

Stephen F. Austin State University

SFA ScholarWorks

---

Faculty Publications

Agriculture

---

1997

## Eyespray Vaccination: Infectivity and Development of Immunity to *Eimeria acervulina* and *Eimeria tenella*

H. David Chapman

*University of Arkansas, Fayetteville*

Tim E. Cherry

*Stephen F Austin State University*

Follow this and additional works at: [https://scholarworks.sfasu.edu/agriculture\\_facultypubs](https://scholarworks.sfasu.edu/agriculture_facultypubs)



Part of the [Agriculture Commons](#), and the [Poultry or Avian Science Commons](#)

[Tell us](#) how this article helped you.

---

### Repository Citation

Chapman, H. David and Cherry, Tim E., "Eyespray Vaccination: Infectivity and Development of Immunity to *Eimeria acervulina* and *Eimeria tenella*" (1997). *Faculty Publications*. 20.

[https://scholarworks.sfasu.edu/agriculture\\_facultypubs/20](https://scholarworks.sfasu.edu/agriculture_facultypubs/20)

This Article is brought to you for free and open access by the Agriculture at SFA ScholarWorks. It has been accepted for inclusion in Faculty Publications by an authorized administrator of SFA ScholarWorks. For more information, please contact [cdsscholarworks@sfasu.edu](mailto:cdsscholarworks@sfasu.edu).

# EYESPRAY VACCINATION: INFECTIVITY AND DEVELOPMENT OF IMMUNITY TO EIMERIA ACERVULINA AND EIMERIA TENELLA<sup>1</sup>

H. DAVID CHAPMAN<sup>2</sup>

Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701

Phone: (501) 575-4870

FAX: (501) 575-4202

TIM E. CHERRY

Stephen F. Austin State University, P.O. Box 13000, Nacogdoches, TX 75962

---

**Primary Audience:** Researchers, Veterinarians, Hatchery and Production Managers

---

## SUMMARY

The infectivity of a coccidiosis vaccine and its ability to immunize chickens against two species of *Eimeria* was examined. The vaccine was administered to newly-hatched chicks by spraying directly onto the eye. The method resulted in a high proportion of chicks infected with *E. acervulina* and *E. tenella*. Vaccinated birds reared in cages in the absence of reinfection did not develop immunity to either species by 4 wk of age, but birds reared in floor pens developed immunity to both *E. acervulina* and *E. tenella*.

**Key words:** Chicken, coccidiosis, *Eimeria*, eyespray, immunity, vaccine

1997 J. Appl. Poultry Res. 6:274-278

## DESCRIPTION OF PROBLEM

Coccidiosis is an important disease of poultry caused by protozoan parasites of the genus *Eimeria*. Live coccidiosis vaccines comprising oocysts of *Eimeria* species are available to immunize chickens and turkeys against this disease. These vaccines are conventionally administered by inclusion in the drinking water or by spraying on the surface of feed when the birds are 3 to 10 days of age. Alternative procedures that have been practiced commercially permit vaccination of newly hatched chicks at the hatchery. One

method involves mechanically spraying a suspension of oocysts directly onto the eyes of chicks as they are conveyed from the hatcher to the chick room. An advantage of this procedure is that it may provide a consistent and uniform dose of vaccine to the individual bird. Vaccination at the hatchery may also result in reduced labor costs and therefore be more cost-effective than vaccinating birds at the farm.

We have shown that it is possible to vaccinate newly hatched turkeys against *Eimeria* species by placing a single drop of a vaccine directly onto the eye [1]. Under commercial

---

<sup>1</sup> Published with permission of the Director, Arkansas Agricultural Experiment Station

<sup>2</sup> To whom correspondence should be addressed

conditions, however, vaccines are applied by a mechanical spraying device. No previous studies have determined the efficiency of this method of vaccination under simulated commercial conditions in the hatchery. The objective of the study was to investigate the infectivity of a coccidiosis vaccine administered to newly hatched chicks by eyespray, and the ability of the vaccine to immunize chickens against two species of *Eimeria*.

## MATERIALS AND METHODS

### VACCINATION

Male chicks (Arbor Acres) were vaccinated at a local hatchery and then transferred to the University of Arkansas poultry farm for the experiments. All chicks had been injected subcutaneously with Marek's vaccine (HVT) prior to administration of the coccidiosis vaccine [2]. One hundred chicks were given Coccivac-D [3] by eyespray following the manufacturer's recommended procedure. One 1,000-dose vial of the vaccine was diluted in 30 mL of distilled water and placed in a conical flask on a magnetic stirrer. The flask was connected to the Immunizer (Biojector II) [3], which was primed until droplets of a consistent size were produced. The head of each chick was held on one side and the Immunizer operated to provide a single drop of the vaccine (30  $\mu$ L) on the eye. All chicks were observed to have taken the vaccine into the nasolacrimal duct.

### INFECTIVITY OF THE COCCIDIOSIS VACCINE

We placed 22 chicks that had been vaccinated and 22 unvaccinated chicks in separate cages in a clean animal room and provided them with a basal chick starter ration that contained no anticoccidial drugs. At 6 and 8 days after vaccination each chick was placed in a plastic bucket for 1 hr and fecal droppings collected. Droppings were mixed in 10 mL of saturated salt solution, an aliquot placed in a McMaster chamber, and the number of oocysts present counted. Oocysts were classified as small (possibly *E. acervulina*) or medium-sized (possibly *E. tenella*).

### FLOOR PEN EXPERIMENT

Vaccinated and unvaccinated chicks were individually identified by attaching a tag with a unique number to the wing. They were then

allocated to floor pens containing new litter (2 pens per treatment; 30 chicks per pen) and provided with a starter and grower ration containing no anticoccidial drugs. Ten days later vaccinated chicks were given amprolium (0.006%) in the drinking water for 48 hr as recommended by the manufacturer [3].

The immune status of the birds was determined when they were 4 wk of age by challenging randomly selected vaccinated and unvaccinated chicks with oocysts of *E. acervulina* or *E. tenella*. Eight birds from each pen (total of 16 birds per treatment) were individually weighed and inoculated with  $2 \times 10^5$  oocysts of *E. acervulina* or  $5 \times 10^4$  oocysts of *E. tenella* per bird. An additional eight chicks per pen were not challenged (unchallenged controls). Seven days later they were weighed once more, killed by CO<sub>2</sub> asphyxiation, the intestines removed, and the duodenum and ceca scored for lesions of *E. acervulina* and *E. tenella* respectively [4]. Data were analyzed by one-way analysis of variance using the PROC ANOVA procedure of SAS software. Means were separated and compared using Duncan's multiple-range test [5].

An additional 60 unvaccinated chicks were reared separately in cages that had been sterilized with steam and given robenidine (33 ppm) in the feed as an additional insurance against accidental infection (susceptible controls). At 25 days of age these birds were transferred to two pens alongside those in the principal study and given unmedicated feed. Three days later they were challenged with oocysts as described above.

Litter samples were collected from the pens of vaccinated and unvaccinated chickens when they were 14 and 21 days of age and the numbers of oocysts present in the samples counted [6].

### BATTERY EXPERIMENT

The effect of vaccination upon the development of immunity in the absence of reinfection was investigated. Thirty vaccinated chicks were placed in battery cages (15 birds per cage) and given unmedicated feed. At 6 days of age, and every 2 days thereafter, birds were transferred to clean cages to reduce the possibility of reinfection by oocysts that had been passed in their feces. Twelve preselected birds were challenged at 4 wk of age with a

mixture of  $1 \times 10^5$  oocysts of *E. acervulina* and  $2.5 \times 10^4$  oocysts of *E. tenella* per bird. An additional 12 birds were not challenged (unchallenged controls). Weight gain from 0–7 days post inoculation and lesions present in the intestines were recorded.

## RESULTS AND DISCUSSION

### INFECTIVITY OF THE COCCIDIOSIS VACCINES

Droppings from individual chicks were examined 6 and 8 days after inoculation of oocysts in order to establish the effectiveness of the eyespray method of vaccine administration. The percentage of birds that produced small or medium-sized oocysts in their droppings is given in Table 1. No oocysts were recovered from the unvaccinated control chicks, indicating that accidental infection had been avoided. Small and medium-sized oocysts were found in the droppings of most birds that had been vaccinated by eyespray (86 and 95% respectively), indicating that it is possible to introduce infections with *Eimeria* species by this route. Patent infections developed in day-old turkey poults when a single drop of the vaccine Coccivac-T [3] was placed directly on the eye [1]. The present results demonstrate that it is also possible to infect day-old chickens with *Eimeria* species when a coccidiosis vaccine is administered by a machine that mechanically sprays oocysts into the eye.

### OOCYSTS IN THE LITTER

The number of oocysts in the litter of birds reared in floor-pens is given in Table 2. Oocysts were present in the litter of vaccinated birds by 2 wk, but none were found in the litter of unvaccinated birds. An increase in the number of oocysts in the litter was observed 3 wk

TABLE 1. Percentage of birds producing oocysts of different sizes in droppings after receiving a live coccidiosis vaccine

VACCINATED <sup>A</sup>	NUMBER OF BIRDS	OOCYST SIZE	
		Small	Medium
		% of Birds that Produced Oocysts	
Yes	22	86	95
No	22	0	0

<sup>A</sup>Coccivac-D given by eyespray

TABLE 2. Numbers of oocysts of *Eimeria* in the litter of birds 2 and 3 wk after vaccination<sup>A</sup>

VACCINATED	AGE OF BIRDS	
	2 Wk	3 Wk
	Oocysts per g of litter	
Yes <sup>B</sup>	49,830	136,000
No	0	17,440

<sup>A</sup>Each observation is the mean for two pens.  
<sup>B</sup>Coccivac-D given by eyespray.

after chicks had been vaccinated, suggesting that recycling of parasites occurred following initial exposure to infection. Oocysts were present in the litter of unvaccinated birds at 3 wk of age. Pens containing the unvaccinated chicks were adjacent to those of the vaccinated birds, so they probably had become infected by accidental exposure to oocysts produced by the vaccinated birds.

### FLOOR PEN CHALLENGE EXPERIMENT

*E. acervulina*. The weight gain of vaccinated and unvaccinated birds that had been challenged with *E. acervulina* was not significantly different from that of the unchallenged controls, and no lesions were present in their intestines (Table 3). In contrast, the weight gain of challenged birds that had been reared in the absence of infection (susceptible controls) was significantly lower than that of the other treatments, and lesions were present in their intestines. This indicates that birds vaccinated by eyespray had developed immunity to *E. acervulina*.

Birds that had not been vaccinated also developed immunity to *E. acervulina*. This species has a high reproductive capacity and is highly immunogenic; it is likely that immunity resulted from exposure to oocysts that had been accidentally transferred from adjacent pens. From a practical point of view, indirect exposure to oocysts may facilitate the development of immunity in a flock of birds.

*E. tenella*. The weight gain of vaccinated birds challenged with *E. tenella* was not significantly different from that of the unchallenged controls, and few lesions were present in their ceca (Table 3). Unvaccinated challenged birds and birds reared in the absence of infection, however, showed a significant reduction in weight gain and lesions were present in their

TABLE 3. Effect of challenge with *Eimeria* species upon weight gain and lesions of chickens that had been vaccinated by eyespray at 1 day of age

VACCINATED <sup>A</sup>	n	CHALLENGE <sup>B</sup>					
		None		<i>E. acervulina</i>		<i>E. tenella</i>	
		Wtg <sup>C</sup> (g)	Lesions <sup>D</sup>	Wtg <sup>C</sup> (g)	Lesions <sup>D</sup>	Wtg <sup>C</sup> (g)	Lesions <sup>D</sup>
Yes	16	540 <sup>a</sup>	0 <sup>c</sup>	503 <sup>a</sup>	0 <sup>c</sup>	492 <sup>a</sup>	0.19 <sup>c</sup>
No	16	533 <sup>a</sup>	0 <sup>c</sup>	504 <sup>a</sup>	0 <sup>c</sup>	420 <sup>b</sup>	1.31 <sup>b</sup>
No <sup>E</sup>	16	484 <sup>a</sup>	0 <sup>c</sup>	354 <sup>c</sup>	2.94 <sup>a</sup>	141 <sup>d</sup>	3.63 <sup>a</sup>

<sup>A</sup>Birds were vaccinated with Coccivac-D by eyespray.  
<sup>B</sup>Birds were not challenged or challenged with  $2 \times 10^5$  oocysts of *E. acervulina* or  $5 \times 10^4$  oocysts of *E. tenella*.  
<sup>C</sup>Weight gain was measured from day 0 to 7 post inoculation.  
<sup>D</sup>Lesions present in the duodenum (*E. acervulina*) or ceca (*E. tenella*)  
<sup>E</sup>Birds had been reared free of infection (susceptible controls).  
<sup>a-d</sup>Means for weight gain or lesions with no common superscript differ significantly ( $P > .0001$ ). Standard error of the mean for weight gain and lesions was 19.0 and 0.18 respectively.

ceca. These results indicate that immunity to *E. tenella* had developed in birds vaccinated by eyespray.

Results of the floor pen challenge study indicate that immunity to *E. acervulina* and *E. tenella* had developed by 4 wk of age. Inclusion of amprolium in the drinking water did not prevent the development of immunity in birds vaccinated by eyespray.

**BATTERY CHALLENGE EXPERIMENT**

The weight gain of vaccinated challenged birds was significantly lower than that of birds that were not challenged (Table 4). Lesions were present in the duodenum and ceca of vaccinated challenged birds, but none were found in the birds that were not chal-

lenged. This indicates that vaccinated birds reared in cages did not develop immunity to *E. acervulina* or *E. tenella*.

The battery experiment was carried out in order to investigate the immunizing potential of eyespray vaccination in the absence of re-infection. Vaccinated birds were transferred to clean cages every 2 days to reduce the possibility of exposure to freshly passed oocysts in the feces. Exposure to oocysts on repeated occasions is known to be important for the induction of immunity to species of *Eimeria* [7]. These results indicate that recycling of parasites will likely be an important factor in the effectiveness of coccidiosis vaccines in chickens under field conditions.

TABLE 4. Effect of challenge with a mixed infection of two species of *Eimeria* upon chickens vaccinated by eyespray at 1 day of age and reared in cages

VACCINATED <sup>A</sup>	CHALLENGE <sup>B</sup>	n	WTG (g) <sup>C</sup>	LESIONS	
				Duodenum	Ceca
Yes	Yes	12	209 <sup>b</sup>	2.36 <sup>a</sup>	3.45 <sup>a</sup>
Yes	No	12	508 <sup>a</sup>	0 <sup>b</sup>	0 <sup>b</sup>

<sup>A</sup>Birds were vaccinated with Coccivac-D by eyespray.  
<sup>B</sup>Birds were not challenged or challenged with  $1 \times 10^5$  oocysts of *E. acervulina* and  $2.5 \times 10^4$  oocysts of *E. tenella*.  
<sup>C</sup>Weight gain was measured from day 0 to 7 post inoculation.  
<sup>a,b</sup>Means within a column with no common superscript differ significantly ( $P > .0001$ ). Standard error of the mean for weight gain and lesions in the duodenum and ceca were 29.0, 0.10, and 0.04 respectively.

---

## CONCLUSIONS AND APPLICATIONS

1. The infectivity of a coccidiosis vaccine to newly hatched chicks, when administered by eyespray at the hatchery, has been demonstrated.
  2. Chicks reared in floor pens developed immunity to *E. acervulina* and *E. tenella* by 4 wk of age when vaccinated by this method.
  3. An increase in the number of oocysts in the litter 3 wk after vaccination indicates that recycling of parasites occurred following initial exposure to infection.
  4. Vaccinated chicks reared in cages did not develop immunity to *E. acervulina* or *E. tenella*, indicating that recycling of parasites is important in the development of immunity.
- 

## REFERENCES AND NOTES

1. **Chapman, H.D.**, 1996. Administration of a coccidiosis vaccine to day-old turkeys via the eye and development of immunity to *Eimeria* species. *Poultry Sci.* 75:1496-1497.
2. Select Labs, 1168 Airport Pkwy, Gainesville, GA 30501.
3. Mallinckrodt Veterinary, Inc., Poultry Health Products Group, Route 113, P.O. Box 537, Millsboro, DE 19966-0537.
4. **Johnson, J. and W.M. Reid**, 1970. Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. *Exp. Parasitol.* 28:30-36.
5. **SAS Institute**, 1988. *SAS/STAT User's Guide*. 1988 Edition. SAS Institute, Inc., Cary, NC.
6. **Chapman, H.D.**, 1992. Immunity to *Eimeria* spp. in broilers reared on nicarbazin and salinomycin. *Poultry Sci.* 71:577-580.
7. **Rose, M.E. and P.L. Long**, 1962. Immunity to four species of *Eimeria* in fowls. *Immunology* 5:79-92.

### ACKNOWLEDGEMENT

The technical assistance of A.B. Hacker is gratefully acknowledged.