Consequences of concurrent *Ascaridia galli* and *Escherichia coli* infections in chickens

By *A. Permin[1],*, J. P. Christensen[1], M. Bisgaard[1]*

[1] Department of Veterinary Pathobiology, The Royal Veterinary and Agricultural University, Stigbøjlen 4, 1870 Frederiksberg C., Copenhagen, Denmark.

Permin A, Christensen JP, Bisgaard M. Consequences of concurrent *Ascaridia galli* and *Escherichia coli* infections in chickens. Acta vet. scand. 2006, 47, 43-53. – Three experiments were carried out to examine the consequences of concurrent infections with *Ascaridia galli* and *Escherichia coli* in chickens raised for table egg production. Characteristic pathological lesions including airsacculitis, peritonitis and/or polyserositis were seen in all groups infected with *E. coli*. Furthermore, a trend for increased mortality rates was observed in groups infected with both organisms which, however, could not be confirmed statistically. The mean worm burden was significantly lower in combined infection groups compared to groups infected only with *A. galli*. It was also shown that combined infections of *E. coli* and *A. galli* had an added significant negative impact on weight gain.

Ascaridia galli; E. coli; interactions; free-range layer chickens

Introduction

Ascaridia galli and *Escherichia coli* are both common causes of infections in confined as well as in free-range poultry productions systems (*Dho-Moulin and Fairbrother,* 1999; *Permin et al*. 1999).

Pathogenic *E. coli* may cause airsacculitis, salpingitis, peritonitis, polyserositis, septicemia and other extra-intestinal diseases in chickens, turkeys and other avian species. However, *E. coli* also constitutes part of the intestinal microflora of healthy birds and most of the diseases associated with *E. coli* are considered secondary to environmental and host predisposing factors (*Dho-Moulin and Fairbrother* 1999). Clinical isolates of avian *E. coli* commonly belong to certain serogroups, i.e. O1, O2 and O78, and to a restricted number of clones (*White et al.* 1993). Experimental infections

have shown that the air-exchange regions of the lungs and the airsacs are important sites of entry of *E. coli* into the bloodstream of birds during the initial stages of infection and that resistance to phagocytosis may be an important mechanism in the development of the disease (*Gross* 1990). It has also been demonstrated that F1 fimbriae are expressed in the respiratory tract, whereas P fimbriae are expressed in the internal organs of infected chickens (*Vidotto et al.* 1990). Unambiguous virulence factors associated with *E. coli* infections in avian species, remain to be identified. Diagnosis of *E. coli* infections is based on the clinical picture, lesions and isolation of *E. coli* (*Dho-Moulin and Fairbrother* 1999).

A. galli may cause anorexia, weight loss, haemorrhages in the intestinal mucosa, obstruction

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* Corresponding author. Tel: +45 35282748. Fax: +45 35283762. E-mail: ape@kvl.dk (A. Permin).

of the intestinal lumen, altered hormone level and eventually death (*Ackert* 1931, *Ikeme* 1971, *Roepstorff et al.* 1999) in a wide range of avian species. The life cycle of *A. galli* is direct with a prepatent time of minimum 28 days under temperate climatic conditions (*Permin et al.* 1998). After ingestion of the infective egg, the egg hatches in the small intestine where the larva embeds in the mucosal layer of the duodenum for a varying period of 3- 56 days depending on age and immunity of the bird (*Herd and McNaught* 1975). After maturation of the worm, it migrates to the intestinal lumen where it lives from intestinal contents and occasionally from host blood. The mature worms copulate and might start producing eggs after 28 days. Diagnosis of *A. galli* is based on faecal isolation of parasite eggs or direct identification of adult worms in the intestine (*Permin and Hansen* 1998).

Few pathogen interaction studies have been carried out in poultry. *Okulewicz and Zlotorzycka* (1985) showed that *A. galli* exerted an inhibiting effect on the natural bacterial micro flora of the intestine of hens. The opposite situation, where the bacterial flora of the intestine inhibited the establishment of *A. galli* was demonstrated by *Stefanski and Przyjakowski* (1967). *Chadfield et al.* (2001) showed an interaction between the intestinal flora and *A. galli*, where the bacterium Salmonella enteriditis was incorporated into the eggs of *A. galli*. This finding, however, is in contrast to the finding of *Baron et al.* (1960), where the eggs of *A. galli* were found to be sterile. To the knowledge of the authors nobody has examined the interaction between *E. coli* and *A. galli* infections in chickens. Consequently this study has been designed to investigate the possible effect of two common concurrent infections; *A. galli* super imposed with *E. coli* and vice versa, on establishment of the respective infections, pathological lesions, mortality and impact on selected production parameters.

Materials and methods

Experimental animals
Female Lohman Brown chickens were used for all experiments. The chickens were purchased as one-day-old chicks and kept for one week in a confined parasite free environment until the experiment. The chickens were given a commercial feed containing 20% protein and water *ad libitum*. The chickens were kept in one flock until infection whereafter they were placed in separate houses.

Infection material
A clinical nalidixic_acid_resistant O78 *E. coli*-isolate originating from broilers suffering from respiratory distress (difficulties in breathing) was grown overnight in an enrichment media (LB-broth) to reach the desired infection dose (*Lee and Arp* 1998). The number of bacteria pr. ml (cfu) was determined by spectophotometry and plate counts.

A. galli eggs were isolated from mature female worms obtained from layers and embryonated in 0.1N sulphuric acid according to the method described by *Permin et al.* (1997b).

Experimental design
In total, three experiments were carried out. The first experiment was conducted to determine the route and dose of the *E. coli* having clinical impact without killing all chickens, a prerequisite for the following two trials. The infection dose and infection route of *A. galli* was set to be 500 embryonated eggs given as a single oral infection in all three experiments according to previous studies by *Permin et al.* (1997a). The last two experiments were designed as 2 x 2 cohort studies, i.e., groups with or without *A. galli* and *E. coli* infections including a control group (*Thrusfield* 1995).

Determination of dose and infection route of E. coli

Fifty-two one-week-old chickens were purchased and were kept for seven days to acclimatise in the new environment. The chickens were divided into 9 groups of which 7 groups each consisted of 6 animals and 2 groups of each 5 animals. The animals were infected as given in Table 1. The primary infection with *A. galli* or *E. coli* took place on day 0 of the experiment. The secondary infection was carried out seven days after the first infection. All chickens were killed on day 14 and subjected to *post mortem* examinations (*Permin and Hansen* 1998).

Final experiments

Based on the results of the first experiment, two further experiments were set up, each with six groups. In total 299 four-week-old Lohman Brown female chickens were used for the experiments. The infection dose of *E. coli* was set to be 10^8 cfu in the two experiments, given as either an oral or a tracheal infection. In all experiments *A. galli* and *E. coli* were given either as single or combined infections. In the case of combined infections the chickens were inoculated with an interval of 7 days between the two infections. In the second experiment the animals were followed for 14 days after the second infection, whereas the animals in the third experiment were followed 4 weeks after the second infection. Group distributions and inoculation schemes are given in Tables 2 and 3.

Parameters measured

All chickens were weighed just as clinical signs were recorded. Furthermore, re-isolation and counting of the nalidixic-acid-resistant *E. coli* (*Lee and Arp* 1998) and *A. galli* larvae/adults (*Permin and Hansen* 1998) was carried out on all animals dying during the experiment and at the end of the experiment. Likewise, pathological lesions, if any, were recorded on all chickens.

Statistical analyses

All data were stored in the statistical programme GraphPad Prism (*GraphPad Software Incorporated* 2000). One-way analysis of variance (ANOVA), chi-square analysis (x^2-analysis) or Students t-test were used to analyse the data.

Results

Determination of dose and infection route of E. coli

Pathological lesions associated with an *E. coli* infection were seen in the group given *E. coli* by tracheal route with 10^8 cfu and in the groups infected with *A. galli* combined with a secondary *E. coli* infection given by tracheal route with a dose of 10^4 or 10^8 cfu. Pathological lesions were observed in four, three and two chickens respectively in these groups (Table 1). However, *E. coli* could only be re-isolated from three of these animals. One animal died in the group infected with *A. galli* and *E. coli* given by tracheal route with 10^4 cfu while two animals died in the group given only a tracheal oral dose of 10^8 *E. coli*. These three animals all tested positive for *E. coli*. A chi-square analysis showed no significant difference in mortality rates between the groups (p>0.05).

A. galli larvae were recovered from all four groups infected with the parasite. An analysis of variance showed that the worm burdens were not significantly different between groups (p>0.05).

The animals were weighed four times during the first experiment. The mean weight gains are given in Figure 1. One week after the first infection with either *A. galli* or *E. coli* differences were seen between the infected groups when compared to the control group (p<0.05), but there was no significant difference between the infected groups. At slaughter (fourth weighing) an analysis of variance showed that the group infected with *A. galli* and subsequently with *E.*

Table 1. Infection groups used for the determination of dose and infection route of *E. coli*

Group	Type of infection	Group size	Pathological findings
Ag+Ec4O	*A. galli* (primary infection) given as oral dose of 500 embryonated eggs + *E. coli* (secondary infection) given as oral dose of 10^4 cfu.	5	No pathological findings
Ag+Ec4T	*A. galli* (primary infection) given as oral dose of 500 embryonated eggs + *E. coli* (secondary infection) given as tracheal dose of 10^4 cfu.	6	1 animal with polyserositis, † 1 animal with pericarditis and doublesided pneumonia
Ag+Ec8O	*A. galli* (primary infection) given as oral dose of 500 embryonated eggs + *E. coli* (secondary infection) given as oral dose of 10^8 cfu.6		No pathological findings
Ag+Ec8T	*A. galli* (primary infection) given as oral dose of 500 embryonated eggs + *E. coli* (secondary infection) given as tracheal dose of 10^8 cfu.	6	1 animal with fibrinopurulent polyserositis 1 animal with pericarditis and purulent double sided pneumonia 1 animal with pericarditis and double sided airsacculitis
Ec4O	*E. coli* given as oral dose of 10^4 cfu.	5	No pathological findings
Ec4T	*E. coli* given as tracheal dose of 10^4 cfu.	6	No pathological findings
Ec8O	*E. coli* given as oral dose of 10^8 cfu.	6	No pathological findings
Ec8T	*E. coli* given as tracheal dose of 10^8 cfu.	6	2 animals with pericarditis and double sided airsacculitis 1 animal with polyserositis and right sided pneumonia, † 1 animal with polyserositis and double sided pneumonia,†
Control	Uninfected control	6	No pathological findings

† the animal died during the first week of the infection trial.

coli given as a tracheal infection with 10^8 bacteria had a significantly lower (p<0.05) weight gain compared to all the other groups. But also the groups infected with *E. coli* given as an oral or tracheal infection with a dose of 10^8 bacteria and the group given *A. galli* and subsequently *E. coli* with 10^4 bacteria had a significantly lower weight gain (p<0.05) at time of slaughter compared to the control group.

Based upon the overall results obtained in one-week-old chickens, which are more susceptible to *E. coli* infections, 10^8 *E. coli* was chosen as

the dose of infection in both oral and tracheal infections and four-week-old chickens were used instead (*Dho-Moulin and Fairbrother* 1999).

Final experiments

In the second experiment *A. galli* was given as the primary infection followed by *E. coli*. The results of the second experiment are outlined in Table 2. Pathological lesions consistent with *E. coli* infections were seen in the group infected with *A. galli* followed by *E. coli* given as a tracheal dose of 10^8 cfu. in addition to the two groups infected by oral or tracheal route with

only *E. coli*. Pathological lesions were observed in four, one and four chickens in these groups, respectively. Pathological lesions were not seen in the group infected with only *A. galli* or in the groups infected with first *A. galli* and subsequently with *E. coli* given as an oral dose of 10^8 cfu.

Mortality was encountered in the groups infected with *E. coli* given as an oral or tracheal infection and in the group with combined *A. galli* and tracheal *E. coli* infection. Mortality due to cannibalism was seen in the control group. A chi-square analysis for differences in mortality showed no significant differences be-

Table 2. Number of chickens, parasitic (*A. galli*), bacterial (nalidixic-acid-resistant O78 *E. coli*) and pathological findings of second experiment with primary *A. galli* infections superimposed by secondary *E. coli* infections.

Group	Type, route and dose of infection	Group size	No of dead animals during experiment	Post-mortem findings at slaughter		
				Pathological changes	Worm burden (±S.D.)	Re-isolation of *E. coli*
Ag	Oral 500 *A. galli* eggs	25	0	25 neg.	5.6±11.0	25 neg.
Ag+Ec8O	Oral 500 *A. galli* eggs + oral *E. coli* with 10^8 cfu	37	0	37 neg.	14.0±18.0	37 neg.
Ag+Ec8T	Oral 500 *A. galli* eggs + tracheal *E. coli* with 10^8 cfu	38	1[1]	1 PS + PC + AS, 1 FPPS +LNC, 1 FPPC, 34 neg.	10.0±14.0	37 neg.
Ec8O	Oral *E. coli* with 10^8 cfu	25	1[2]	24 neg.	0	24 neg.
Ec8T	Tracheal *E. coli* with 10^8 cfu	25	4[3]	21neg.	0	21neg.
Control	Uninfected control	25	3[4]	22 neg.	0	22 neg.

AS=airsacculitis; FP=fibrinopurulent; L=liver; neg=negative; NC=necrosis; PC=pericarditis; PS=polyserositis; SP=spleen;
[1] One animal died after the 2nd infection testing positive for a nalidixic-acid-resistant *E. coli* in liver and spleen and with necrosis of the spleen.
[2] One animal died after first infection with polyserositis, but was negative for bacteriology.
[3] Four animals died after the infection with *E. coli*; three animals tested positive for a nalidixic-acid-resistant *E. coli* in liver and spleen. Of these two animals had fibrinopurulent pericarditis, one had polyserositis and the remaining *E. coli* negative chicken had fibrinopurulent salpingitis.
[4] Three animals died due to cannibalism, but had no other pathological findings.

Table 3. Number of animals, parasitic (*A. galli*), bacterial (nalidixic-acid-resistant O78 *E. coli*) and pathological findings of third experiment with primary *E. coli* infection superimposed by secondary *A. galli* infection

Group	Type, route and dose of infection	Group size	No of dead animals during experiment	Post-mortem findings at slaughter		
				Pathological changes	Worm burden ($\pm$S.D.)	Re-isolation *of E. coli*
Ag	Oral 500 *A. galli* eggs	20	0	20 neg.	0.8$\pm$1.4	20 neg.
Ec8O+Ag	Oral *E. coli* with 10^8 cfu + oral 500 A. galli eggs	21	0	21 neg.	0.3$\pm$0.5	21 neg
Ec8T+Ag	Tracheal *E. coli* with 10^8 cfu + oral 500 *A. galli* eggs	21	$7^{1,4}$	14 neg.	0.1$\pm$0.3	14 neg.
Ec8O	Oral *E. coli* with 10^8 cfu	21	0	21 neg.	0	21 neg.
Ec8T	Tracheal *E. coli* with 10^8 cfu	21	$6^{2,4}$	15 neg.	0	15 neg.
Control	Uninfected control	20	0	20 neg.	0^3	20 neg.

[1] Seven animals died after the second infection with *A. galli*. All were positive for a nalidixic_acid_resistant O78 *E. coli* and had extensive pathological changes, all with airsacculitis and fibrinopurulent pericarditis.
[2] Six animals died after the first infection with *E. coli*. All were positive for a nalidixic_acid_resistant O78 *E. coli* and five chickens had pathological with airsacculitis and fibrinopurulent pericarditis. One chicken had no pathological changes.
[3] Few larvae were recovered in the uninfected groups.
[4] Significantly more animals died compared to the remaining groups.

tween the groups (p>0.05). Pure isolates of *E. coli* were obtained from liver and spleen from the group with combined *A. galli* and tracheal *E. coli* infection and in the group infected with only tracheal *E. coli*. At slaughter, *A. galli* larvae were isolated from the three groups initially infected with *A. galli*. A significantly lower worm burden was seen in the *A. galli* group compared to the combined groups (p<0.01). It was not possible to recover the nalidixic-acid-resistant O78 stain used for inoculation of the birds at time of slaughter.

The mean weight gains for all groups are given in Figure 2. After the first infection with *A. galli* until the second infection a slight weight depression was seen in all groups including the control group. An analysis of variance between all groups at time of the first and second infec-tion showed no significant difference between the groups (p>0.05). However, after the second infection (and for the remaining time of the experiment), with *E. coli* given as a tracheal or oral infection, a significantly lower weight gain was seen in these groups compared to all other groups (p<0.05).

In the third experiment *E. coli* was given as the primary infection followed by *A .galli*. The results of the third experiment are outlined in Table 3. Pathological changes due to *E. coli* were only found in the two groups given *E. coli* by tracheal route, one of which was additionally infected with *A. galli*. In this group seven animals died after the secondary infection with *A. galli,* while only six chickens died in the group infected tracheally with *E. coli*. All were positive for *E. coli* and had extensive pathological

changes corresponding to *E. coli* infections. A $\varkappa^2$-analysis showed that a significantly higher number of animals died in these two groups compared to the others (p=0.056).

At slaughter larvae were recovered from all groups infected with *A. galli.* A t-test revealed that there were significantly lower worm burdens in the combined infection groups compared to the group only infected with *A. galli* (p<0.05). The nalidixic-acid-resistant O78 strain used for inoculation of the birds was not recovered at the time of slaughter.

The mean weight gains are given in Figure 3. A weight depression was seen for the two groups infected with *E. coli* given as a tracheal primary infection. Additional weight loss was observed

for the group infected additionally with *A. galli.* An analysis of variance between all groups at the time of the first infection showed no significant difference in weight gain between the groups (p>0.05). However, after the infection with *E. coli* there was a significant difference between the groups infected first with *E. coli* by tracheal route and the group infected secondly with *A. galli* (p<0.05) compared to the other groups. The weight gain for the group infected with only with *E. coli* by tracheal route was significantly lower (p<0.05) two weeks after infection. At the time of slaughter there was a significant difference between the group tracheally infected with *E. coli* followed by *A. galli* compared to the other groups (p<0.05) whereas the
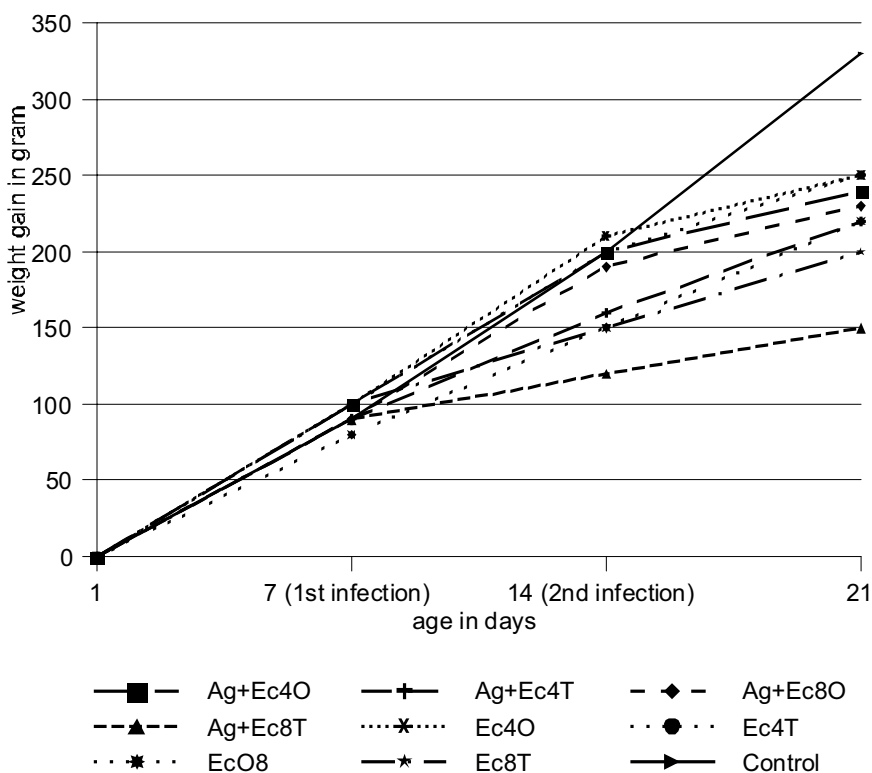

Figure 1. Average weight gain of the 9 groups in experiment 1 (determination of dose and infection route of *E. coli*) where Ag = *A. galli,* Ec= *E. coli,* O=oral, T=tracheal, 4=10^4 cfu and 8=10^8 cfu.

single infected *E. coli* group had a weight gain similar to the other groups (p>0.05).

Discussion

In total, three experiments were carried out to examine the effect of various combinations of *A. galli* and *E. coli* infections in growing chickens of layer type. Characteristic pathological lesions due to *E. coli* were seen in all the groups tracheally infected with *E. coli* as previously described by *Dho-Moulin and Fairbrother* (1999) and *Nakamura et al.* (1985), while lesions were absent in those inoculated orally. Pathological lesions were not observed in relation to the *A. galli* infection. This is presumably due to the rather low worm burdens observed in

the chickens (*Ikeme* 1971, *Permin et al.* 1997a). The combined infections of *E. coli* and *A. galli* did not produce more pathological lesions, which is unexpected as simultaneous parasitic infections often create more severe pathological lesions (*Christensen et al.* 1987). However, a trend for increased mortality rates was seen in the groups infected with the two pathogens, but it was not confirmed statistically.

Significantly different worm burdens were isolated from the intestinal tract of the *A. galli* and *E. coli* infected groups compared to the *A. galli* infected groups. With *A. galli* given first followed by an oral or a tracheal *E. coli* infection, significantly higher worm burdens were observed in both groups. *Johnson and Reid* (1973)
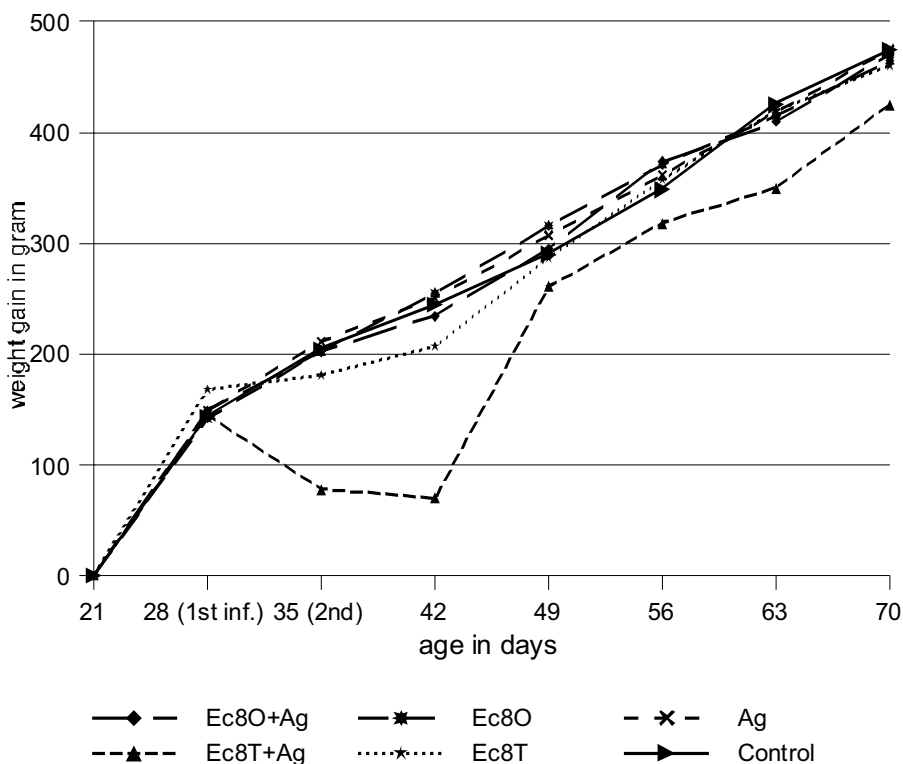

Figure 2. Weight gain in final (2nd experiment) where *A. galli* was given as the primary infection and *E. coli* was given as the second infection and where Ag = *A. galli,* Ec= *E. coli,* O=oral, T=tracheal and 8=10^8 cfu.

had similar results on the establishment of *A. galli* when chickens were infected with *Bacillus subtilis* and *B. cereus.* With a tracheal or oral *E. coli* infection given first followed by an *A. galli* infection the opposite situation was observed. Other studies have shown, that in antibiotic-sterilized chickens, the presence of *B. mesentericus, B. megatherium* and *Lactobacillus acidophilus* in the intestine inhibited the establishment of *A. galli* (*Stefanski and Przyjakowski* 1967) whereas *Okulewicz and Zlotorzycka* (1985) showed that *A. galli* inhibited the natural bacterial micro flora of the intestine of hens. The mechanisms for these phenomena are not known, but possibly related to the development of immunity. A recent paper by *Pritchard*

and Brown (2001) has indicated that although cellular response mechanisms of bacteria and parasites are related to each their pathway (Th2 for parasites and Th1 for bacteria) there is a balance between the two pathways. Thus a parasite infection might favour the Th2 cell development and indirectly suppress the establishment of bacteria, or vice-versa.

Furthermore, lower worm burdens were detected in the third experiment which ran for additional weeks. Similar observations were made by *Tongson and McCraw* (1967) where a nonspecific age related immunity developed in growing chickens around the age of 3 months. The mechanism could be a self-cure mechanism as recently described in chickens in rela-
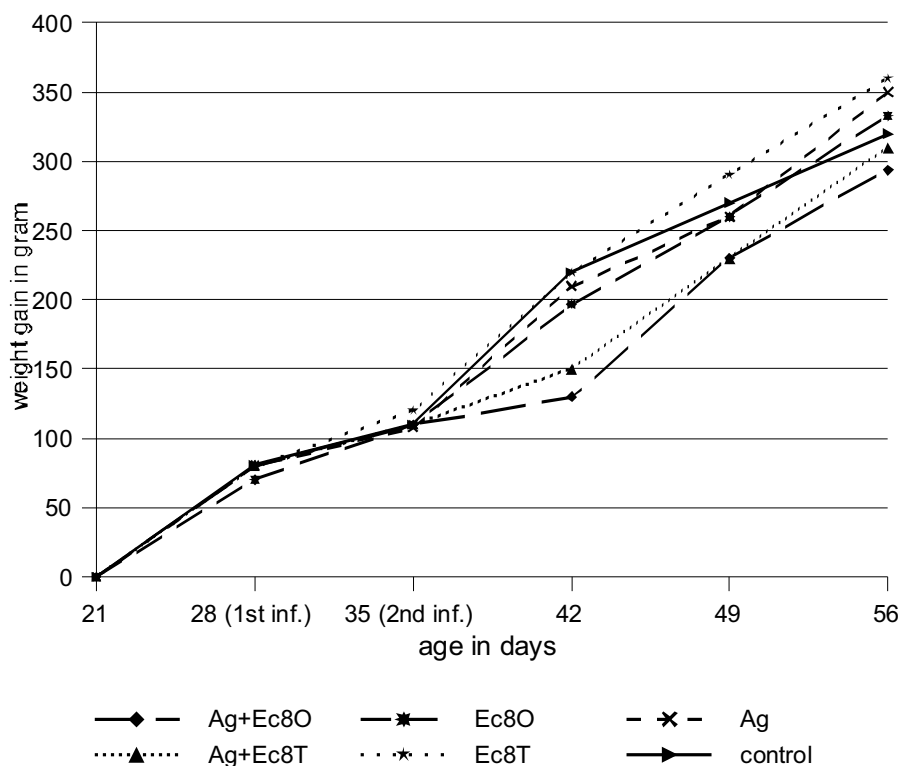

Figure 3. Weight gain in final experiment (3rd experiment) where *E. coli* was given as the primary infection and *A. galli* was given as the second infection and where Ag = *A. galli,* Ec= *E. coli,* O=oral, T=tracheal and 8=10^8 cfu.

tion to *A. galli* (*Permin and Ranvig* 2001). *Balic et al.* (2000) discussed the expulsion of trichostrongyle nematodes after primary infections in rodents speculating that the mechanism behind is genetically related as also described by *Behnke et al*. (1992). The expulsion is mainly seen in rodents and not in larger animals (*Balic et al.* 2000).

The nalidixic-acid-resistant *E. coli* strain was only isolated from the chickens which died during the experiment and not from any of the animals at slaughter. Similar findings have been reported by *Leitner and Heller* (1992), who could not isolate an orally inoculated nalidixic-acid-resistant O78 from the trachea or from the blood in stressed turkeys. However, in orally infected broiler chickens, stress resulted in bacteremia and mortality.

In the experiments significantly lower weight gains were seen in the groups given *E. coli* as a tracheal infection. Weight depression as a result of tracheal *E. coli* infections is in accordance with the findings of other researchers (*Dho-Moulin and Fairbrother* 1999). It was further shown that the combined infection with *A. galli* had a significant added negative impact on weight gain. Interestingly a primary infection with *A. galli* followed by an oral infection with *E. coli* also had a significantly negative impact on the weight gain.

Young birds (4-8 weeks) may have a brief period of anorexia and depression after infection with *E. coli* followed by acute septicaemia with mortality. However, weight depression was also seen after an oral *E. coli* infection when *A. galli* eggs were given as the primary infection. This may be related to damage of the intestinal mucosa leading to loss of blood and, probably, establishment of a secondary infection such as *E. coli* (*Herd and McNaught* 1975). Likewise, infections with *A. galli* have been reported to cause reductions in the growth rate, weight loss and mortality in broilers (*Ackert and Herrick*

1928, *Reid and Carmon* 1958, *Ikeme* 1971, *He et al.* 1990). The severity of the intestinal lesions may depend on the number of worms established in the intestine (*Ikeme* 1971). However, in this study only moderate weight losses were seen due to the parasite and only in the very young birds (1-3 weeks) whereas the older birds apparently were able to compensate for the infection. This is in contrast to earlier findings (*Ackert and Herrick* 1928, *Reid and Carmon* 1958). *Permin and coworkers* (unpublished) have observed similar findings in growing chickens where the animals apparently compensated for the loss due to the parasites by an increased feed intake.

The findings of this study indicate a negative relationship between concurrent infections of *E. coli* and *A. galli*. The mechanisms behind the observed relationship are not known, but might be related to immune mechanisms (*Pritchard and Brown* 2001). *Leitner and Heller* (1992) investigated the potential of pathogenic *E. coli* to penetrate the bloodstream via the intestinal mucosa in normal and stressed turkeys and chickens, but did not examine this in relation to stress caused by parasites. Their studies showed that, in orally infected turkeys, the pathogenic bacteria (a nalidixic-acid-resistant O78) remained in the intestine where it replaced 10% to 50% of the native coliform flora. But in orally infected broiler chickens, stress resulted in bacteremia and mortality. In our study significant weight depressions were seen in the orally infected chickens, which indicates that *A. galli,* when given as a primary infection, has an damaging effect on the intestinal mucosa (*Herd and McNaught* 1975) enabling *E. coli* to establish when it is given as an oral infection. However, an increased mortality was not seen. An additional effect of *A. galli* was seen in the group secondarily infected with *A. galli.* This could be related to an immunosuppressive effect of *A. galli* (*Malviya et al.* 1988, *Sharma* 1997, *Roepstorff et al*. 1999).

Conclusion

In conclusion combined infections of *A. galli* and *E. coli* can have a significant impact on confined and free-range chickens keeping in mind that both infections are common in such production systems. Further studies are needed in order to determine the underlying mechanisms of combined infections of *A. galli* and *E. coli* in growing and adult chickens.

Acknowledgements

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