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The Pathogenic Effects of a UK field Isolate of *Eimeria tenella* in Modern Broiler and Layer Chickens

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ABSTRACT

Trials were conducted in groups of 14 day old Ross Broilers and Lohmann Brown laying birds experimentally infected with 350, 1,250, 5,000, 20,000, 80,000, 320,000, or 1,280,000 sporulated oocysts of a field strain of *Eimeria tenella*. The oocyst output, weight gain and performance, clinical signs and mortality were recorded for 14 days post infection. Birds were necropsied for lesion scoring and histopathological examination. Performance was related to the level of challenge with mortality of up to 80% occurring in birds infected with 20,000 oocysts and above. There was good correlation between clinical signs and post mortem findings, with severe lesions present in birds in the more heavily infected groups. Caecae were engorged and distended with bloody caecal cores. Histopathological examination confirmed the presence of increasing numbers of parasites from day 6 onwards, with massive epithelial destruction and mucosal sloughing in the heavily infected groups.

INTRODUCTION

Coccidiosis is an important disease of chickens world-wide and is recognised as a problem of intensification (Reid 1990). Its control relies almost exclusively on chemotherapy through the incorporation of anticoccidials in the feed. Besides the continual expense of using drugs, one of the major problems to the poultry industry has been the emergence of resistant strains of coccidia. In many countries anti-coccidial efficacy is constantly monitored using a number of parameters. One of the more frequently used laboratory based methods is the Anticoccidial Sensitivity Test

(AST) in which the efficacy of a number of anticoccidial drugs is evaluated against field isolates in an in-vivo system. A number of modifications to this method exist but all basically involve artificially challenging parasite-naive birds. Challenged and uninfected control birds are then monitored for performance, faecal oocyst output and clinical and pathological effects (Chapman and Shirley 1989). Monitoring of resistance indices relies on knowledge of the pathogenic effects of the seven main species of *Eimeria* affecting chickens and the lesions the individual species produce (Long and Reid 1982). In the UK, much of the information used for these investigations was undertaken in light hybrid layer strain birds (Joyner and Norton 1969). Genetic selection and improvement, over the last two decades, has resulted in broiler birds with improved growth rates and feed conversion, and egg-laying strains of birds with increased prolificacy (Law and Payne, 1990). It is well established that different strains of birds vary in their susceptibility to coccidia (Long 1968). Anecdotal evidence also suggests that there has been a shift in the prevalence of indigenous populations of coccidia in the field due to changes in poultry management and drug usage (Marshall, personal observations). *Eimeria necatrix* and *E. brunetti* were considered major pathogens over 20 years ago but are now only rarely seen in broilers in the UK (Norton, personal communication). *E. tenella* remains a highly pathogenic species and is often incriminated in coccidiosis outbreaks.

This study represents one of a series of trials aimed at investigating the performance, parasitological, clinical and pathological effects of a field strain of prevalent poultry coccidia in modern strains of broiler and laying birds. This particular study was designed to investigate the dose-response of a field strain of *E. tenella* in young susceptible Ross Broilers and Lohmann Brown layers.

MATERIALS AND METHODS

Experimental birds

Female Ross Broiler and Lohmann Brown layer chicks were obtained from the hatchery at day-old and reared coccidia free until two days prior to the start of the trials (day -2). Chicks were housed in groups of 200 on wire-floored cages (2.3m^3, 200 birds/5.38m^2). The birds were maintained at an initial temperature of 30 °C with 14 hrs of light and 10 hrs of red light. Drinking water was provided via gravity feed nipple drinkers, and birds were fed a modified standard ration after Ryley and Betts (1973) with the addition of Vitamin K. This was provided ad libitum in trough feeders fitted to the outside of the cage. At 12 days, replicate groups were transferred to smaller wirefloored cages (1.0 m^3, 10 birds/m^2). Feed and water was provided as before and the birds maintained at an ambient temperature of 25 °C with 14 hrs of daylight.

Parasite culture and challenge

Oocysts of *E. tenella* were originally isolated from a broiler unit in the south of England in 1988, and had been maintained in the laboratory by periodic passage in young chicks fed an anticoccidal-free diet. For each of the trials, fresh oocysts were prepared by the method of Norton and Hein (1976) and used within two weeks after sporulation. The oocysts were counted using a Fuchs Rosenthal haemocytometer (Hawksley, London) and stored in 2% potassium dichromate until required for use. Individual birds were orally infected with *E. tenella* oocysts, diluted to the correct dose, by direct administration into the crop in 1 ml volumes using a sterile springloaded syringe fitted with a ball-ended canula.

Experimental design

Separate identical trials were carried out on Ross Broilers and Lohmann Brown laying birds. Two days before inoculation (day -2), chicks were randomised by weight into eight treatment groups as shown in Table 1.

Table 1. Experimental design

Group	Infection
1	None
2	350 oocysts *E.tenella*
3	1,250 oocysts *E.tenella*
4	5,000 oocysts *E.tenella*
5	20,000 oocysts *E.tenella*
6	80,000 oocysts *E.tenella*
7	320,000 oocysts *E.tenella*
8	1,280,000 oocysts *E.tenella*

Groups of 10 birds were then given 350, 1,250, 5,000, 20,000, 80,000, 320,000, or 1,280,000 oocysts at 14 days of age. All birds were individually weighed on days -2, 0 and then on days 3 to 14 post infection (PI) when the experiments were terminated. Mortalities were recorded daily, and post mortem examinations carried out. The total faecal output from each group was collected daily, and the numbers of oocysts per gram of faeces were determined by a modified MacMaster salt flotation technique, MAFF (1986). Two birds, from each dose level, were euthanased and necropsied on days 6, 7 and 8 and lesion scores were performed on caecae using the method of Johnson and Reid (1970). Smears were taken from the caecal mucosa and examined for the presence of parasite stages. Caecae were also examined histopathologically following fixation in Bouin's solution, embedding in wax, sectioning at 5 µm, and staining with hematoxylin and eosin (H&E). Total mucosal thickness (TMT) of the caecal wall was measured, for each of the birds examined, using a method similar to that

described by Pout (1967). Mucosa and submucosa thickness for each histological section, was measured at 10 different points, using an eyepiece graticule. A total mucosal thickness ratio (TMTR), for each of the infected groups, was calculated by dividing the mean TMT, by the mean TMT of the uninfected controls.

RESULTS

In both types of birds (broilers and layers), clinical signs were first noted in the heavily infected groups (groups 7 and 8) on 3 days post infection. The birds appeared depressed and were anorexic. Groups 4 to 6 showed similar signs on day 4, although with a lesser degree of severity. Mortalities ranged from 0 to 80% as shown in Table 2, with the majority of deaths occurring on days 5 and 6. Post mortem examinations showed all birds with empty small intestines and typical haemorrhagic lesions in the caecae. Clinical signs were recorded in all these groups until day 7, when the surviving birds became more alert, and their appetites returned. These observations corresponded well with the weight gains (Table 2).

Table 2. Mean weight gain and mortality in broilers and layers infected with *Eimeria tenella*

Broilers

Group	Mortality	Mean weight gain (day 4 - 7)	Mean weight gain (day 0 - 14)	Percentage weight gain (day 4 - 7) compared to UIC	Percentage weight gain (day 0 - 14) compared to UIC
1	0/10	128.90	607.60	100.00	100.00
2	0/10	129.20	654.70	100.23	107.75
3	0/10	119.50	650.00	92.71	106.98
4	0/10	99.80	589.20	77.42	96.97
5	4/10	51.83	521.77	40.21	85.87
6	7/10	1.77	481.80	1.37	79.30
7	4/10	-14.17	402.97	-10.99	66.32
8	8/10	50.70	509.00	39.33	83.77

Layers

Group	Mortality	Mean weight gain (day 4 - 7)	Mean weight gain (day 0 - 14)	Percentage weight gain (day 4 - 7) compared to UIC	Percentage weight gain (day 0 - 14) compared to UIC
1	0/10	36.70	185.00	100.00	100.00
2	0/10	26.30	182.00	71.66	98.38
3	0/10	20.00	178.90	54.50	96.70
4	0/10	7.70	161.60	20.98	87.35
5	1/10	-20.91	113.63	-56.98	61.42
6	5/10	-6.90	129.00	-18.80	69.73
7	5/10	-15.00	107.00	-40.87	57.84
8	8/10	-14.30	96.00	-38.96	51.89

UIC; uninfected controls

Weight gain

There was no significant effect compared with the uninfected controls on weight gain in broilers which received up to 5,000 oocysts, and in layers up to 1,250 oocysts ($p = 0.64$ and 0.91, respectively). Of the birds which received 1.28×10^6 oocysts and survived, broilers gained 87% of the weight of the uninfected controls compared to 54% for the surviving layer birds.

Oocyst output

In layers, the uninfected control group acquired an adventitious infection and produced low numbers of oocysts from days 8 to 12, but the broilers remained uninfected. All infected groups produced large numbers of oocysts from day 6 onwards. In broilers, a few oocysts were produced on day 5 by the four groups which received the higher levels of infection. The greatest numbers of oocysts were produced by the groups which received the fewest numbers of parasites (i.e. groups 2 and 3 which received 350 and 1,250 oocysts per bird respectively). Group 8, which received the highest dose of 1,280,000 oocysts per bird, produced the fewest oocysts of all the groups. Overall, the broilers produced higher numbers of oocysts in all groups. Oocyst production for broilers and layers is summarised in Table 3.

Table 3. Oocyst output

Group	Infection	Chichen	Total oocysts per gram of faeces ($x10^6$)	Total oocysts per bird ($x10^6$)
1	None	Broiler	0.00	0.00
		Layer	0.01	0.18
2	350	Broiler	0.53	94.15
		Layer	1.46	47.97
3	1,250	Broiler	1.21	79.51
		Layer	2.55	70.29
4	5,000	Broiler	1.78	121.12
		Layer	1.47	37.87
5	20,000	Broiler	1.61	88.86
		Layer	1.75	26.92
6	80,000	Broiler	1.80	56.51
		Layer	1.00	25.71
7	320,000	Broiler	1.22	59.03
		Layer	1.49	22.61
8	1,280,000	Broiler	0.68	38.55
		Layer	0.36	4.87

Lesion scores

Lesions and parasites were observed in all infected groups with an increase in the numbers of parasite stages as the dose-level increased. Grossly, lesions ranged from none in group 2 (350 oocysts) to extreme haemorrhagic cores in all groups receiving 20,000 oocysts or more (groups 5 to 8) (Table 4). The walls of the caecae appeared thickened in some infected groups but were very thin and friable in birds in groups 7 and 8.

Histopathological findings

The histopathological observations related to both the level of infection and the interval post infection. Infected birds showed varying degrees of oedema, hyperaemia and congestion of the caecae with inflammation of the mucosa, epithelial erosion and luminal core formation and haemorrhage.

At 6 days post infection, birds infected with between 350-5,000 oocysts (Groups 2-4) showed oedema, hyperaemia and cellular infiltration of the caecal mucosa (TMTR Broilers 1.43-2.86, Layers 1.84-2.49). Second generation schizonts were visible within the caecal mucosa of all groups and both types of birds. Moderate numbers of gametocytes and some oocysts were present within surface and crypt epithelial cells in layers, but were less numerous in broiler birds. Both broiler and layer birds infected with over 20,000 oocysts (Groups 5-8) showed increasing epithelial damage and haemorrhage within the caecae. Sections taken from broiler birds in the most heavily infected group that survived challenge (320,000 oocysts), showed a flattened mucosa (TMTR 1.12), as a result of severe epithelial erosion and sloughing. Many gametocyte stages were present in surviving epithelial cells and the lamina propria was infiltrated with inflammatolry cells comprising mainly lymphocytes and eosinophils. Widespread crypt destruction was evident with surviving crypts hyperplastic, and containing inflammatory cells and exudate. The lumen contained a haemorrhagic necrotic exudate with large numbers of oocysts, epithelial cells and erythrocytes. A similar picture was seen with laying birds although the most severe sloughing occurred in birds infected with 80,000 oocysts (TMTR 1.34). Layers infected with 320,000 had severe mucosal damage and thickening but sloughing had not occurred.

At 7 and 8 days post infection, the observed histological changes were not dissimilar to those observed on day 6. Infected birds showed oedema, hyperaemia and cellular infiltration of the caecal mucosa. Epithelial loss and destruction had produced flattened mucosae in many of the infection groups (TMTR 1.13 to 2.08 in broilers and 1.12 to 2.33 in layers on day 7, 1.09 to 3.49 in broilers and 1.41 to 1.88 in layers on day 8) although the group of broilers given 20,000 oocysts had a much higher TMTR (3.49) on day 8 due to massive oedema in the submucosa. Large numbers of gametocytes and some oocysts were present within surface and

Table 4. Post mortem observations and lesion scores

Group	Strain	Infection	Observations	Lesion score
1	Broiler	None	No lesions or parasites seen	0
	Layer	None	No lesions or parasites seen	0
	Broiler	350	Few scattered petechiae, no thickening of mucosa on day 6. Numerous pwtechiae, bloody contents and mucosal thickening days 7 and 8. Day 6: few gametocytes and oocysts on smears.Days 7 and 8: very numerous gametocytes and oocysts on smears.	1 2/3
	Layer	350	Few scattered petechiae, no thickening of mucosa on day 6. Numerous pwtechiae, bloody contents and mucosal thickening days 7 and 8. Day 6: few to numerous gametocytes. one oocyst seen on smears.Days 7 and 8: very numerous gametocytes and oocysts on smears.	1 3
3	Broiler	1,250	Numerous petechiae, bloody contents and mucosal thickening all days. Days 6, 7 and 8: numerous gametocytes and oocysts on smears.	3
	Layer	1,250	Numerous petechiae, bloody contents and mucosal thickening all days. Haemorrhagic caecal cores on day 8. Days 6, 7 and 8: numerous to very numerous gametocytes and oocysts on smears.	3/4
4	Broiler	5,000	Numerous petechiae, bloody contents and mucosal thickening all days. Days 6, 7 and 8: numerous gametocytes and oocysts on smears.	3
	Layer	5,000	Large haemorrhagic cores in caecae days 6 and 7. Days 6 and 7: numerous to very numerous gametocytes and oocysts. surviving birds available for examination on day 8.	4
5	Broiler	20,000	Large haemorrhagic cores in caecae all days. Days 6, 7 and 8: very very numerous gametocytes and oocysts.	4
	Layer	20,000	Large haemorrhagic cores in caecae all days. Days 6: numerous schizonts, gametocytes and oocysts. Day 7: few gametocytes and oocysts. Day 8: numerous oocysts, huge caecal core in 1 bird.	4
6	Broiler	80,000	Large haemorrhagic cores in caecae all days. Days 6, 7 and 8: very very numerous gametocytes and oocysts.	4
	Layer	80,000	Large haemorrhagic cores in caecae days 6 and 8.Days 6: very numerous schizonts, merozoites, gametocytes and oocysts. Day 7: not sampled. Day 8: few oocysts, huge caecal core.	4
7	Broiler	320,000	Large haemorrhagic cores in caecae all days. Thin and friable walls. Days 6: very numerous schizonts, gametocytes and oocysts. Day 7 and 8: numerous gametocytes and oocysts.	4
	Layer	320,000	Large haemorrhagic cores in caecae days 6 and 7. Thin and friable walls. Days 6: very numerous schizonts, merozoites, gametocytes and oocysts. Day 7: numerous gametocytes and oocysts. Day 8: no surving birds available for examination.	4
8	Broiler	1,280,000	Large haemorrhagic cores in caecae day 6. Thin and friable walls. Days 6: numerous merozoites and gametocytes. Day 7 and 8: no surving birds available for examination.	4
	Layer	1,280,000	No surving birds available for examination.	-

crypt epithelial cells of both groups of birds on both days with many sections showing haemorrhagic thickened caecal cores containing large numbers of oocysts.

DISCUSSION

The trials were designed to investigate the clinical and pathogenic effects of a field isolate of *E. tenella* in modern broiler and layer chickens and obtain information on the dose-response and lesions produced in infected birds. Six parameters have been used to assess the comparative pathogenicity of the recently isolated strain of *E. tenella* in modern chickens. These are clinical signs, mortality, weight gain, oocyst output, lesion scores, and examination of tissue sections for histopathological changes. The results from these trials show that these aspects taken together can help to build up an accurate picture of the relative pathogenicity of different strains of parasite, and also their effect in different strains of bird. Table 4 summarises the relative pathogenicity of the field strain in broilers and layers, and shows that when mortality, lesion scores and clinical signs are compared there is no difference between the two strains of bird. However, the broiler strain's weight performance is significantly ($p<0.0001$) better than the layer birds over the whole period of the trial, and the broilers required a higher infective dose level of oocysts to produce the same degree of weight loss. During the critical disease period between days 4 and 7 as few as 350 oocysts caused a 28% decrease in the weight gain in the layer birds. 5,000 oocysts were required to cause a similar decrease in broiler birds (i.e. an 8-fold increase in challenge dose). Groups 5 to 8, which received between 20,000 and 1.28×10^6 oocysts showed a severe decrease in weight gain in both strains of birds. The broilers only gained between -11 and 40% of the controls. The layers were much more severely affected and showed a gain between -19 and -57%. Oocyst output is not a reliable measure of pathogenicity, but can be used to assess the potential challenge burden available to a flock. Broilers produced much higher numbers of oocysts compared to the layer birds (possibly due to increased caecal size in these birds), although both strains of bird produced very high numbers even when challenged with low numbers of oocysts.

Under intensive husbandly conditions in the UK, broiler chickens are reared almost exclusively in large deep-litter buildings with a high stocking density. This has frequently caused problems with coccidiosis, and the large numbers of oocysts produced by this present *E. tenella* strain suggest that modern broilers could be exposed to caecal coccidiosis especially if strict control strategies are not rigorously applied. Although caecal coccidiosis does not appear to be a major problem in laying birds reared in the traditional cage systems, the switch to egg production in perchery and free-range systems, may increase the incidence of infection and disease, especially in birds in which immunity to the parasites has not been fully

developed during earlier growth phase. Histopathological examinations from these trials indicate that lesions on days 6, 7 or 8 post infection are comparable and therefore lesion scores can be performed on any of these days. Parasite stages appeared to be at their most numerous on day 7 and for lesion scoring this may be the most convenient and optimal day for caecal lesion scoring.

When these current results in layer birds are compared with those obtained by Joyner and Norton (1969) (Table 5) in light hybrid chicks, it can be seen that although not all parameters were measured, the results with the recent field isolate of *E. tenella* reported here, suggest that this strain exerted less apparent pathogenic effects on modern birds, when mortality and clinical signs are compared.

Table 5. Comparison with results obtained by Joyner and Norton, 1969.

	dose	mortality (%)	total oocysts per bird (x 10^6)
Joyner and Norton	20,000	80	7.20
(Weybridge strain	80,000	80	5.80
of *E. tenella*)	320,000	50	1.90
	20,000	40	88.86
Broiler	80,000	70	56.51
	320,000	40	59.03
	20,000	10	26.92
Layer	80,000	50	25.71
	320,000	50	22.61

As no bodyweight data was reported, it is not possible to make direct performance comparisons. However, challenge levels using the Weybridge strain for chemotherapy experiments in the 1980's were much higher that those used with the current field isolate (Catchpole, personal observation). The Weybridge strain of *E. tenella* used by Joyner and Norton in their trials, was a well established "laboratory strain" and had been passaged through chickens for over 20 years prior to the experiments. It is not known the effects that repeated passage had on the parasite pathogenicity although several authors have suggested that laboratory selection may result in a reduction in pathogenicity (Joyner and Norton 1969; Long 1970). Strain variation in pathogenicity has also been reported for different isolates of *E. tenella* (Joyner and Norton 1969).

The oocyst output of the field strain in both broiler and layer strains appeared to be much higher than the Weybridge laboratory strain used by Joyner and Norton

(1969) in the birds challenged with 20,000 to 320,000 oocysts. This could either be a reflection of the higher reproductive potential of this *E. tenella* strain, or the decreased mortality and clinical signs would suggest that there is less tissue damage and therefore more parasites are able to develop. Light hybrids were a laying strain and therefore it is not possible to make comparisons with results from broilers.

The results of this dose-response study provide information on a field strain of *E. tenella* in terms of a number of parameters including mortality, clinical and pathological lesions, performance data and oocyst output in modern strains of broiler and layer birds. This data in conjunction with the histopathological findings and lesion scores should allow for better evaluation and standardisation of the anticoccidial sensitivity test for monitoring the effectiveness of control strategies in the field.

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