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Effect of an experimental *Eimeria tenella* invasion upon an artificial *Salmonella* Typhimurium infection in broiler-chickens

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ABSTRACT

The effect of an experimental *Eimeria tenella* invasion upon an artificial *Salmonella* Typhimurium infection was studied in 200 broiler-chickens. The birds were divided into 5 experimental groups. The first group was challenged with *S.* Typhimurium for three consecutive days. By the 4th day, the chickens were infected once with sporulated *E. tenella* oocysts. The second group was infected once with *E. tenella* and 24 h later - infected with *S.* Typhimurium during three consecutive days. The third group of birds was submitted to a single *E. tenella* infection. The birds from group IV were infected with *Salmonella* for three days whereas chickens from group V were not infected (negative controls). The highest values of parameters, determining the severity of the epidemy (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 with birds with mixed infection, with *Eimeria* infection coming first 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*, *Eimeria tenella*, broiler-chickens, oocysts

Introduction

Both wild and domestic birds together with synantropic rodents, are the principal natural source of *Salmonella* spp. Most commonly, various serovars of *S. enterica* subsp. *enterica* are isolated. The prevalent representatives as *S.* Enteritidis, *S.* Typhimurium, *S.* Isangi, *S.* Anatum etc. predominate (SATO, 1999; HANSEN et al., 2000; MULDER and SIMONS, 2001).

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The foodstuffs of avian origin (eggs and meat) are among the primary sources of salmonellosis in humans (BRYAN and DOYLE, 1995; HAFEZ, 1999; HAFEZ and MAZAHERI, 2000).

They develop most frequently as foodborne toxi infections with a various progress and outcome. According to BRYAN (1981), the *Salmonella* serotypes, isolated from humans, correlated with those detected in birds. Similar data are reported in Bulgaria too (DIMITROV et al., 1974; MILEV and SERBEZOVA, 1970).

The factors promoting the appearance and the development of salmonelloses in domestic birds are numerous. In intact flocks the infection is imported from the environment, most commonly via the forage especially when it is contaminated with animal excrements. As a result, a clinical manifestation of the infection is observed or, usually in older birds, a *Salmonella* carriership occurred.

The morbidity rate in birds - *Salmonella* carriers, depends on different factors, with *Eimeria* infections being among the important ones (ARAKAWA et al., 1981; BABA et al., 1985; STEPHENS and VESTAL, 1966). Cases of the concurrent infection, in which salmonellosis occurs in *Eimeria*-infected birds are not uncommon. In previous studies of ours, we outlined the role of *E. tenella* for the severity of *S.* Enteritidis (KOINARSKI et al., 1998) and *S.* Isangi infections (KOINARSKI et al., 1999).

Our studies are related to the effect of *E. tenella* on *S.* Typhimurium infection, in which the most important fact was the presence of the highest amount of bacteria in caeca, where *E. tenella* is usually localized too.

A concurrent disease in chickens caused by *S.* Typhimurium and *E. tenella* was reported by several investigators (TAKIMOTO et al., 1984; MORISHIMA et al., 1984; ARAKAWA et al., 1985; BABA et al., 1985). Various aspects of the problem were studied but the results were often conflicting.

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

Materials and methods

Birds. Two hundred newly hatched broiler chickens were obtained from a controlled *Salmonella*-free poultry hatchery. Their bacteriological status was monitored prior to the study. They were divided into 5 groups:

The birds from Group I were challenged with *S.* Typhimurium at the age of 3 days for three consecutive days via oral application of 1 mL bacterial suspension with a density

of 2.5×10^6 colony forming units (CFU). By the 4th day, the chickens were invaded once with 6×10^4 sporulated oocysts (*E. tenella*).

The second Group of 40 birds (at the age of 5 days) was once infected with sporulated *E. tenella* oocysts at the same dose and 24 h later - infected with *S.* Typhimurium for three consecutive days at the same dose as in Group I.

Group III included 40 birds at the age of 5 days that were submitted to a single *E. tenella* infection without challenge with *Salmonella*.

Group IV (40 birds at the age of 3 days) was treated for 3 consecutive days only with *Salmonella* suspension with the forementioned density.

Group V (negative controls) consisted of 40 birds that were neither treated with *Eimeria* nor with *Salmonella*, housed in the same conditions.

Challenging agents. E. tenella strains, isolated from naturally infected birds, enriched through 3-week-old birds and cultivated according to GAWAIN et al. (1981). The oocyst culture used for infection was tested for sterility by inoculation of 1 mL in McConkey agar in Petri's dishes.

The *S.* Typhimurium strain was isolated from a field case in newly hatched chickens 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 a restored pathogenic potential ($2.5.10^6$-5.10^6 CFU). The enrichment and cultivation of *S.* Typhimurium isolate were done with a Selenite broth and McConkey agar (Becton Dickonson) according to QUINN (1997).

Studied parameters. During the experiment, the birds were given a standard forage without supplement of antibiotics or anticoccidials. They were housed on a slat floor under conditions, minimizing the risk of spontaneous *Salmonella* and/or *Eimeria* infections.

In all experimental and control groups of birds, the parameters morbidity rate, death rate, lethality, duration of *Salmonella* carriership, live weight changes, forage expenditure per unit weight gain were determined for 4 weeks. In dead or euthanized birds (10 birds each week during 4 weeks), the presence of *Salmonella* in parenchymal organs and caeca (QUINN, 1997) as well as the lesion index (LI) and the oocyct index (OI), (CUCKLER, 1959) have been determined. The euthanasia was performed via cervical dislocation.

The data were statistically processed using one way analysis of variance (ANOVA) and Cl - confidence limits. A traditional method of calculation and comparison of the confidence limits - α = are sin $\sqrt{p}$ ($n<500$; level of possibility 0.9545) was used.

Results and discussion

The data for epidemiological parameters are presented in Table 1. The highest epidemiological intensity was observed in Group II, where the *Eimeria* invasion preceded

the experimental *Salmonella* infection. The morbidity rate for this group was 77.5% and the lethality - 64.5%. This is explicable because of the invasive nature of protozoa against the intestinal epithelium, allowing an easy adhesion, penetration and subsequent dissemination of *Salmonella*e in parenchymal organs, that reflects in a higher lethality. It must be emphasized that those two parameters could be hardly controlled in spontaneous diseases, so most of authors prefer to interpret only the cumulative mortality (STEPHENS and VESTAL, 1966). In our experiment, the latter reached its highest values in Group II - 50%.

Table 1. Epizootological parameters in experimental and control broiler-chickens (n = 40 in each group)

Group	Diseased birds	MR %	Cl *	L %	Cl	DR %	Cl
I	28	70.0	55 ÷ 93	42.8	28 ÷ 58.2	30.0	17.0 ÷ 45.9
II	31	77.5	62.8 ÷ 88.5	64.5	49.2 ÷ 78.3	50.0	34.7 ÷ 65.2
III	18	45.0	30.1 ÷ 60.4	44.4	29.5 ÷ 59.8	20.0	9.2 ÷ 33.6
IV	12	30.0	17.0 ÷ 45.9	33.3	19.7 ÷ 49.4	10.0	2.7 ÷ 21.1
V	0	0	0	0	0	0	0

MR = morbidity rate, %; L = lethality, %; DR = death rate, %; CL = confidential limits.

The values of the studied parameters in Group I were somewhat lower that could be due to the time, necessary for penetrating the intestinal barriers prior to their damage by *Eimeria*. In both groups of chickens with mixed infection however, those parameters were higher compared to chickens with monoinfection (either with *Eimeria* or *Salmonella*).

Table 2. Presence of *S.* Typhimurium in livers and caeca of broiler-chickens

Group	Days after invasion							
	7		14		21		28	
	liver	caecum	liver	caecum	liver	caecum	liver	caecum
I	2/10	6/10	3/10	5/10	0/10	1/10	0/10	2/10
II	4/10	9/10	6/10	10/10	2/10	4/10	0/10	4/10
III	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
IV	0/10	5/10	3/10	4/10	0/10	0/10	0/10	1/10
V	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

Table 2 shows the data for the degree and the duration of *Salmonella* carriership in caeca of chickens from challenged groups. It is evident that the carriership was the most

prolonged in chickens from Group II, preliminary infected with *Eimeria*. The *Salmonella* organisms were detected in caeca even during the control euthanasia by post infection week 4, whereas in parenchymal organs there were detected by post infection day 21 at last.

Table 3. Dynamics of change in lesion index (LI) and oocyst index (OI) in broiler-chickens (results are expressed as Mean ± SE)

Group	Days after invasion							
	7		14		21		28	
	LI	OI	LI	OI	LI	OI	LI	OI
I	28.5± 0.19^{A3}	32.3± 0.15^{A3}	28.1± 0.16^{A3}	30.1± 0.17^{A}	0	12.5± 0.06^{B3}	0	
II	35.4± 0.03^{A3}	34.5± 0.17^{A3}	32.3± 0.17^{A3}	34.0± 0.23^{A3}	0	25.0± 0.11^{B3}	0	24.0± 0.14^{B3}
III	30.0± 0.26	25.1± 0.09	10.0± 0.11	22.0± 0.16	0	single	0	single
IV	0	0	0	0	0	0	0	0
V	0	0	0	0	0	0	0	0

$^{A}P<0.01$; $^{B}P<0.001$vs the other challenged group; $^{1}P<0.05$; $^{2}P<0.01$; $^{3}P<0.001$ vs group III (non-challenged)

The most extensive carriership and excretion of *Salmonella* were observed during the first two weeks after the challenge. The most susceptible chickens were those from Group II. This demonstrates that the preliminary invasion with *Eimeria* resulted in a severe infection, manifested by a massive colonization of caeca and residence in parenchymal organs (caecum) that could be latent or manifested depending on the level of infection. This corresponds with data already reported (BABA et al., 1985; MORISHIMA et al.,1984). The challenge dose we used resulted primarily in carriership that was more frequently expressed as a clinical disease with a lethal issue in chickens from Group II, seen from lethality values (Table 1).

The degree and the duration of *Salmonella* carriership corresponded to the data of other investigators. Thus, BABA et al. (1985) showed that by the first 24 h, the number of *Salmonella* in caeca of *Eimeria*-infected chickens was not higher than those in non-invaded chickens, but afterwards, *Eimeria*-infected chickens were characterized by sharply elevated caecal *Salmonella* counts, a cause for the subsequent excretion. According to TAKIMOTO et al. (1984) and MORISHIMA et al. (1984), the excretion of *Salmonella* lasts more than 2 weeks. The authors have not followed the duration of carriership to the end, but stated that it was more extensive in chickens with concurrent infection. The causes for the longer periods of carriership in birds with mixed infection/invasion are not clear as stated by ARAKAWA et al.

(1981), but STEPHENS and VESTAL (1966) assumed that they were caused by the damaged intestinal wall. We also support the hypothesis of those authors, although their model was performed with *S.* Typhimurium/E. *necatrix*. BABA et al. (1985) presumed that the cause for the massive reproduction of *Salmonella*e in the caeca of *E. tenella*-infected birds was the decreased concentrations of volatile fatty acids and the increased oxidation-reduction potential in intestines.

Table 4. Dynamics of change in average live weight (g) and the weight gain (%) vs the negative control group in broiler-chickens (results are expressed as Mean ± SE)

Group	Days after invasion						
	Baseline	5		12		21	
	Average live weight	Average live weight	Weight gain	Average live weight	Weight gain	Average live weight	Weight gain
I	105 ± 3.16	202.6 ± 0.92[1]	81.5	381.4 ± 2.13[2]	74.3	647.0 ± 2.74[2]	86.6
II	91.5 ± 2.18	181.6 ± 1.18[2]	75.7	302.6 ± 2.12[2]	50.3	523.0 ± 1.93[2]	70.0
III	96.5 ± 2.33	209.5 ± 1.33[1]	94.9	331.8 ± 1.93[2]	50.9	628.0 ± 2.33[2]	84.0
IV	101.9 ± 2.2	200.4 ± 1.13[1]	82.7	346.3 ± 2.27[2]	60.8	680.0 ± 2.15[2]	91.0
V	100.0 ± 2.24	219.5 ± 1.26	100.0	460.0 ± 3.65	100.0	747.0 ± 2.17	100.0

[1]P<0.01; [2]P<0.001 vs group V (negative controls)

The results reflecting the dynamics of changes of the lesion index and the oocyst index are shown in Table 3. The comparison among groups revealed that those parameters were the highest in chickens with concurrent infection/invasion, namely in birds from Group II (initially infected with *E. tenella* and subsequently challenged with *Salmonella*). Moreover, the difference was present as early as the first control study and persisted up to the end of the experimental period of 4 weeks, an indication for a unremitting infection. In chickens with *Eimeria* monoinfection (Group III), the lesions during the second control investigation were few, correlating with the oocyst index as well. The 3rd and the 4th control examinations (days 21 and 28) showed no lesions and only single oocysts were detected - a sign for a remitting infection. According to STEPHENS and VESTAL (1966), in chickens infected with E. *necatrix* and *S.* Typhimurium those changes in the intestinal tract were extended up to the 21st day. The other investigators having worked upon this problem and cited by us, have not followed out those parameters, that, in our opinion, are important for the elucidation of the severity of the epidemiological process and its duration.

Tables 4 and 5 present the data for the changes in the live weight, the weight gain vs the control (untreated) group and the feed conversion ratio (FCR). The lowest live body weight, respectively the lowest percentage of weight gain were observed in birds from Group II compared to controls. The respective values for Group I were similar. The values

for Groups III and IV (with *Eimeria* and *Salmonella* monoinfections in that order) were similar one to another, but lower vs untreated birds (Group V). The data correlated with FCR values as well.

Table 5. Feed conversion ratio (FCR) in broiler-chickens (results are expressed as Mean ± SE)

Group	Days after invasion		
	5	12	21
I	1.8 ± 0.04[2]	1.8 ± 0.04[2]	1.9 ± 0.04[2]
II	2.2 ± 0.05[2]	2.3 ± 0.05[2]	2.5 ± 0.06[2]
III	2.1 ± 0.04[2]	2.1 ± 0.04[2]	2.2 ± 0.06[2]
IV	2.0 ± 0.03[2]	1.8 ± 0.04[2]	1.8 ± 0.03[2]
V	1.4 ± 0.03[2]	1.3 ± 0.02	1.2 ± 0.02

[1]P<0.01; [2]P<0.001 vs group V (negative controls)

The feed conversion ratio was the highest in Group II compared to all other groups, thus evidencing the severity of mixed infection, when *Eimeria* invasion preceded the *Salmonella* challenge.

This study as well as previous studies of ours (KOINARSKI et al., 1998) on *S.* Typhimurium showed the more severe infection and the more prolonged carriership occurred in chickens when *Eimeria* invasion came before the *Salmonella* infection. This must be considered when eimeriosis is detected in growing chickens, especially when the population is positive to *Salmonella* or there is a risk for an exogenous infection.

The analysis of data allowed us to conclude that the most extensive *Salmonella* carriership during the first 2 weeks was in birds with concurrent infection, particularly when the *Eimeria* invasion preceded the *Salmonella* infection. The values of parameters characterizing the intensity of epidemiological process - morbidity rate, lethality and cumulative mortality in those chickens were the highest. The lesion index and the oocyst index were the most elevated too, whereas the economical parameters - the worst.

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SAŽETAK

Učinak pokusne invazije parazitom *Eimeria tenella* na umjetno izazvanu infekciju serovarom *Salmonella* Typhimurium istraživan je na 200 tovnih pilića. Pilići su bili podijeljeni na pet pokusnih skupina. Prva skupina bila je inficirana serovarom *S.* Typhimurium u tijeku tri uzastopna dana. Četvrtoga dana pilići su bili jednokratno invadirani sporuliranim oocistama *E. tenella*. Druga skupina jednokratno je bila invadirana parazitom *E. tenella*, a 24 sata nakon toga inficirana serovarom *S.* Typhimurium u tijeku tri uzastopna dana. Treća skupina pilića podvrgnuta je jednokratnoj invaziji parazitom *E. tenella.* Četvrta skupina bila je inficirana salmonelama u tijeku tri dana, dok je peta bila neinficirana kontrolna skupina. Najveće vrijednosti pokazatelja težine zaraze (pobol, smrtnost i kumulativni pomor) utvrđene su u skupini u kojoj je invazija parazitom *E. tenella* prethodila infekciji salmonelama. Također je ustanovljeno da su salmonele najčešće i najduže bile izdvajane iz parenhimskih organa pilića s mješovitom infekcijom u kojoj su pilići najprije bili invadirani parazitima, a potom inficirani salmonelama. U toj skupini dokazane su najviše vrijednosti oocista i najveći indeksi lezija te postignuti najlošiji rezultati obzirom na ukupnu težinu i dnevni prirast pilića.

Ključne riječi: *Salmonella* Typhimurium, *Eimeria tenella*, tovni pilići, oociste