

Effect of *Eimeria adenoeides* Challenge upon the Course of an Experimental *Salmonella* Enteritidis Infection in Turkey Poults

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Abstract: The aim of the present study was to perform a controlled experiment in order to evaluate the predisposing role of *Eimeria adenoeides* invasion on the appearance, development and severity of a *Salmonella* Enteritidis infection, with both infectious agents being field isolates obtained from spontaneous cases of the disease. Furthermore, we aimed to examine the principal epidemiological parameters of the mixed infection.

The effect of an experimental *Eimeria adenoeides* invasion upon an artificial *S. Enteritidis* infection was studied in 160 turkey poults. The birds were divided into 4 experimental groups. Group 1 was infected with *Eimeria adenoeides*. Group 2 was infected once with *Eimeria adenoeides* and 24 h later infected with *S. Enteritidis* during 3 consecutive days via feed. The birds in group 3 were infected with *Salmonella* for 3 days whereas those in group 4 were not infected (negative controls). The highest values of parameters determining the severity of the epidemic (morbidity rate, lethality, cumulative mortality) were observed in the group where the *Eimeria* invasion preceded the *Salmonella* infection.

The most frequent and most prolonged isolation of *Salmonella* from parenchymal organs was also observed in birds with mixed infection, with *Eimeria* infection coming before salmonellosis. The same group was characterized with the highest values of oocyst and lesion indexes as well as with the worst economic results—lowest live body weight and lowest daily weight gain.

Key Words: *Salmonella* Enteritidis, *Eimeria adenoeides*, turkey poults, oocysts, production parameters, infection

Introduction

The annual losses caused by diseases transmitted by birds to humans in the USA amount to 813 million dollars (1). According to Maciorowski et al. (2), the paratyphoid is still a public health concern on a worldwide scale. It is known that domestic and wild birds are primary carriers of salmonellae (3-5). Very often, those are meat-type and egg-type poultry. According to Kasbohrer (6), the principal serotypes causing salmonellosis in humans are *S. Enteritidis* and *S. Typhimurium*, both serotypes being responsible for 80% of salmonellosis cases in Germany. Furthermore, there is indisputable evidence that turkey foodstuffs are also a common source of salmonellosis in humans (6).

Hafez and Jodas (7) reported that salmonellosis in turkeys are distributed all over the world and cause considerable economic losses related mainly to the high death rate in turkey poults up to the age of 4 weeks. Out of 535 turkey flocks examined, 9.5% were carrying salmonellae (8). *S. Enteritidis* was detected in 8 out of 1460 turkey flocks (9).

While during the first 2-3 weeks the course of salmonellosis in birds, including turkeys, is more severe, afterwards carriership is more frequently observed. Among the carrier birds, the infection could be predisposed by several factors, generally resulting in physiological and morphological changes in the intestinal tract. In this regard, *Eimeria* infections are particularly important (10,11).

In turkeys, coccidiosis caused by several eimeria species is one of the most significant diseases (12,13). However, *Eimeria adenoeides* is the most pathogenic species in turkeys, causing the heaviest economic losses as a result of the high mortality rate, the reduced weight gain, and last, but not least, the decreased resistance of infected birds to other diseases (14-16).

It is known that infection with *E. adenoeides* causes considerable histopathological changes in the ceca of infected turkeys (17). On the other hand, the highest bacterial counts are found in the cecum compared to the other parts of the alimentary tract (18). This influences the opportunity for the emergence of a mixed infection.

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The data on dual natural and experimental infections with salmonella and coccidia in chickens are plentiful, but in turkeys they are relatively scarce.

The aim of the present study was to evaluate the predisposing role of *E. adenoides* on the course of *Salmonella* infection in turkey poults after experimental infection.

Materials and Methods

Birds

One hundred sixty newly hatched (BUT-9) meat turkey poults were obtained from a controlled *Salmonella*-free poultry hatchery. Prior to the experiment, their bacteriological status was determined with particular emphasis on the presence of salmonellae.

During the entire experiment, the turkey poults received a standard feed without supplement of antibiotics or coccidiostatics. They were housed on a slat floor under conditions minimizing the risk of spontaneous *Salmonella* and/or *Eimeria* infection.

Challenging agents

Eimeria adenoides strains were isolated from naturally infected turkeys, enriched through 3-week-old turkeys, cultivated using the routine methods (19) and stored in 2.5% potassium bichromate solution in a refrigerator (4 °C). The sporulated oocysts were administered orally via an ingluvial tube. The oocysts used for infection was tested for sterility by inoculation of 1 ml on MacConkey agar as well as on blood agar.

The *Salmonella* Enteritidis strain was obtained from a field case in newly hatched turkey poults and was stored in broth medium. Prior to the infection, it was enriched twice through albino mice and reisolated from parenchymal organs after the death of laboratory animals with restored pathogenic potential (20). The enrichment and cultivation of the *S. Enteritidis* isolate were done on a selenite broth and MacConkey agar (Becton Dickinson). The biochemical identification and serotyping were done according to the routine bacteriological methods.

Experimental design

The turkeys were divided into 4 groups, each containing 40 birds. The birds from group 1 were infected orally with 8×10^4 sporulated *Eimeria adenoides* oocysts per bird at 5 days of age.

Group 2 was once infected with sporulated *Eimeria adenoides* oocysts at the same dose also at 5 days of age, and 24 h later infected orally with 2.5×10^5 colony forming units (CFU) per bird with *S. Enteritidis*, for 3 consecutive days via feed. This was carried out by calculating the amount of feed consumption per bird and per day and mixing with *S. Enteritidis*.

Group 3 included 40 turkey poults at the age of 6 days, treated for 3 consecutive days only with *Salmonella* suspension with the aforementioned dose.

Group 4 (negative controls) consisted of 40 turkey poults infected neither with *Eimeria* nor with *Salmonella*, housed under the same conditions.

Investigated parameters

Over 3 weeks the postinfection morbidity rate, lethality and mortality rate were calculated. Lethality is the number of birds that died out of those that showed clinical signs. We determined mortality as the number of dead birds to the total number of birds in the experimental group.

The presence of *S. Enteritidis* in parenchymal organs (liver, spleen, cecum) was investigated by 3-week serial studies. At weekly intervals 6 birds per group were euthanized by cervical dislocation and the liver, spleen and cecum were bacteriologically investigated for the presence of *S. Enteritidis*. Briefly, the sample was inoculated into selenite-F broth incubated at 37 °C/48 h and then streaked on MacConkey agar and incubated for a further 24 h.

During the experiment, the live body weight (individually) and amount of forage expenditure were determined in order to establish the weight gain (individually) and feed conversion ratio (FCR, for group).

We determined the lesion scores in the cecum (21) and the oocyst index (22). Because this is described for chickens we made some corrections for *E. adenoides* infection in turkeys:

-Lesion index (the maximum is 4) was examined using the following scheme:

assessment 1 – scarce petechial hemorrhages on the mucosal surface and slight thickening of the intestinal (cecum) mucosa.

assessment 2 – a few hemorrhages up to pinhead size on the mucosal surface, edema and thickening of the intestinal mucosa

assessment 3 – many hemorrhages up to pinhead size on the mucosal surface, edema and thickening of the intestinal mucosa, degenerative changes in the mucosal epithelium, ceca contain necrotic cheese-like debris.

assessment 4 – many hemorrhages up to pinhead size on the mucosal surface, edema and pronounced thickening of the intestinal mucosa, strong degenerative changes in the mucosal epithelium, ceca are full of necrotic cheese-like debris containing lots of oocysts and blood traces.

-Oocysts index (the maximum is 40) was examined using the following scheme:

assessment 0 — 0 to 0.1 million oocysts in the intestine (ceca) for turkey

assessment 1 — 0.1 million to 1 million oocysts

assessment 10 — 1 million to 5 million oocysts

assessment 20 — 5 million to 10 million oocysts

assessment 40 — more than 10 million oocysts

The lesion scores (LS) (21) and the oocyst index (OI) (22) were determined weekly in 6 euthanized birds and some of the dead birds (from the first week 4 dead birds, from the second week 2 and from the third week 1).

The experiment was approved by the Committee on Animal Experimentation at Trakia University, Stara Zagora, Bulgaria, and was performed according to the recommendations of Directive 86/609/EC of November 24, 1986.

The data were statistically processed by one-way analysis of variance (ANOVA). All results are presented as mean \pm standard error of mean (SEM). The differences were considered significant at the $P < 0.05$ level.

Results

The data for the epidemiological parameters morbidity rate, lethality and mortality rate are presented in Table 1.

The highest morbidity rate was observed in turkeys with mixed *Eimeria/Salmonella* infection (group 2, 62.5%), where the mortality rate was 52.5%. A lower morbidity rate was estimated in turkeys infected only with *Eimeria adenoeides* (37.5%), where the mortality rate was 15%. In the group of turkeys infected only with *S. Enteritidis* (group 3), the morbidity rate was 12.5%, whereas the mortality rate was 5%. The highest lethality (%) was observed in turkeys with mixed infection (84%).

Table 2 shows the bacteriological investigations of the degree and duration of *S. Enteritidis* carrierships in the liver, spleen and cecum in turkey poult from challenged groups. It is evident that carriership was the most prolonged and *S. Enteritidis* was isolated from the livers, spleens and ceca in turkey poult from group 2, where the *Eimeria* infection predisposed the *S. Enteritidis* infection. The most extensive carriership and excretion of *S. Enteritidis* were observed during the first week after the challenge. During the second week after the challenge, the number of susceptible turkeys was smaller for reisolation from the liver and spleen but from the cecum it remained comparatively high (5 out of 6 examined). During the last examination (day 21), *S. Enteritidis* was reisolated from the cecum and in one turkey from the spleen. In turkeys with *Salmonella* monoinfection, the reisolation of *S. Enteritidis* was lower, as shown by the data in Table 2, and, during the third week, the bacteriological studies of the viscera in this group were negative.

Table 1. Comparative data for some basic epidemiological parameters in turkeys infected by *E. adenoeides* and by *S. Enteritidis*.

Group	Morbidity rate		Confidence limits	Lethality		Confidence limits	Mortality rate		Confidence limits
	%	n		%	n		%	n	
1	37.5	15	23-53.1	40	6	25.2-56.7	15	6	5.6-27.8
2	62.5	25	46.8-76.9	84	21	53.3-91.1	52.5	21	47.9-59.7
3	12.5	5	1.6-18.5	40	2	25.2-56.7	5	2	0-9.7
4	0	0	0	0	0	0	0	0	0

Group 1 – infected with *Eimeria adenoeides*; group 2 - infected with *Eimeria adenoeides* and *S. Enteritidis*; group 3 – infected with *S. Enteritidis*; group 4 – healthy, untreated.

Table 2. Reisolation of salmonellae from the viscera (liver, spleen, cecum) in turkey poults infected with *E. adenoeides* and *S. Enteritidis*.

Group	Days postinfection								
	7			14			21		
	liver	spleen	cecum	liver	spleen	cecum	liver	spleen	cecum
1	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
2	4/6	3/6	5/6	3/6	2/6	5/6	0/6	1/6	3/6
3	2/6	2/6	3/6	1/6	1/6	2/6	0/6	0/6	0/6
4	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

Group 1 – infected with *Eimeria adenoeides*; group 2 - infected with *Eimeria adenoeides* and *S. Enteritidis*; group 3 – infected with *S. Enteritidis*; group 4 – healthy, untreated.

The production traits in turkeys challenged with both *E. adenoeides* and *S. Enteritidis* as well as in the groups with mono-infections are presented in Tables 3 and 4.

Table 3 shows that the highest live body weight was observed in non-challenged birds from group 4 and the lowest in group 2, submitted to a double challenge. The live body weight in the turkeys from group 1 (infected with *E. adenoeides*) was similar 2 and 3 weeks after the challenge. The birds from group 3 that survived the *Salmonella* mono-infection did not exhibit significant differences in the body weight compared to group 4 (negative controls).

During the first postinfection week, weight gain was lowest in birds with double infection (group 2, Table 4). During the second and third weeks after the challenge, an obvious tendency towards weight gain reduction was present, although the differences among groups 1, 2 and

3 were not statistically significant. This tendency was maintained up to the end of the experimental period in turkeys from group 2.

The results for the FCR shown in Table 5 demonstrate that, in birds where the *Eimeria* infection preceded the challenge with *Salmonella* (group 2), this parameter had the highest values during the entire study. Compared to non-infected birds (group 4), FCR values were relatively high during the second week in groups with either *Eimeria* or *Salmonella* mono-infections (groups 1 and 3, respectively). During the third experimental week, no differences between the negative controls and the turkeys that survived the respective mono-infections were observed.

The investigations of the changes in lesion scores in the intestinal mucosa and the oocyst index are shown in Table 6. Both parameters had higher values in the group with mixed infection (group 2) compared to the mono-infected

Table 3. Dynamics of the live weight (g) in turkeys infected with *E. adenoeides* and/or *S. Enteritidis*.

Group	Days postinfection								
	Baseline	N	7	N	14	N	21	N	
1	89.0 ± 5.2	40	130.0 ± 8.2*	30	185.0 ± 12.4*	20	369.0 ± 4.3	10	
2	83.3 ± 1.2	40	113.0 ± 15.1*	30	175.0 ± 15.1**	20	354.0 ± 5.7*	10	
3	85.6 ± 3.3	40	136.5 ± 10.2	30	198.0 ± 9.2	20	406.0 ± 8.6	10	
4	90.0 ± 1.8	40	136.8 ± 3.3	30	201.4 ± 11.1	20	400.0 ± 10.1	10	

*P < 0.05; **P < 0.01 (vs. group 4)

Group 1 – infected with *Eimeria adenoeides*; group 2 - infected with *Eimeria adenoeides* and *S. Enteritidis*; group 3 – infected with *S. Enteritidis*; group 4 – healthy, untreated.

Table 4. Weight gain (g) in turkeys infected with *E. adenoeides* and/or *S. Enteritidis*.

Group	Baseline body weigh	Days postinfection						
		N	7	N	14	N	21	N
1	89.0 ± 5.2	40	41.0 ± 1.1*	30	55.0 ± 3.4	20	184.0 ± 11.1	10
2	83.0 ± 1.2	40	30.0 ± 2.3**	30	62.0 ± 5.1	20	179.0 ± 10.8	10
3	85.6 ± 3.3	40	51.1 ± 3.4	30	62.2 ± 3.4	20	208.0 ± 11.1	10
4	90.0 ± 1.8	40	46.5 ± 3.1	30	64.9 ± 3.8	20	198.0 ± 10.3	10

*P < 0.05; **P < 0.01 (vs. group 4)

Group 1 – infected with *Eimeria adenoeides*; group 2 - infected with *Eimeria adenoeides* and *Salmonella* Enteritidis; group 3 – infected with *Salmonella* Enteritidis; group 4 – healthy, untreated.

Table 5. Feed conversion ratio (FCR) in turkeys infected with *E. adenoeides* and/or *S. Enteritidis*.

Group	Days postinfection		
	7	14	21
1	2.92	2.31	1.63
2	4.12	2.53	1.78
3	2.25	2.36	1.03
4	2.68	2.20	1.26

Group 1 – infected with *Eimeria adenoeides*; group 2 - infected with *Eimeria adenoeides* and *Salmonella* Enteritidis; group 3 – infected with *Salmonella* Enteritidis; group 4 – healthy, untreated.

group. The highest differences were observed during the first week after the challenge ($P < 0.05$). During the second week, the changes in the intestinal wall were irrelevant, without statistical significance although the oocyst discharge in this group was higher ($P < 0.05$). The third examination revealed no lesions in the intestinal wall in either group infected with *Eimeria*. In monoinfected turkeys, single oocysts were still visible on the observation field but in birds challenged twice the discharge of oocysts was still considerable.

Discussion

The high intensity of the epidemic process established in turkeys from the second group, where the *Eimeria* infection preceded the challenge with *Salmonella*, could be explained by the destructive effect of parasites on cecal mucosa, observed in earlier studies (17). The infection with parasites

allows easy adhesion, penetration and subsequent dissemination of salmonellae in parenchymal organs, resulting in a higher morbidity rate and lethality. Those parameters were very difficult to check and therefore the authors working upon the associated infections in chickens with eimeriosis determined only the death rate (23).

The most frequent carriership of salmonellae in the twice challenged birds from group 2, which was also the most prolonged vs. monoinfected birds, showed that the preliminary infection with *Eimeria* parasites resulted in a severe infection manifested by massive colonization of the cecum and following detection of salmonellae in the liver and spleen that could be latent or manifested. A similar increase in *S. Typhimurium* in the liver and cecum is also reported by Arakawa et al. (10) and Takimoto et al. (24) in chickens infected with *E. tenella*, and by Quin et al. (25) in chickens infected with *E. tenella* and *S. Enteritidis*.

According to Baba et al. (11,26), the factors favoring the survival and enhancement of salmonellae in the intestinal lumen are the free fatty acids' concentration and the oxidation reduction potential. Having studied those factors in chickens infected with *E. tenella*, the authors concluded that the increased oxidation reduction potential as well as the strong reduction in free fatty acids' levels favored the enhancement of salmonellae in the intestinal lumen.

In birds that survived the associated infection, a considerable reduction in the average live weight and weight gain as well as a higher FCR were detected vs. untreated turkeys. In previous studies of ours on broiler chickens inoculated with *E. tenella/S. Enteritidis* (27), similar results were obtained in the group where the

Table 6. Dynamics of changes in lesion scores (LS) and in the oocyst index (OI) in turkeys infected with *E. adenoeides* and/or *S. Enteritidis*.

Group	Postinfection days								
	7			14			21		
	LS	OI	n*	LS	OI	n*	LS	OI	n*
1	2.8 ± 0.2	32.0 ± 3.5	10	1.1 ± 0.1	10.2 ± 2.1	8	0	Single	6
2	3.2 ± 0.2*	38.0 ± 2.8**	10	1.2 ± 0.1	18.5 ± 3.4*	8	0	10.5 ± 0.4	7
3	0	0		0	0		0	0	
4	0	0		0	0		0	0	

*P < 0.05; **P < 0.01 (vs. the group with monoinfection)

*The number includes 6 euthanized birds, and the rest died spontaneously

Eimeria infection preceded the salmonellosis. We assume that those data correlated with the results of monitored epidemic parameters and outlined the features of a very intense epidemic process. Although the cited authors (10,11,24-26) have not studied these parameters, we think that they are necessary in order to show the severity of both associated infections and monoinfections.

Our results about the cecal lesion scores and the oocyst index showed that, as early as the seventh postinfection day, *E. adenoeides* caused considerable injuries to the intestinal lumen. The data showed that, in the group challenged twice, the parameters were very high and the time for expression was protracted compared to that in monoinfected turkeys. The values of those parameters evidence a severe infection. According to Holdsworth et al.

(28), lesion scores and oocyst indexes are important for assessment of the severity of infection with *Eimeria* in birds, including turkeys. Similar data are reported by Stephens and Vestal (23) in chickens infected with *E. tenella* and inoculated with *S. Typhimurium*, while the other investigators that have worked upon this problem did not monitor this parameter.

Our results about the course of *S. Enteritidis* infection in turkeys preceded by *E. adenoeides* infection led us to conclude that the dual infection was accompanied with adverse effects on production parameters, and prolonged the salmonella colonization in the viscera and cecum. The changes in the intestinal mucosa, the oocyst discharge and the poor production traits are indicative of a severe infection in turkeys with dual infection.

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