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NECROTIC ENTERITIS POTENTIAL IN A MODEL SYSTEM USING *CLOSTRIDIUM PERFRINGENS* ISOLATED FROM FIELD OUTBREAKS

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Necrotic enteritis is an enteric disease of avian species caused by the anaerobic bacterium Clostridium perfringens*. The disease is regularly controlled in the broiler chicken industry through the use of antimicrobials in feed, but is re-emerging in areas such as Europe where there is a ban on antimicrobials as growth promoters. In order to study prospective therapies, researchers must be able to reproduce this disease in a controlled environment, but this is not always possible due to differences in the pathogenicity of* C. perfringens *strains. The objective of this study was to test the potential of 5 isolates (SNECP43, 44, 47, 49 and 50), taken from field cases of necrotic enteritis, at recreating the disease in a controlled challenge experiment.*

This study found *that only one of the five* C. perfringens *challenge isolates (SNECP50) from necrotic enteritis outbreaks was able to consistently reproduce the disease in this experimental model*. Researchers found that:

- This isolate caused 33.7% necrotic enteritis-associated mortalities in the challenged birds, and produced an average lesion score of 3.5 in the diseased birds (Table 1).
- All of the other isolates taken from field cases of necrotic enteritis were unable to produce significant mortalities, and would not be suitable for use in future challenge studies with this model, when mortality is a parameter of interest.
- PCR and PFGE analysis helped to further characterize these isolates and revealed the absence of the *cpb2* toxin gene as well, thus questioning its importance in necrotic enteritis development.
- The difference in virulence between SNECP43 and SNECP50 demonstrates that a high degree of genetic relatedness does not necessarily imply analogous virulence in a challenge model.

Evidence for study results was gained when SNECP43 and 50 were derived from a common clone, with SNECP50 passed *in vivo* and SNECP43 subcultured *in vitro*. Four hundred birds were divided into 16 pens, with 3 pens each receiving one of 5 treatments, with one control pen. Day-old birds were raised on a high wheat-based diet to promote necrotic enteritis development, and were challenged with between 3.4×10^9 and 3.2×10^{11} CFU of *C. perfringens* in feed over 24 hr starting on day 13 of the challenge experiment. Lesion scores were assessed on two birds per pen sacrificed on day 17 and on any dead birds during the 25-day study. Growth performance was assessed up to 25 days, and mortality recorded throughout. Only SNECP50 produced necrotic enteritis mortalities significantly different ($P \leq 0.05$) from the control. The five isolates were also typed using pulsed-field gel electrophoresis to assess their genetic relatedness. All epidemiologically unrelated isolates were deemed genetically unrelated, while SNECP43 and 50 differed by only a single minor band. Toxin type was assessed using PCR, and was also used for the detection of the gene encoding the β2-toxin.

Because of the current absence of other specific virulence attributes, the virulence level of *C. perfringens* strains should not be predicted on the basis of genetic relatedness to known virulent strains, but only upon testing in an adequate *in vivo* challenge model.

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Table 1. Correlation coefficients for correlations between growth performance parameters, lesion score, and mortality (*P* values of correlations in parentheses).

	Variable	BW0	BW25	FC	LS	NEM
BW0	Body weight (kg) 0 days					
BW25	Body weight (kg) 25 days	−0.047 (0.864)				
FC	Feed conversion (kg feed/kg gain)	−0.249 (0.352)	−0.38 (0.147)			
LS	Lesion score	−0.089 (0.744)	−0.627 (0.009)	0.336 (0.204)		
NEM	NE mortality %	−0.013 (0.962)	−0.532 (0.034)	0.581 (0.018)	0.643 (0.007)	
TM	Total mortality %	0.177 (0.513)	−0.458 (0.074)	0.402 (0.123)	0.65 (0.006)	0.926 (<0.0001)