

Evaluation of coccidial lesions in the ceca of broilers challenged with *Eimeria tenella* using digital image analysis

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BACKGROUND

- Coccidiosis is a major gastrointestinal disease that causes significant economic losses in the broiler industry.
- Pathogenicity often determined via visual scoring of lesions during necropsy.
 - This has been the standard since it was first validated by Johnson and Reid in 1970.
 - For species *E. tenella*, it is performed on a categorical scale of 0-4.
 - It is a reliable but relatively subjective assessment.
- Visual scoring has its challenges.
 - It can be very time intensive to sufficiently score and diagnose a flock.
 - Similarly infected individual birds can vary drastically in lesion severity.
 - Subjective assessment leaves much room for inter and intra observer variation
- There is need for a more objective method of assessment.
 - Imaging technology could satisfy that need while reducing labor and time inputs.
 - Advancements in technologies have paved the road for increasing the functionality and diversity of imaging utility for other subjects.

OBJECTIVE

Evaluate the use of a digital image analysis method to quantify coccidial lesion severity in the ceca of broilers challenged with *Eimeria tenella*.

MATERIALS & METHODS

- 288 male Ross 708 broilers were raised in 24 battery cages.

Age	# Birds	Event
21	144 + 144	Treatments administered via oral gavage
Challenge		<ul style="list-style-type: none"> Challenge: 1 mL of 10³ oocysts of <i>E. tenella</i> Control: 1 mL saline
24	24	Digital images taken
3 Days PC	(2/cage)	
26	24	Digital images taken
5 Days PC	(2/cage)	
28	24	Digital images taken
7 Days PC	(2/cage)	

- Both ceca were then gently washed, dried, and photographed using a remote-controlled, mounted digital camera set-up.
- The collected digital images were analyzed using the ImageJ software.

- In ImageJ, the following measures were taken for each individual cecum and averaged between the left and right ceca:

- Ceca length and width (cm)
- Ceca total surface area (cm²)
- Total lesion surface area (TLA, cm²)
- Lesion area percent (TLA%) as a percentage of total surface area

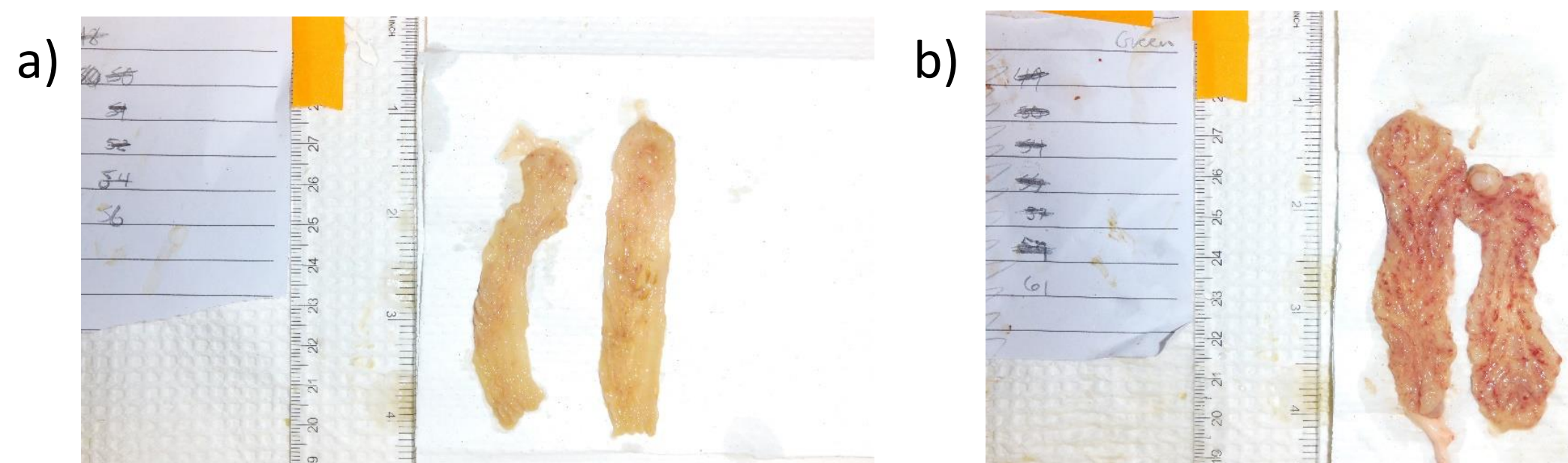


Figure 1: Digital images of paired ceca from a a) control bird and b) challenge bird from 5 days PC. The infected ceca show clear coccidial lesions throughout the organs.

- Lesions were quantified using a red color threshold analysis on a standardized hue-saturation-brightness (HSB) scale.
- Lesions were also visually scored for comparison with the digital data.

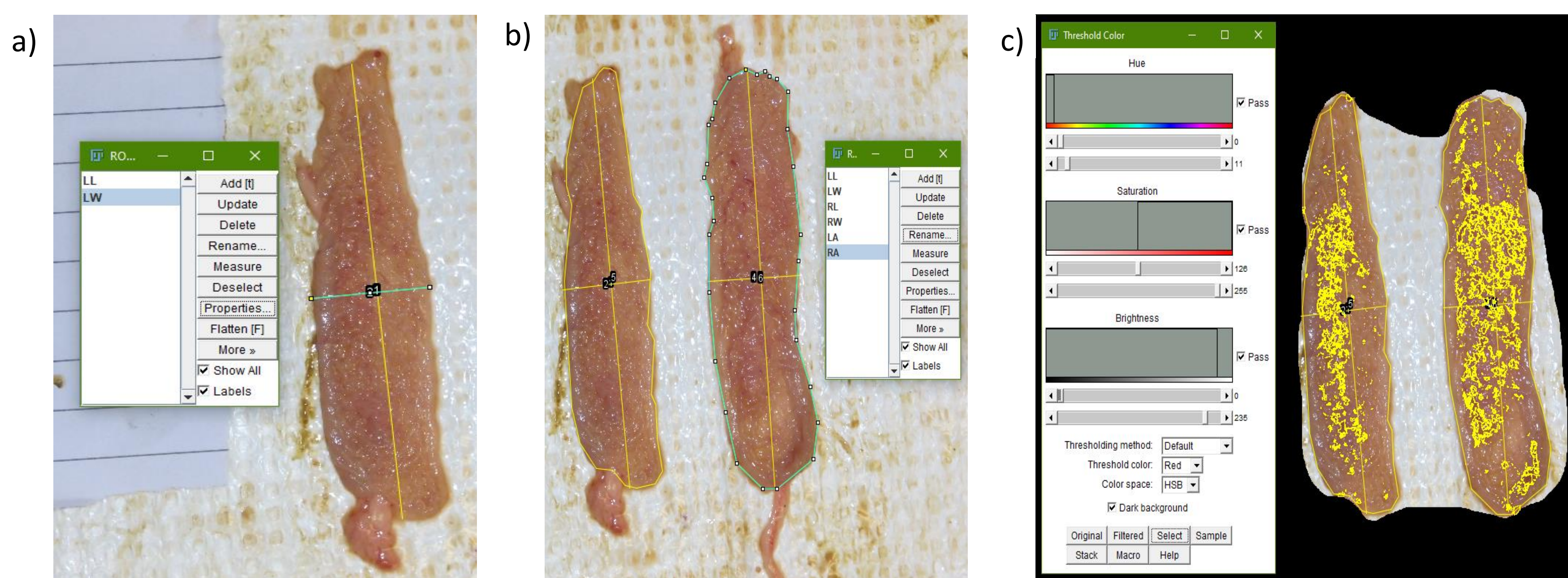


Figure 2: Screen captures of the digital measurements taken of the ceca from a challenged bird. a) Measuring length and width of a cecum using the line tool after setting the pixels:cm scale in ImageJ. b) Measuring the total surface area of the ceca using the polygon tool. c) Measuring the total lesion area of the ceca using a red color threshold analysis on a standardized hue-saturation-brightness (HSB) scale. The thresholded color, representing lesions, are outlined in bright yellow throughout the area of the ceca.

STATISTICAL ANALYSIS

- SAS v. 9.4 (GLIMMIX procedure) was used to analyze the effects of treatment (challenge vs control) and days PC on all measures and their interactions were reported.
- Left and right ceca measures were averaged for analysis.
- Measures were analyzed as continuous variables and means were separated with LSMean.
- Lesion scores had a Poisson distribution and the values were back transformed for the results.
- Pearson's pairwise correlations were analyzed on raw data. Data was considered significant at $P \leq 0.05$.

RESULTS

- Result graphs are pictured with days post challenge on the X axis and recorded measure on the Y axis. Control is shown in red, while challenge is shown in yellow.

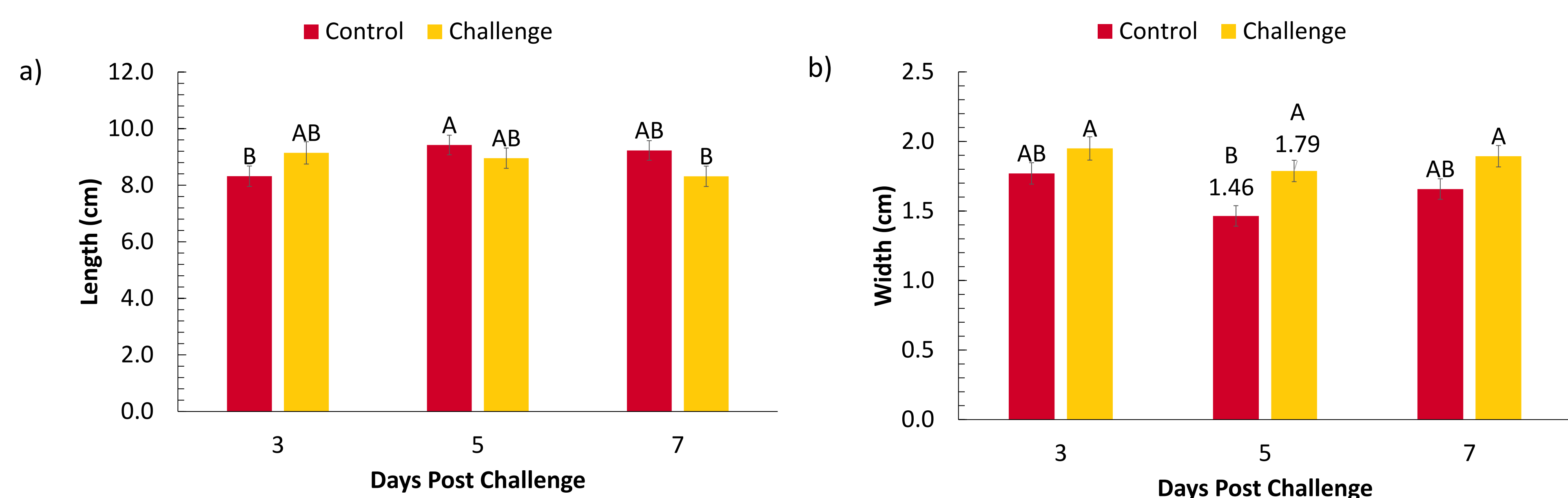


Figure 3: a) Minimal differences were observed in ceca length measurements. b) Challenge birds had wider ceca 5 days post challenge in comparison to control birds ($P \leq 0.03$; 1.79 cm vs. 1.47 cm). Ceca width was similar between control and challenge birds 3 and 7 days post challenge.

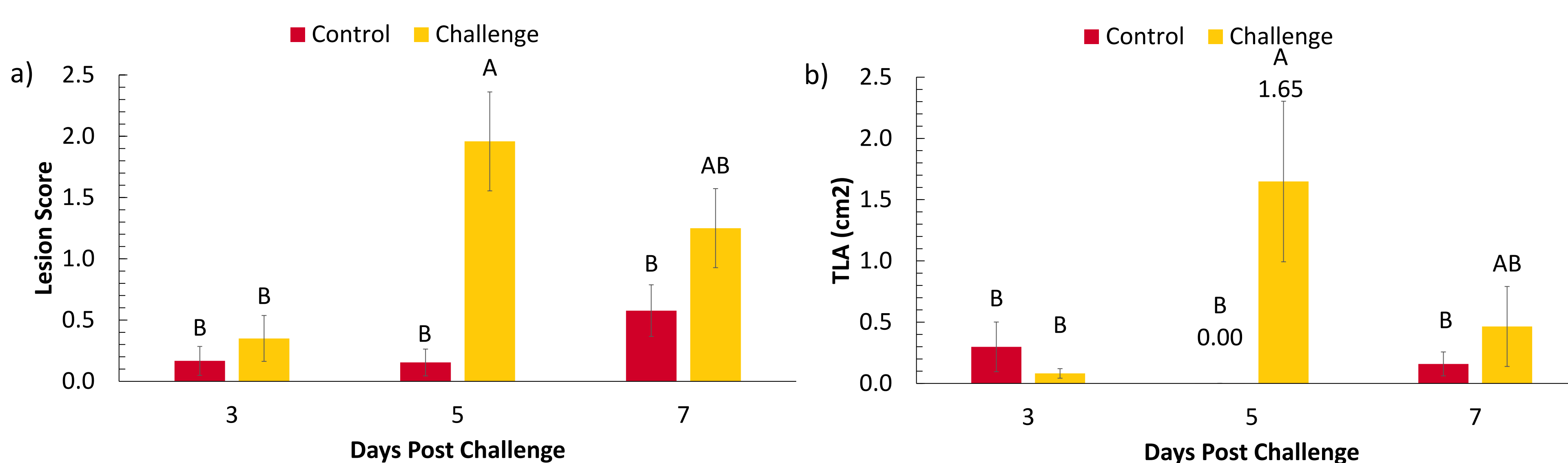
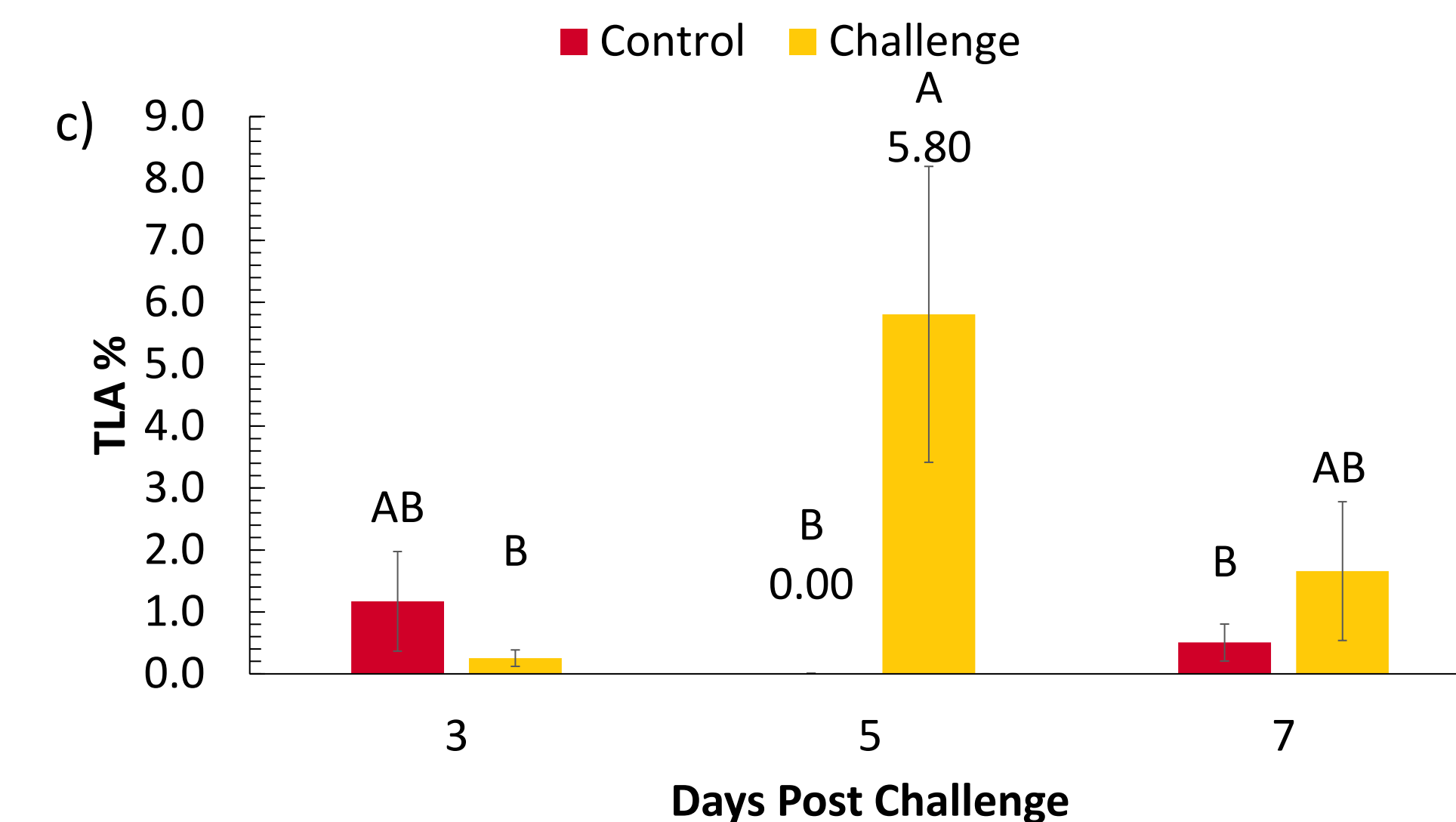


Figure 4: a) Lesion severity was similar between treatment groups on days 3 and 7 post-challenge. Day 5 post-challenge lesion severity was significantly greater in challenge birds compared to control birds (2). b) Total lesion area was greatest 5 days post challenge in challenge birds in comparison to all other days and treatments (1.65 cm²; $P \leq 0.05$). c) Challenge birds 5 days post challenge additionally had significantly different total lesion area percent (5.80%; $P \leq 0.05$). Lesion area percent for ceca with a lesion score of 2 had the highest lesion area percent (1.84%) compared to scores of 1 (0.28%) and 0 (0.03%; $P < 0.0001$).



CORRELATIONS (r)

- As lesion score increased, ceca length decreased, ceca width increased, and lesion area decreased

	Lesion Score	Length, cm	Width, cm	Total Lesion Area, cm ²	Total Lesion Area, %
Lesion Score	1.00	-0.35	0.38	0.62	0.60
Length, cm		1.00	-0.36	-0.25	-0.28
Width, cm			1.00	0.30	0.27
TLA, cm ²				1.00	0.99
TLA, %					1.00

CONCLUSION & IMPLICATIONS

- Ceca lesions were the most severe for challenge birds 5 days post challenge.
 - Highest lesion scores, lesion area, and percent lesion area
 - Wider but shorter ceca, possibly due to coccidial damage to the cecal wall
 - Positive correlation between lesion score and ceca width
 - Negative correlation between lesion score and ceca length
- The use of an imaging software provided digital values for lesion severity which reflected those of the visual lesion scoring method.
 - A moderate positive relationship between lesion score and total lesion area was found.
 - Digital image technology has the potential to serve as a practical diagnostic-active tool to determine the pathogenicity of coccidia.
- The use of imaging software to assess infection could be applied to the creation of practical diagnostic devices or apps.

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