

Research Note—

Sensitivity of Field Isolates of *Eimeria* to Monensin in the TurkeyH. D. Chapman^A and T. Rathinam^ADepartment of Poultry Science, University of Arkansas, Fayetteville, AR 72701

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SUMMARY. A method previously described by Jeffers and Bentley, involving calculation of a growth and survival ratio and optimal anticoccidial activity index, was used to investigate the sensitivity of 23 field isolates of *Eimeria* obtained from turkey flocks to the ionophorous antibiotic monensin. Isolates were obtained from litter and intestinal samples from several major turkey-growing regions of the United States, and in most cases contained at least two species of *Eimeria*. A mixture of strains that had been maintained in the laboratory for many years in the absence of exposure to anticoccidial drugs was found to be sensitive to monensin. Six of the field isolates were judged sensitive, seven partially resistant, and ten resistant to the drug, judged by the Jeffers and Bentley criteria. This is the first report of the acquisition of resistance to monensin in isolates of *Eimeria* from turkey flocks in the United States.

RESUMEN. *Nota de Investigación*—Sensibilidad a la Monensina de aislados de campo de *Eimeria* en el pavo.

Para investigar la sensibilidad a la monensina de 23 aislados de campo de *Eimeria* obtenidos de lotes de pavos, se usó un método descrito previamente por Jeffers y Bentley que involucra el cálculo de la proporción de crecimiento y sobrevivencia y el índice de actividad anticoccidiana óptima. Los aislados fueron obtenidos de la cama y de muestras de intestinos a partir de varias regiones importantes de crianza de pavos en los Estados Unidos y en la mayoría de los casos contenían al menos dos especies de *Eimeria*. Se encontró que una mezcla de cepas que habían sido mantenidas en el laboratorio por muchos años en ausencia de exposición a drogas anticoccidianas, fue sensible a la monensina. Seis de los aislados de campo fueron considerados sensibles, siete parcialmente resistentes y 10 resistentes a la droga, de acuerdo con el criterio de Jeffers y Bentley. Este es el primer reporte de la adquisición de resistencia a la monensina en aislados de *Eimeria* de lotes de pavos en los Estados Unidos.

Key words: *Eimeria*, monensin, resistance, turkey

Abbreviations: GSR = growth and survival ratio; OAA = optimum anticoccidial activity

Control of coccidiosis in turkeys is achieved principally by the inclusion of anticoccidial drugs in the feed. In the chicken, this has resulted in the widespread development of drug-resistant strains of *Eimeria* (4) but there have been very few reports of the development of drug resistance in the turkey. One of the most widely used drugs for the control of coccidiosis in turkeys is monensin, an ionophorous antibiotic that has been shown to be effective against *Eimeria adenoides*, *Eimeria meleagridis*, and *Eimeria gallopavonis* (7,15), and which is included in the feed at concentrations ranging from 59.5 to 99.2 ppm (2). In 1980, Jeffers and Bentley reported that 7 of 16 isolates of *E. adenoides* and *E. meleagridis* obtained from turkey flocks in Canada where monensin had previously been used were resistant to the drug, but that all 22 isolates obtained from the United States were sensitive (10). There appear to have been no recent published reports of the sensitivity of *Eimeria* species to monensin in the turkey, and only a few studies for other anticoccidial drugs (12,17). In this study the effect of monensin upon field isolates of *Eimeria* obtained from several major turkey-producing regions in the United States was investigated.

MATERIALS AND METHODS

Animals and husbandry. Female turkey poults (Nicholas breed) were obtained from a local hatchery, transferred to an isolation building, and reared in brooder cages at a stocking density of 257 cm²/poult until 9 days of age. Husbandry and management followed guidelines for the

care and use of agricultural animals in agricultural research (1) and experiments were approved by the University of Arkansas animal care and use committee. At 9 days of age poults were transferred to a test facility and randomly allocated to clean grower cages (five poults/cage). Poults were fed a corn/soybean meal diet formulated according to starter feed requirements for turkey poults. The experimental rations comprised feed in which monensin (Coban 60[®]; Elanco Animal Health, Indianapolis, IN) was incorporated at a concentration of 99.2 ppm, or unmedicated feed. Analysis of random feed samples indicated that monensin was present within acceptable limits of the intended concentration.

Parasites. Turkey production personnel were requested to collect surface litter samples (approximately 1 pound in weight) from turkey farms at different geographical locations when poults were between 3 and 6 wk of age. Samples were collected from random locations within a house and labeled with the name of the farm and date of collection. They were received over a period of approximately 9 mo and were given a unique identification based upon the seven states from which they originated. Two isolates from North Carolina were obtained from intestines of young poults that had been killed because of suspected enteric infection; any oocysts present were harvested from their ceca. Oocysts of *Eimeria* were isolated from samples following laboratory procedures described for the recovery of oocysts from feces or ceca (20). A commercial vaccine containing a mixture of species (Coccivac-T[®], stated to contain oocysts of *Eimeria dispersa*, *E. adenoides*, *E. gallopavonis*, and *E. meleagridis*) was also propagated in turkey poults. Oocysts in this mixture (referred to as isolate L1) had been propagated for many years in a commercial laboratory (Schering Plough, Animal Health Corp., Millsboro, DE) in the absence of exposure to anticoccidial drugs.

Propagation. The possible presence of multiple species, of varying pathogenicity, in field isolates of *Eimeria* complicates the interpretation of results and therefore it was considered desirable to limit the species

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present by excluding *E. meleagridis* and *E. gallopavonis*. *Eimeria meleagridis* was excluded by propagating oocysts in turkeys that had been rendered solidly immune to this species. The validity of this procedure depends upon the absence of cross-immunity demonstrated between *E. meleagridis* and other species that infect the turkey, such as *E. adenoides* (18). Immunity to *E. meleagridis* was first induced by inoculating poults reared in strict isolation at 7, 14, and 21 days of age with 2×10^5 , 1×10^5 , and 0.5×10^5 oocysts, respectively, of a pure line of this species that was derived from a single oocyst (14). The absence of oocysts in the feces following challenge is widely used as a stringent measure of immunity to *Eimeria* species. Feces of these poults were, therefore, examined daily following the third inoculum, and after three successive zero counts, the absence of oocysts indicated that the birds were immune to *E. meleagridis*. Poults were then transferred to clean cages and used to propagate the isolates. This procedure was carried out for the isolates tested in Experiments 2 and 3 but was not possible for isolates used in Experiments 4 and 5.

The prepatent period of *E. gallopavonis* (time after inoculation of oocysts to the production of new oocysts in the feces) is reported to be 144 hr (9). This species was therefore eliminated from all isolates, and from Coccivac-T[®], by infecting two poults with each culture and collecting any oocysts produced in their feces from 120 to 136 hr after inoculation.

Evaluation of resistance. In an initial experiment, the commercial mixture (L1) was tested to investigate the methodology to be employed and whether strains that had not been exposed to monensin would be sensitive to the drug. Four subsequent experiments (Experiments 2–5) were then conducted in which four to seven field isolates were examined. For each experiment, all isolates had been propagated within the previous month and were therefore of similar age.

The procedure used to evaluate monensin sensitivity was based upon that described by Jeffers and Bentley (10). Two replicates of five 9-day-old poults were weighed, allocated to cages, and given either medicated or unmedicated feed. Two days later they were infected with a maximum of 100,000 oocysts of each isolate. An additional two replicates were not medicated and uninfected, respectively (controls). Poults in each cage were weighed again 6 days after infection and the gain in weight calculated. Any mortality was recorded. These two parameters were expressed in a growth and survival ratio (GSR) defined as the cage weight at trial termination (day 6) plus the weight of any dead birds, divided by the cage weight when placed on the drug (day 2). The percentage of optimum anticoccidial activity (% OAA) for each treatment was then calculated to determine monensin efficacy as follows: % OAA = (average GSR of infected medicated group – average GSR of infected unmedicated group) / (average GSR of uninfected controls – average GSR of infected unmedicated group) \times 100. The criterion used by Jeffers and Bentley to denote monensin resistance, namely that the % OAA be $\leq 50\%$, was used in this study (10). In addition, an isolate was deemed partially resistant if the % OAA was 51%–74%, and sensitive if the % OAA was $\geq 75\%$.

Analysis. GSR values for medicated and unmedicated infected poults for each experiment were analyzed by one-way analysis of variance using the PROC ANOVA procedure of SAS software (19). Means were separated and compared using Duncan's multiple-range test.

RESULTS

Response of turkey isolates to monensin. The average GSR of medicated infected and unmedicated infected poults, and the % OAA derived from these values, is presented in Table 1. Within each experiment, significant differences in GSR of medicated poults was observed indicating variation among isolates in the response to monensin. Using the criterion of % OAA, the laboratory mixture was judged sensitive to monensin. Six field isolates were judged sensitive, seven partially resistant, and 10 resistant to the drug. Significant differences were observed in the GSR of unmedicated poults indicating that the isolates varied in pathogenicity.

DISCUSSION

There have been many investigations of the drug sensitivity of field isolates of *Eimeria* in the chicken, in which a variety of different methods and criteria have been utilized to evaluate resistance (4,5), but there have been few recent studies conducted in the turkey. In the experiments reported here the methodology used by Jeffers and Bentley was employed in order to facilitate comparison with their earlier work (10). Jeffers and Bentley utilized two replicates of five poults/treatment, and measured weight gain and mortality of infected and uninfected birds over an 8-day period beginning 2 days prior to inoculation. These criteria were expressed in a GSR from which they calculated the % OAA for each isolate examined. This index was used to determine the sensitivity of 38 field isolates of *Eimeria* from turkey flocks to monensin. They infected birds with doses ranging from 0.4×10^5 to 3.5×10^5 oocysts, but in this study an attempt was made to standardize the dose given to poults, which in most cases was 1×10^5 oocysts/bird.

Jeffers and Bentley utilized mixed infections of *E. meleagritidis* and *E. adenoides*, and identified these species by microscopy and gross examination of intestines (10). *Eimeria meleagritidis* develops principally in the small intestine and *E. adenoides* mainly in the ceca (16), but it should be noted that other species develop in these locations that cannot readily be distinguished based upon morphological criteria such as size of the oocyst (8). In the present study, *E. meleagritidis* was excluded in some experiments by propagation of cultures in poults solidly immune to that species. *Eimeria gallopavonis* was excluded by collecting oocysts prior to 144 hr, the prepatent period reported for this species of *Eimeria* (9). Medium-sized, ellipsoidal oocysts of similar dimensions to *E. adenoides* ($26 \times 17 \mu\text{m}$), and smaller ovoid-shaped oocysts of similar size to those of *E. meleagritidis* ($19 \times 16 \mu\text{m}$), were observed in varying proportions in most isolates (Table 1).

Considerable variation in the pathogenicity of field isolates was noted in the unmedicated poults despite the exclusion of *E. meleagritidis* and *E. gallopavonis* from the cultures. This could be a reflection of the presence of other species, different proportions of the species present, or strain variation in the pathogenicity of individual species present in the cultures.

Jeffers and Bentley investigated the efficacy of monensin against isolates obtained from turkey flocks from May 1976 to November 1978, shortly after the drug was introduced for the control of coccidiosis (10). They compared isolates from flocks in Canada (Ontario and Quebec) that had a previous history of monensin usage with isolates from flocks in the United States (Minnesota and Virginia), where the drug had not previously been employed. They found that whereas seven of 16 Canadian isolates were resistant to monensin, all 22 isolates from the United States were sensitive. In this study, it was not possible to compare isolates from flocks with and without prior exposure to monensin because this drug has been very widely used in the field. Therefore a mixture of species that had been maintained in the laboratory for many years (Coccivac-T[®]) was employed in an initial experiment to determine the efficacy of monensin against coccidia that had not been exposed to the drug. The results indicate that this mixture was sensitive to monensin. It seems, therefore, reasonable to conclude that the loss in sensitivity of those isolates judged resistant, or partially resistant, was the result of exposure to monensin in the field.

Experiments involving the intentional selection of resistance to monensin in *E. adenoides* have not been reported, but resistance was induced in *E. meleagritidis* following four generations of selection in medicated turkeys (11). It was concluded that the latter species possesses the ability to readily develop resistance to the polyether antibiotic anticoccidials under appropriate conditions of drug exposure. It should be noted that in chickens, resistance has been induced to monensin in

Table 1. The effect of monensin upon the GSR and % OAA of a laboratory mixture and field isolates of turkey coccidia.

Isolate ^A	% Oocysts ^B		Oocyst dose/poult	GSR ^C		% OAA ^D	Resistance category ^E
	Small	Medium		Infected medicated	Infected unmedicated		
L1	33	67	1 × 10 ⁵	Experiment 1 2.345	1.721	94.3	S
A1	95	5	1 × 10 ⁵	Experiment 2 1.546 ^c	1.230 ^d	27.4	R
A2	84	16	1 × 10 ⁵	1.898 ^{ab}	1.603 ^b	93.1	S
A3	56	44	1 × 10 ⁵	2.194 ^a	1.484 ^c	79.0	S
A4	90	10	1 × 10 ⁵	1.860 ^{bc}	1.303 ^d	52.5	PR
A5	95	5	1 × 10 ⁵	1.819 ^{bc}	1.227 ^d	51.3	PR
A6	100	0	1 × 10 ⁵	1.848 ^{bc}	1.744 ^a	59.1	PR
C1	50	50	1 × 10 ⁵	Experiment 3 1.728 ^a	1.367 ^a	97.3	S
V1	15	85	1 × 10 ⁵	1.465 ^{ab}	1.013 ^{bc}	62.3	PR
V2	20	80	8.2 × 10 ⁴	1.272 ^b	0.732 ^c	53.7	PR
P1	32	68	1 × 10 ⁵	1.641 ^{ab}	1.320 ^{ab}	76.8	S
M1	43	57	1 × 10 ⁵	1.275 ^b	0.885 ^c	45.7	R
K1	35	65	1 × 10 ⁵	1.829 ^a	1.500 ^a	72.3	PR
M2	49	51	1 × 10 ⁵	Experiment 4 1.942 ^a	1.475 ^a	92.6	S
M3	63	37	1 × 10 ⁵	1.398 ^{bc}	1.250 ^b	17.5	R
M4	65	35	1 × 10 ⁵	1.405 ^{bc}	1.276 ^b	47.3	R
M5	33	67	1 × 10 ⁵	1.357 ^c	1.091 ^{bc}	30.0	R
K2	56	44	8.4 × 10 ⁴	1.479 ^{bc}	1.134 ^{bc}	82.3	S
K3	100	0	1 × 10 ⁵	1.141 ^c	1.037 ^c	11.0	R
V3	36	64	1 × 10 ⁵	1.717 ^{ab}	1.560 ^a	37.5	R
N1	16	84	1 × 10 ⁵	Experiment 5 1.839 ^a	1.561 ^a	48.9	R
N2	5	95	7.6 × 10 ⁴	1.739 ^a	1.737 ^a	0.5	R
V4	47	53	8.5 × 10 ⁴	1.877 ^a	1.648 ^a	47.5	R
V5	7	93	1 × 10 ⁵	1.804 ^a	1.408 ^a	54.8	PR

^AL1 = laboratory maintained mixture; isolates preceded with capital letters A, C, K, M, N, P, and V were from Arkansas, California, Kansas, Missouri, North Carolina, Pennsylvania, and Virginia, respectively.

^BPercentage of small oocysts (approximately 19 × 16 μm in size), and percentage of medium-sized oocysts (approximately 26 × 17 μm) in each isolate.

^CGrowth and survival ratio (GSR) = cage weight at termination/cage weight at initiation. Data are expressed as the average of two replicates/treatment. For each experiment, values in a column followed by different lowercase superscripts are significantly different ($P \leq 0.05$).

^D% OAA is the percentage of optimum anticoccidial activity. % OAA = (GSR of infected medicated birds - GSR of infected unmedicated birds) / (GSR of uninfected unmedicated birds - GSR of infected unmedicated birds) × 100.

^EAn isolate was characterized as sensitive to monensin (S) when the % OAA was ≥75%, partially resistant (PR) when % OAA was 51%–74%, and resistant (R) when % OAA was ≤50%.

the laboratory (3) and that there have been many reports of resistance in isolates of *Eimeria* from the field (e.g. 6,13).

Monensin is one of the most widely used anticoccidial agents included in the feed of turkeys for the control of coccidiosis and thus exposure to this drug has been widespread. Presently, in the United States approximately 70% of meat-type turkeys are reared with monensin in at least one of their feeds (unpubl. obs.). The finding that the majority of field isolates have acquired resistance or partial resistance to this drug might therefore be anticipated. Of the 23 isolates examined, the majority were judged resistant or partially resistant to monensin. Resistance was identified in isolates from Arkansas, Kansas, Missouri, North Carolina, and Virginia. This is the first report of acquired resistance to monensin in coccidia from turkey flocks in the United States.

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