Research note: Lateral transmission of *Histomonas meleagridis* in turkey poults raised on floor pens

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ABSTRACT Histomoniasis is caused by the protozoa *Histomonas meleagridis* (**HM**) that are laterally transmitted among birds leading to high mortality in commercial flocks. This study tested an HM infection model assessing the lateral transmission of HM in turkey poults raised on floor pens. Day (d)-old female turkey poults (n = 320) were individually wing-tagged and allocated to one of four treatment groups (4 floor pens/group and 20 poults/pen) based on the percentage of poults inoculated with HM: 1) 10% (HM10); 2) 20% (HM20); 3) 30% (HM30); and 4) 40% (HM40). On d 9, seeder poults intracloacally received a 1 mL inoculum/bird containing ~80,000 histomonads. Poults were individually weighed on d 0, 9, and 25 and feed intake recorded on per pen basis. On d 25, all birds were euthanized by cervical dislocation and ceca and liver were evaluated for HM lesions. Data were analyzed using JMP (Pro16) and significance ($P \leq 0.05$) between treatments were determined by LSD test. Mortality was 7.63%, 12.5%, 21.58%, and 20.59%, while transmission rates from inoculated to non-inoculated birds were 62.5%, 57.5%, 92.43%, and 78.75% in HM10, HM20, HM30, and HM40 groups, respectively. Average daily feed intake was proportionally reduced with the increasing number of inoculated poults from HM10 to HM40. Average daily gain was significantly lower in HM30 and HM40 poults compared to those in HM10 and HM20 during the postchallenge period (d 10−25). Therefore, we herein report the successful lateral transmission of HM among turkey poults raised on floor pens. This research model closely resembles commercial field conditions and affords a much-needed platform for conducting relevant basic and applied research on histomoniasis in poultry.

Key words: *Histomonas meleagridis*, lateral transmission, floor pen, blackhead, turkey poults

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INTRODUCTION

Histomonas meleagridis is an important parasite of poultry with significant negative effects on production especially in turkeys and broiler breeders (Clark and Kimminau, 2017; Liebhart et al., 2017). *H. meleagridis* is the responsible agent of histomoniasis (blackhead disease) (Hess et al., 2015; Mitra et al., 2018). This parasite resides in the ceca and disrupts the cecal lining and eventually transmits to the liver via blood (McDougald and Fuller, 2005). Field cases of histomoniasis in turkeys are characterized by high morbidity, as parasites spread from bird to bird throughout the flock via lateral transmission, as well as high mortality (Clark and Kimminau, 2017). The lack of an effective drug or solution to prevent or alleviate the negative effects of histomoniasis has driven a prompt need to research this disease (Barros et al., 2020). Disease models investigating the pathogenesis of *H. meleagridis* in turkeys and chickens have been developed to find potential means (nutritional, managerial, etc.) to alleviate its negative effects. These models use various approaches to infect the birds, including intracloacal inoculation of all experimental birds with either *H. meleagridis* or *Heterakis gallinarum* eggs, which are the vectors for *H. meleagridis* (Hauck and Hafez, 2013; Beer et al., 2022).

Despite these efforts, the real challenge in commercial settings is the lateral transmission of *H. meleagridis* from bird to bird. Not all recent research models consider this important transmission phenomenon; therefore, for studying blackhead, researchers individually inoculate all experimental birds. Previous studies aimed to clarify the introduction of the protozoan parasite into a flock and the transmission between birds using molecular tools (Hess et al., 2015). One of the only reports on successful lateral transmission of *H. meleagridis* was in a battery cage model (McDougald and Fuller, 2005), which did not mimic filed conditions with birds raised on the floor. Earlier trials explored lateral transmission

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of *H. meleagridis* in floor pens; however, with only one replicate pen per treatment with low number of birds (Hu and McDougald, 2003; Landman et al., 2015).

Accordingly, current models are not practical for understanding disease progression in commercial settings that could facilitate managerial strategies to prevent the lateral transmission of *H. meleagridis* and/or develop practical approaches to mitigate the disease. In addition, inoculating all experimental birds is labor intensive and not always feasible especially in large scale experiments. Development of a model that closely resembles commercial field conditions is a critical step in better understanding blackhead disease and provides much-needed opportunities for conducting relevant basic and applied research on histomoniasis in poultry. Therefore, the objective of this trial was to develop a research model for the lateral transmission of *H. meleagridis* in turkeys raised on the floor.

MATERIALS AND METHODS

Treatments and Housing

A total of 320, day (d)-old female turkey poults were wing-tagged, individually weighed, and allocated to one of four treatment groups (4 floor pens/group and 20 poults/pen) based on the percentage of poults inoculated with *H. meleagridis*: 1) 10% (HM10); 2) 20% (HM20); 3) 30% (HM30); and 4) 40% (HM40). On d 9, seeder poults received 1 mL inoculum/bird containing ~80,000 histomonads (UGA Buford strain) using a blunt-tipped pipette inserted about 3 cm into the cloaca. Poults were raised on floor pens (1.1 m^2) lined with fresh wood shavings with free access to water and commercial type mash starter feed during d 0 to 25. All the experimental procedures were conducted based on IACUC procedures of the University of Georgia.

Performance, Mortality, and Lesion Scoring Criteria

Poults were individually weighed on d 0, 9, and 25 and feed intake recorded on per pen basis. Mortality was checked and recorded twice daily, and performance including average daily gain (**ADG**), and average daily feed intake (**ADFI**) were adjusted for mortality. Furthermore, dead birds were necropsied and examined for histomoniasis lesions. On d 25, all birds were euthanized by cervical dislocation, and ceca and liver were inspected for HM lesions based on a scale of 0 to 4 (McDougald and Hu, 2001). Birds with no lesions were considered non-infected while those with a lesion score of 1 or above were considered infected.

H. meleagridis *Culture*

Frozen isolates of *H. meleagridis* (UGA Buford strain) were thawed in a water bath at 41°C, and cultured in modified Dwyer's medium consisting of rice flour and 10% horse serum in medium 199 with Hanks' balanced salt solution (Sigma) at 41°C. Every 2 to 3 d, culture flasks were split into 2 flasks containing fresh media. On inoculation day, all fresh cultures were pooled and histomonads

Table 1. Effect of number of birds, inoculated intracloacally with *Histomonas meleagridis* on day (d) 9, on performance of turkey poults.

	Treatments[1]				*Statistics*	
Parameter[2]	HM10	HM20	HM30	HM40	*SEM*[3]	*P value*[4]
d 0–9						
ADFI	17.97	18.75	17.69	17.08	*0.49*	*0.178*
ADG – overall	12.19	12.37	13.82	13.24	*0.42*	*0.101*
d 10–25						
ADFI	48.59	46.76	45.38	40.81	*2.32*	*0.162*
ADG – overall	29.75[a]	30.64[a]	24.25[b]	23.78[b]	*1.72*	*0.027*
ADG – inoculated birds	11.39[ab]	17.02[a]	6.15[b]	14.70[a]	*2.61*	*0.006*
ADG – non-inoculated birds	31.52[b]	33.55[a]	30.00[bc]	29.37[c]	*0.63*	*<0.001*
d 0–25						
ADFI	37.38	36.55	34.93	32.05	*1.61*	*0.149*
ADG – overall	23.33	23.98	20.32	19.93	*1.18*	*0.069*
ADG – inoculated birds	12.23[ab]	15.60[a]	9.91[b]	14.34[a]	*1.33*	*0.030*
ADG – non-inoculated birds	24.59[b]	25.87[a]	24.39[b]	23.65[b]	*0.45*	*0.012*

[a-c]Within each row, means with different letters are significantly different.

[1]A total of 320 d-old female turkey poults were individually wing-tagged and allocated to one of four treatment groups (4 floor pens/group and 20 poults/pen) based on the percentage of poults inoculated with *Histomonas meleagridis* (HM): 1) 10% (HM10); 2) 20% (HM20); 3) 30% (HM30); and 4) 40% (HM40).

[2]ADFI: average daily feed intake; ADG: average daily gain.

[3]*SEM*: pooled standard error of means.

[4]*P-value*: based on one-way ANOVA.

Table 2. Effect of number of birds, inoculated intracloacally with *Histomonas meleagridis* on day (d) 9, on turkey poults mortality, lateral transmission rate and lesion scores.

	Treatments[1]				*Statistics*	
Parameter	HM10	HM20	HM30	HM40	*SEM*[2]	*P value*[3]
Mortality (%)	7.63	12.5	21.58	20.59	*5.31*	*0.245*
Infection rate (%)						
Overall	62.5[bc]	57.5[c]	92.43[a]	78.75[ab]	*6.44*	*0.009*
Inoculated birds	100	87.5	100	93.75	*6.50*	*0.495*
Non-inoculated birds	58.32[b]	50[b]	89.15[a]	70.27[ab]	*8.60*	*0.037*
Ceca lesion scores						
Overall	0.97[c]	1.05[c]	2.45[a]	2.00[b]	*0.15*	*<0.001*
Inoculated birds	3.62	3.12	3.91	3.16	*0.25*	*0.090*
Non-inoculated birds	0.68[c]	0.53[c]	1.82[a]	1.22[b]	*0.11*	*<0.001*
Liver lesion scores						
Overall	0.40[b]	0.60[b]	1.16[a]	1.33[a]	*0.18*	*<0.001*
Inoculated birds	3.50	3.0	3.83	2.78	*0.32*	*0.084*
Non-inoculated birds	0.05[b]	0.00[b]	0.00[b]	0.34[a]	*0.06*	*0.007*

[a-c]Within each row, means with different letters are significantly different.

[1]A total of 320 d-old female turkey poults were individually wing-tagged and allocated to one of four treatment groups (4 floor pens/group and 20 poults/pen) based on the percentage of poults inoculated with *Histomonas meleagridis* (HM): 1) 10% (HM10); 2) 20% (HM20); 3) 30% (HM30); and 4) 40% (HM40).

[2]*SEM*: pooled standard error of means.

[3]*P-value*: based on one-way ANOVA.

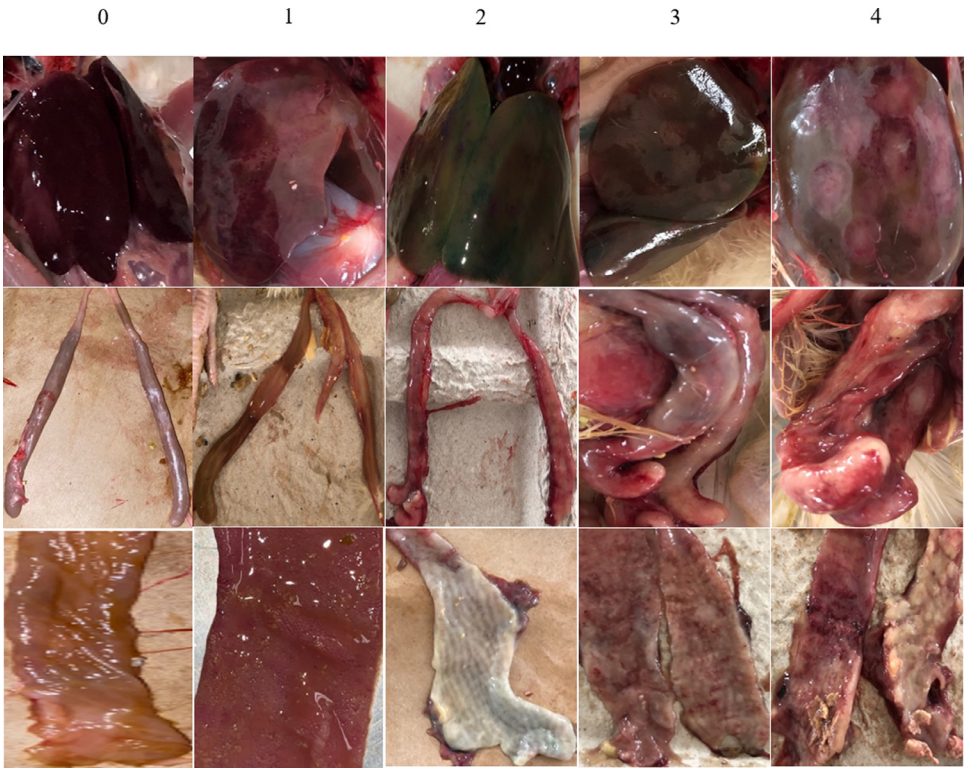

Figure 1. Histomoniasis lesions for liver and ceca based on a scale of 0 to 4.Ceca and liver were inspected for *Histomonas meleagridis* lesions based on a scale of 0 to 4 as previously described (Hu et al., 2004). A total of 320 d-old female turkey poults were individually wing-tagged and allocated to one of four treatment groups (4 floor pens/group and 20 poults/pen) based on the percentage of poults inoculated with *Histomonas meleagridis* (HM): 1) 10% (HM10); 2) 20% (HM20); 3) 30% (HM30); and 4) 40% (HM40).

were counted using a Neubauer blood cell chamber and a cell counter and kept at 41°C until inoculation.

Statistical Analysis

Data were analyzed using JMP (Pro16) and significance ($P \leq 0.05$) between treatments were determined by LSD test.

RESULTS AND DISCUSSION

Performance data are shown in Table 1. ADG was significantly lower in HM30 and HM40 groups compared to HM10 and HM20 during the postchallenge period (d 10 −25). ADFI was proportionally reduced from HM10 to HM40, and was directly associated with the increase in the number of poults inoculated per group.

Mortality, infection rate, and ceca and liver lesion scores on d 25 are shown in Table 2. Mortality was 7.63%, 12.5%, 21.58%, and 20.59% in HM10, HM20, HM30, and HM40 groups, respectively, with the first histomoniasis mortality occurring in the inoculated seeder birds on d 9 post inoculation. Morbidity became common in all the groups as the infection progressed. HM was successfully transmitted from inoculated to non-inoculated birds with infection rates of 62.5%, 57.5%, 92.43%, and 78.75% in HM10, HM20, HM30, and HM40 groups, respectively. Necropsy of the dead birds revealed lesions typical of histomoniasis in the liver and ceca (Figure 1). HM30 and HM40 had significantly greater lesion scores in the liver compared to HM10 and HM20. The highest lesion scores in the ceca belonged to HM30 and they were significantly different from other groups. Furthermore, cecal lesion scores were significantly higher in HM40 compared to HM10 and HM20. These results are in line with previous reports of blackhead disease indicating the successful lateral transmission of *H. meleagridis* from bird to bird in turkey poults raised on floor pens (Hu and McDougald, 2003). Others reported transmission of *H. meleagridis* from bird to bird in mixed sex turkey poults using qPCR, although there was no replication in that experiment (Landman et al., 2015).

Therefore, we herein report the successful lateral transmission of *H. meleagridis* among turkey poults raised on floor pens with 30% seeder birds as the best ratio. This research model closely resembles commercial field conditions where parasites are laterally transmitted through 'cloacal drinking' and affords a much-needed platform for conducting relevant basic and applied research on histomoniasis in poultry.

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DISCLOSURES

The authors declare no conflict of interest.

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