.6 \pm 0.1)$ ) than birds reared with 0% cage floor coverage ( $\ln(16.4 \pm 0.1)$ ), 20% cage floor coverage ( $\ln(12.1 \pm 0.2)$ ), or 60% cage floor coverage ( $\ln(11.7 \pm 0.2)$ ).

Least square means of lesion scores for each treatment group for each scoring region (upper intestine, middle intestine, lower intestine and cecum) are presented in Table 4. No statistically significant differences between the treatment groups were found for the middle and lower intestinal regions for the ANOVA analysis. The GLIMMIX model was unable to converge for the two aforementioned regions. Statistically significant differences ( $p<0.05$ ) between treatments were only detected in the upper intestinal and cecal regions for both the ANOVA and GLIMMIX analyses (Table 4). In the upper intestinal region, birds reared with 40% cage floor coverage had the lowest mean lesion score of  $0.2 \pm 0.10$  compared to  $1.3 \pm 0.11$ ,  $0.6 \pm 0.11$  and  $1.1 \pm 0.10$  for the 0, 20 and 60% treatment groups, respectively, for both the ANOVA and GLIMMIX analyses. In the cecal region, birds reared with cage floor coverage had statistically significant lower mean lesion scores than birds reared with no coverage for both the ANOVA and GLIMMIX analyses (Table 4). However, there were no statistically significance differences among the 20, 40 and 60% treatment groups. The overall lesion score frequencies for each treatment (Fig. 4) illustrate that birds housed with 40% cage floor coverage had more lesions scores of 0 (95.1%) compared to the 0, 20 and 60% treatment groups (54.7%, 83.6% and 75.0%, respectively).

## DISCUSSION

Efficacy of live coccidiosis vaccination is inextricably linked to poultry management. There are two main management factors that impact successful vaccination with live coccidiosis vaccines: 1) vaccine administration

(usually at the hatchery)-synchronous, uniform exposure to a small controlled dose of vaccinal oocysts and 2) environmental control in the barn-encouraging cycling of vaccinal oocysts through several low-level trickle infections until protective immunity is elicited (Long and Millard, 1979; Reid, 1990; Shirley, 1993).

A common method of live coccidiosis vaccine administration is the hatchery spray cabinet delivery method (Chapman, 2000; Vermeulen *et al.*, 2001; Chapman *et al.*, 2002). This delivery method largely bypasses the flock hierarchy as oocyst uptake may occur through the intra-ocular route, direct ingestion or ingestion via preening (as promoted by coloured dye) (Chapman, 2000; Caldwell *et al.*, 2001; Vermeulen *et al.*, 2001; Chapman *et al.*, 2002). However, the actual uniformity of vaccinal oocyst uptake may not be reliable as only ingestion of the coloured dye, not the number of oocysts, can be practically assessed after each vaccination at the hatchery. This shortcoming of hatchery spray vaccination can be addressed through environmental transmission of parasites from infected (vaccinated) to uninfected (missed during vaccination) birds in the barn (Velkers *et al.*, 2012a).

A live coccidiosis vaccine administered to the flock, without additional management in the barn, may provide a "protective base" to initiate control of endogenous parasite development and generate partial immunity (Chapman, 1978, 1999). To achieve successful protective immunity for mixed *Eimeria* species infection, management techniques must allow for prolonged oocyst cycling with the minimum numbers of infective oocysts required to elicit this immunity (Joyner and Norton, 1976; Nakai *et al.*, 1992; Parry *et al.*, 1992; Stiff and Bafundo, 1993; Williams, 2002; Chapman *et al.*, 2005a, b). Once the bird has developed protective immunity to a particular *Eimeria* species/strain it is assumed to persist for the bird's lifetime; protective immunity against multiple *Eimeria* species lasting at least 18 weeks has been demonstrated experimentally (Long and Millard, 1979).

A combination of fecundity of the ingested species (Chapman *et al.*, 2002), exposure to oocysts (via contaminated feces or other vectors) (Reyna *et al.*, 1983) and the environment (including housing) (Chapman *et al.*, 2002; Price, 2012) may impact the transmission rate in field conditions (Velkers *et al.*, 2010a, 2012b). Live coccidiosis vaccine administration determines which parasite species infect the birds; in-barn management affects the environment indirectly controlling the fecal-oral cycling and subsequent re-exposure of birds to oocysts. For a particular bird in a flock or cage, a suboptimal dose of vaccine may be sufficient for protective immunity if adequate cycling of oocysts in the barn environment among birds is maintained (Velkers *et al.*, 2012a). Thus, the method of

housing must be taken into account because birds reared on floor would have increased access to cycling vaccinal oocysts compared to birds reared in a conventional cage system (Price, 2012). The means of achieving low-level, repeated prolonged exposure to vaccinal strains of parasites to confer long-lasting immunity against homologous challenge in cage-reared pullets has not been well defined but covering a portion of the cage flooring with resilient fibre trays appears to achieve this goal.

Previous studies have noted that the use of a flat material can assist with oocyst cycling in caged pullets (Soares *et al.*, 2004); however, the breadth of coverage most efficient at enhancing protective immunity in live vaccinated caged-reared pullets had not been explored systematically until now. The current study controlled for variation of live coccidiosis vaccine uniformity by using oral gavage as the vaccine administration method. In this way, the effect of area of cage floor coverage with the trays on resultant oocyst cycling and protective immunity could be tested independently. Throughout the treatments (live vaccination with different percentage cage floor coverage), oocyst output was enumerated to discover the temporal oocyst shedding patterns. Interestingly, birds reared in cages with 40 and 60% floor coverage shed detectable oocysts for at least 6 days longer than birds reared with 0 or 20% coverage; these data suggest that the parasites are 'cycling' for longer in these groups compared with the 0 and 20% floor coverage groups. Perhaps, introducing a tray into the cage environment increases the probability of a bird ingesting contaminated infectious oocysts from feces; however, the dynamics of parasite transmission among caged birds with and without coverage has yet to be explored.

Although, mean body weights immediately post-treatment decreased as cage floor coverage increased, post-treatment weights for all treatment groups were at the upper end of the normal body weight range for a pullet at 42 days of age (see Lohmann-LSL management guide, Anonymous, 2005). Body weights were last measured at 48 days of age and therefore compensatory growth previously demonstrated in birds following coccidial infections (Fitz-Coy and Edgar, 1992; Chapman, 2000; Chapman *et al.*, 2002; Williams, 2002; Nollet *et al.*, 2007; Lee *et al.*, 2009) could not be measured.

Challenge infections with a modest homologous oocyst inoculation at 42 days of age were used to assess the relative protective immunity elicited during the various treatments. Birds vaccinated by oral gavage and reared on 40% cage floor coverage with the durable fibre trays over 5 weeks allowed for significantly reduced total oocyst output (Fig. 3) as well as lower lesion scores for the upper and cecal intestinal regions (Table 4)



after challenge infection compared to similarly vaccinated birds reared without cage floor coverage. Notably, inoculated birds reared with 40% cage floor coverage experienced a 99.7% reduction in oocysts shed relative to inoculated birds reared with 0% cage floor coverage after challenge. Although use of lesion scores as a measure of the effectiveness of anticoccidial drugs is well-established (Chapman, 1998), use of lesion scores to assess protective immunity is controversial (Williams and Catchpole, 2000; Chapman *et al.*, 2005b). Lesion scores obtained in immunized birds following challenge can be unreliable because immunopathological (inflammatory) changes to the mucosa can mimic and be confused with lesions associated with parasite development (Chapman *et al.*, 2005b). Nonetheless, the lesion scores after challenge correlated well with the mean total oocyst output results in the present study (Table 4). Post-challenge, the mean body weight gains, accounting for significant differences between body weights prior to challenge, was not biologically significant and were within the normal range for a pullet around 48 days old, as determined by the Lohmann-LSL management guide (Anonymous, 2005). The modest challenge dose may not have been large enough to impact the pullets' weight gains; thus, post-challenge weight gains were an unreliable measure of protective immunity in this study. Preventing or controlling clinical coccidiosis may become a bigger challenge in intensive poultry production in the face of legislation limiting prophylactic anticoccidial use; avoiding coccidiosis in such conditions will require a long-term solution for coccidiosis control. For these reasons, further research is needed to develop practical methods that will expand the utility of live coccidiosis vaccination in a wide range of poultry production environments. The findings of the present study demonstrate that proactive poultry environmental management can be used to enhance the success of live coccidiosis vaccination in caged birds. We demonstrated that 40% coverage of cage floors with durable fibre trays for an extended duration (~5 weeks) had a significant and beneficial impact on the success of live coccidiosis vaccination with cage-reared pullets. Birds housed with 40% coverage of their cage floors developed the strongest protective immunity against challenge. Animal welfare scores post-treatment were modest suggesting that the direct impact on the birds of the cage flooring modification was clinically irrelevant. This "easy to use", relatively inexpensive and environmentally friendly modification requires no additional labour after the day of pullet placement because the fibre trays disintegrate and fall through the wire floor into the manure disposal system after approximately 5 weeks. Adoption of this environmental management tool may enhance the success of live vaccination against coccidiosis in cage-reared poultry.

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