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**Infection and excretion of Salmonella Enteritidis in two different chicken lines with concurrent Ascaridia galli infection.**

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Infection and excretion of *Salmonella* Enteritidis in two different chicken lines with concurrent *Ascaridia galli* infection.

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Infection and excretion of *Salmonella* Enteritidis in two different chicken lines with concurrent *Ascaridia galli* infection.

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### Abstract

Studies on the impact of interaction of *Salmonella enterica* serovar Enteritidis and the parasitic nematode *Ascaridia galli* with the avian host were undertaken with particular emphasis on infection and excretion of these pathogens in two different layer lines. A total of 148 salmonella free day-old chickens (73 Hellevad and 75 Lohmann Brown) were randomly divided into five groups for each line. Group 1 served as an uninoculated control group. Groups 2 and 3 were infected with *A. galli* and *S. Enteritidis*, respectively. Group 4 was first infected with *S. Enteritidis* and subsequently with *A. galli* and *vice-versa* for group 5. The number of chickens excreting *S. Enteritidis* was significantly higher ( $p < 0.001$ ) in the groups infected with both *S. Enteritidis* and *A. galli* compared with those only infected with *S. Enteritidis* over time. Furthermore, over time excretion of *S. Enteritidis* was significantly higher ( $p < 0.001$ ) in the group first infected with *S. Enteritidis* and subsequently with *A. galli* compared with the group infected in the reverse order. No significant differences were observed between the two lines concerning excretion of *S. Enteritidis* over time in any groups ( $p = 0.61$ ,  $p = 0.73$ ,  $p = 0.31$ ). *A. galli* established itself significantly better ( $p = 0.02$ ) in the group first infected with *A. galli* and subsequently with *S. Enteritidis* compared to the group infected in the reverse order. Furthermore, the *A. galli* infection rate was significantly higher ( $p = 0.02$ ) in Hellevad chickens compared to Lohmann Brown chickens at the end of the experiment.

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**Keywords:** Free-range-chickens,  
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 interactions

## Introduction

Danish table egg production has traditionally been based on battery cage and deep litter production systems. However, today table eggs from free-range and organic systems account for approximately twenty per cent of the marketed eggs in Denmark as a result of the increased interest for organic products and improved animal welfare (Anonymous, 2005a). Free-range chickens are regularly infected with endoparasites (Permin *et al.*, 1999) and may also more easily acquire a range of bacterial and viral diseases because of their free access to out-door areas and resulting lack of biosecurity compared to conventional indoor systems where a high level of biosecurity can be obtained (Christensen *et al.*, 1999; Permin *et al.*, 2002). In addition, more effective cleaning and disinfection between stocks can be achieved in conventional indoor systems.

In relation to public health, *Salmonella enterica* serovar Enteritidis represents one of the most important bacterial infections in layers, and is one of the most frequently isolated serotypes of *Salmonella* from poultry. Introduction of the European Directive on food-borne zoonoses (Anonymous, 1992) and national legislation has led to considerable reduction in the number of *Salmonella* infected flocks in Denmark (Anonymous, 2005b) which has been translated into a reduction in the number of cases of food poisoning due to *S. Enteritidis* in Denmark (Anonymous, 2005b). *S. Enteritidis* rarely results in disease in adult chickens. However, the asymptomatic colonization of the alimentary tract of poultry may result in human food poisoning cases associated with consumption of contaminated eggs.

*Ascaridia galli* infection is commonly observed in layers in conventional indoor, as well as in free-range and organic systems (Permin *et al.*, 1999). Its short direct life cycle and the resistance of its eggs favour infections under floor and free-range production systems (Permin *et al.*, 1998a) where the chickens are not separated from their faeces. A cross-sectional prevalence study of

gastrointestinal helminths in Denmark has shown that the flock prevalence of *A. galli* is 100% in free-range and organic systems compared to 25% in confined indoor deep litter production systems (Permin *et al.*, 1999). Furthermore, it has been shown that the eggs of *A. galli* might transfer *Salmonella* (Chadfield *et al.*, 2001) thus representing an increased risk of the persistence of *Salmonella* in the environment.

Recent studies of interactions between *A. galli* and *Pasteurella multocida* or *Escherichia coli* infections in chickens have shown that dual infections favour the establishment of bacterial infections and result in more severe disease than is seen with the bacterial infection alone (Dahl *et al.*, 2002; Permin *et al.*, 2006). Although interactions between gastrointestinal helminths and other pathogens appear to be important, little research has so far been carried out within this field. Control of food-borne human salmonellosis inevitably includes control of the infectious agent in the animal host. Thus, understanding the mechanisms of *Salmonella* infection, including intestinal colonisation, invasion, excretion and persistence in layers dually infected with *A. galli* is essential in order to identify appropriate measures to reduce infection of flocks and public health risk. The objective of the present study was to investigate the interaction of dual infections of *A. galli* and *S. Enteritidis* on the colonization and infection with these agents, including subsequent excretion and persistence of *S. Enteritidis* in two different layer lines. Layer lines used for free-range table egg production, including Lohmann Brown, have been selected for high performance in individual cages without considering their ability to adapt to free-range and organic production systems. In addition to Lohmann Brown chickens, this study included Hellevad chickens, which are assumed to be better adapted to organic production systems (Worm, 2003).

## Materials and Methods

**Experimental animals and housing facilities.** Hellevad (a cross of New Hampshire and White Leghorn lines) and Lohmann Brown chickens were used in the study (Worm, 2003). Seventy-three day-old Hellevad chickens were obtained from a small Danish hatchery while 75 day-old Lohmann Brown chickens were obtained from a commercial hatchery. Both hatcheries and parent flocks tested negative under the Danish *Salmonella* Control Programme (Feld *et al.*, 2000; Wegener *et al.*, 2003). Before infection chickens were placed together in a cleaned and disinfected house. They were neither vaccinated nor beak trimmed. Before and throughout the experiment, the chickens had free access to water and a standard commercial antibiotic-free premixed starter feed containing a coccidiostat (monensin-natrium) at a concentration of 100 mg/kg. At 11 days of age the chickens were wing-banded and randomly allocated into five groups of 15 for each line with the exception of two groups of Hellevad chickens which consisted of 14 chickens. Each group was placed in a clean, disinfected house with a floor area of 6 m<sup>2</sup> and access to an outside pen by day. Three days prior to the primary infection at the age of 19 days, the *Salmonella* status of all chickens was evaluated by examining pools of faecal swabs for each group by standard bacteriological culture procedures performed as described later under bacteriological examinations.

**Experimental design.** The experiment was designed as a 2 x 2 + 1 cohort study (Thrusfield, 1995) in which group 1 for each line was kept as uninfected controls. Groups 2 and 3 were infected with *A. galli* and *S. Enteritidis*, respectively. Group 4 was first infected with *S. Enteritidis* and one week later with *A. galli* and *vice-versa* for group 5. The experiment was terminated 70 days after the primary infection.

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**Infections.** *S. Enteritidis* phage type 4, isolated from a naturally infected Danish commercial layer flock was used in this study (Aabo *et al.*, 2002). It was stored in Luria Bertani (LB) broth containing 15% glycerol at  $-80^{\circ}\text{C}$ . The strain was cultured aerobically overnight at  $37^{\circ}\text{C}$  on blood agar base (Oxoid, Denmark) with 5% citrated bovine blood. Five colonies were subsequently inoculated into LB broth and incubated aerobically overnight at  $37^{\circ}\text{C}$  with shaking. The challenge inoculum was prepared from the overnight broth culture which was serially diluted with physiological saline to a concentration of approximately  $1 \times 10^6$  colony-forming units (cfu) of bacteria per 0.1 ml. Confirmation of inoculate doses was verified by viable counts of serial tenfold dilutions on LB agar.

*A. galli* eggs were isolated from the uterus of mature worms obtained from *Salmonella*-free naturally infected commercial layers. Embryonated eggs were prepared by cultivation of the eggs in 0.1 N sulphuric acid as described previously (Permin *et al.*, 1997b). Prior to infection the infectivity of the eggs were assessed visually by motility of larvae.

The experimental design is outlined in Table 1. All chickens in group 1 (control group) were sham infected orally with 0.1 ml 0.9% saline at the age of 19 days and 26 days. Chickens in group 2 were infected orally with 1000 +/- 50 infective *A. galli* eggs at the age of 19 days. All chickens in group 3 were infected orally with  $1 \times 10^6$  cfu *S. Enteritidis* at the age of 19 days. Both groups 2 and 3 were sham infected orally with 0.1 ml 0.9% saline at the age of 26 days. Group 4 was infected with  $10^6$  cfu *S. Enteritidis* at 19 days old and 7 days later with 1000 +/- 50 infective *A. galli* eggs and *vice-versa* for group 5. The same dose of *A. galli* infective eggs was used in a comparable study with concurrent infections with *Pasteurella multocida* and *A. galli* (Dahl *et al.*, 2002). The specific age of the chickens was chosen to ensure intestinal colonization of *S. Enteritidis* without significant morbidity or mortality (Gast and Beard, 1989; Gorham *et al.*, 1991) and the two pathogens were

given with one week interval to ensure a possible host response to the primary infection before the secondary infection.

**Body weight.** The body weight of individual chickens was recorded at 0, 7, 14, 21, 35, 49, 63 and 70 days after the primary infection.

**Bacteriological Examinations.** To assess faecal excretion of *S. Enteritidis* cloacal swabs were collected weekly from each chicken for bacteriology. The swabs from groups 1 and 2 (*S. Enteritidis* non-infected groups) were pooled in groups of five for each chicken line and each group but bacteriological examination was performed individually on chickens in groups 3, 4 and 5 (*S. Enteritidis* infected groups). The first time swabs were collected from group 5, these were pooled as for group 1 and 2. All samples were checked for *Salmonella* using a method originally described by De Smedt (1986) with minor modifications. Briefly, after overnight incubation in buffered peptone water (BPW) (Merck, Germany) at 37°C, three drops of the pre-enrichment culture was inoculated onto Modified Semisolid Rappaport-Vassiliadis (MSRV) (Oxoid, Denmark) agar plates, and incubated at 42°C for 18-24 hours. Bacteria putatively identified as *Salmonella* on MSRV, were subcultured on modified Brilliant Green Agar (Oxoid, Denmark) and incubated overnight at 37°C, followed by plating of typical colonies on blood agar incubated overnight at 37°C. Suspect *Salmonella* colonies were finally confirmed by slide agglutination using polyvalent anti-*Salmonella* serum (SSI, Copenhagen, Denmark). At the end of the experiment, selected isolates were serotyped according to Kauffmann (1972) for verification of *S. Enteritidis*.

**Parasitological examinations.** The excretion of *A. galli* eggs was monitored weekly from week 4 post infection by evaluating the faecal egg excretion using a modified McMaster method adapted to

detect minimum egg counts of 20 eggs per gram (EPG) of faeces (Permin and Hansen, 1998).

Faecal samples from groups 1 and 3 (*A. galli* non-infected groups) were pooled in one sample for each chicken line and each group at all sampling dates. Samples from the *A. galli* infected groups (groups 2, 4 and 5) were pooled in one sample for each group and each chicken line at the first sampling date (28 days p.i.). Thereafter, faecal samples were collected and examined as individual samples from the *A. galli* infected groups (groups 2, 4 and 5). For this procedure, each chicken was housed separately in a cage for a short time and fresh droppings were taken from the cage floor.

At the end of the experiment all chickens were sacrificed and subjected to *post mortem* examination during which all intestinal tracts were collected. The intestinal tracts were dissected longitudinally and the contents were washed in two sieves, the smallest with a mesh aperture of 38 µm. The sieve retentate was examined for the presence of mature and immature stages of *A. galli* using a stereomicroscope at 40x magnification. The numbers of immature and adult worms were counted and all adult worms were sexed by morphological parameters.

**Statistical analysis.** A repeated measurements model (Diggle, 1988) was chosen to investigate the effect of the different groups on the body weight gain for the two chicken lines and to investigate the possibility of an interaction between the treatment groups and the chicken lines and interaction between chicken lines and time and with baselines as covariates. To fit the model the raw weight data were log transformed. Data on *S. Enteritidis* excretion in the groups were analysed by logistic repeated measurement with individual weekly records as statistical units. The model included treatment groups and chicken line as main effect and chicken line and time as interactions.

A Kruskal-Wallis test was performed to compare the worm burden (larvae + adult *A. galli*) between the three *A. galli* infected groups, separately for Hellevad and Lohmann Brown chickens, respectively. The Kruskal-Wallis test was chosen due to non normal distribution of the raw data

with no apparent way to transform to Gaussian distributed data. Logistic regression was carried out to investigate the probability for *A. galli* to establish and survive in the intestines of the chickens being related to the main effects of treatment groups and chicken lines and the possibility that there is an interaction between the main effects.

## Results

**Weight gain.** All chickens gained weight during the experimental period and no interactions between chicken line and treatment groups were observed ( $p = 0.62$ ). For both lines the control group (group 1) performed significantly ( $p < 0.05$ ) better than the groups infected with either *A. galli* (group 2) or *S. Enteritidis* (group 3) and the group infected first with *S. Enteritidis* and subsequently with *A. galli* (group 4). For both lines no significant difference ( $p = 0.06$ ) was demonstrated between the control group (group 1) and the group infected first with *A. galli* and subsequently with *S. Enteritidis* (group 5) or between the groups dually infected with *S. Enteritidis* and *A. galli* in reverse order ( $p = 0.1078$ ) (data not shown).

**Excretion of *S. Enteritidis*.** Before inoculation, no *Salmonella* bacteria were detected in the pooled cloacal swabs from any of the groups. In addition, *Salmonella* was not isolated from any cloacal swabs from group 1 (control group) or group 2 (infected with *A. galli* only) throughout the experiment.

Faecal excretion rates of *Salmonella* for the groups inoculated with *S. Enteritidis* are given in Table 2. The number of chickens excreting *S. Enteritidis* in each group varied considerably between sampling dates. The number of birds excreting was higher in groups 4 and 5 (infected with *S. Enteritidis* and *A. galli*) and the birds eliminated the infection more slowly than those in group 3

(infected with *S. Enteritidis* only) for both lines. The highest *Salmonella* shedding rate for Hellevad chickens in group 3 was 40%, and excretion stopped between 49 and 56 days after infection. In the same group the highest shedding rate for Lohmann Brown chickens was less than 30% and excretion stopped between days 28 and 35 post infection, except for a single chicken. The highest percentage of chickens shedding *Salmonella* (100%) and the longest excretion time (i.e. to the end of the experiment) was observed for those infected with *S. Enteritidis* at the age of 19 days and subsequently infected with *A. galli* at the age of 26 days (group 4). The Lohmann Brown chickens given the dual infection in the reverse order (group 5) appeared to stop excreting *Salmonella* by 56 p.i. and only one Hellevad chicken was excreting *Salmonella* by 63 days p.i. In total, excretion of *S. Enteritidis* was demonstrated in 46.7% (7/15), 100% (14/14) and 100% (14/14) of Hellevad chickens from groups 3, 4 and 5, respectively, compared with 40.0% (6/15), 100% (15/15) and 100% (15/15), respectively, for Lohmann Brown chickens. No statistically significant difference was observed between Hellevad and Lohmann Brown chickens in any of the groups ( $p=0.61$ ,  $p=0.73$ ,  $p=0.31$ ).

A statistically significant difference ( $p<0.001$ ) in the incidence of faecal excretion was observed between group 3 and groups 4 and 5 for both lines. Furthermore, the incidence of faecal excretion was statistically higher ( $p<0.001$ ) for the group infected first with *S. Enteritidis* and subsequently with *A. galli* (group 4) than the group infected in the reverse order (group 5) for both lines.

***A. galli* egg excretion.** As there was no obvious way of transforming the raw EPG data to produce acceptable normally distributed data no statistical test was performed for comparison of groups or lines. Control chickens (group 1) and chickens infected only with *S. Enteritidis* (group 3) remained negative for *A. galli* eggs in the pooled faecal samples throughout the experiment. All pooled

samples from the *A. galli* infected groups (groups 2, 4 and 5) taken 28 days p.i. were negative. The mean EPG and the percentage of positive samples in the different groups on the sampling dates is given in Table 3. Positive faecal egg counts (FEC) were detected the first time individual samples were taken (35 days p.i.) in group 2 for both lines and for Hellevad chickens in group 5. The Lohmann Brown chickens in group 5 had the first positive FEC 42 days p.i. In group 4, the Hellevad chickens were detected positive for the first time 49 days p.i., while no Lohmann Brown chickens were detected positive at any sampling dates. In total, 13.3% (12/90), 12.9% (9/70) and 29.3% (25/84) of the samples from the Hellevad chickens were found positive for *A. galli* eggs in groups 2, 4 and 5, respectively. *A. galli* eggs were shed on at least one sampling date in 40.0%, 35.7% and 71.4% of the Hellevad chickens in group 2, 4 and 5. In total, 17.8% (16/90), 0% (0/90) and 18.9% (17/90) of the droppings from the Lohmann Brown chickens were found positive for *A. galli* eggs in groups 2, 4 and 5, respectively. *A. galli* eggs were shed on at least one sampling date in 46.7% and 60.0% of the chickens in group 2 and 5 for Lohmann Brown chickens.

**Worm counts.** Occasionally, expelled worms were found in faecal droppings of the chicken. At slaughtering, adult female worms were harboured in 20.0% (3/15), 21.4% (3/14) and 57.1% (8/14) of the Hellevad chickens of groups 2, 4 and 5, respectively and in 5/15 (33.3%), 2/15 (13.3%) and 5/15 (33.3%) of the Lohmann Brown chickens of groups 2, 4 and 5, respectively. The total numbers of *A. galli* (larvae + adult) recorded at the end of the experiment are shown in Table 4. There was great variability in the number of worms per chicken. The Kruskal-Wallis test showed no significant differences ( $p=0.07$ ) in the total number of *A. galli* (larvae + adults) between the three Hellevad groups infected with *A. galli*. Likewise, no significant difference ( $p=0.40$ ) was observed between the three Lohmann Browns groups infected with *A. galli*. However, logistic regression showed that the probability of *A. galli* to establish was significantly higher ( $p = 0.02$ ) in the group

that was first infected with *A. galli* and subsequently with *S. Enteritidis* (group 5) compared with the group infected in the reverse order (group 4) and *A. galli* established and survived differently in Hellevad and Lohmann Brown chickens, independently of the groups, as the infection rate of *A. galli* to establish was significantly higher ( $p = 0.02$ ) in Hellevad chickens compared to Lohmann Brown chickens. No interaction between chicken line and treatment group was demonstrated ( $p = 0.54$ ). In the *A. galli* infected groups no other gastrointestinal parasites except *A. galli* were demonstrated. All chickens in the non-infected groups remained free of *A. galli* and other gastrointestinal parasites throughout the experimental period.

**Clinical observations, mortality and pathological changes.** No signs of clinical disease were observed during the study. During the observation period no mortality was observed in any group and gross post-mortem lesions were not observed at necropsy. Two Hellevad chickens from group 3 escaped from the outside pen before the experiment was finished.

## Discussion

Neither clinical signs nor mortality were observed in Hellevad and Lohmann Brown chickens infected with *S. Enteritidis* in the present study. This confirms previous studies (Gast and Beard, 1989; Gorham *et al.*, 1991) showing that susceptibility to disease of chickens infected with paratyphoid *Salmonella* spp. decreases rapidly during the first week after hatching. In addition, no clinical signs and gross lesions were observed in the *A. galli* and control groups, or in the groups with dual infection of *S. Enteritidis* and *A. galli*. Low level infections with *A. galli* do not normally cause clinical signs (Permin *et al.*, 1998a). Reduction in growth rate has previously been associated with *A. galli* (Ackert and Herrick, 1928; Reid and Carmon, 1958; Ikeme, 1971a; Ikeme 1971b;

Permin *et al.*, 1998b) but in this study no clear conclusions could be drawn on the effect of *A. galli* on weight gain.

The low level of *Salmonella* infection observed in the Hellevad and Lohmann Brown chickens infected only with *S. Enteritidis* was also seen in a preliminary pilot study with a similar groups of animals (data not shown). In the present study both the percentage of chickens shedding *Salmonella* and the duration of excretion were significantly influenced by concurrent *A. galli* infection. For both lines of chicken the number of birds excreting *S. Enteritidis* was significantly higher in the group infected first with *S. Enteritidis* and subsequently with *A. galli* than in the group infected in the reverse order.

To the best of our knowledge this is the first experimental study to demonstrate that *A. galli* may play an important role in determining the outcome of a concurrent *Salmonella* infection. The consequences on the persistence of *Salmonella* in the environment through infected *A. galli* eggs (Chadfield *et al.*, 2001) are obvious but the mechanisms leading to an asymptomatic *Salmonella* carrier state and the higher rate of *Salmonella* shedding in the dual infected groups remains to be elucidated. Concurrent infections with *A. galli* and other bacteria have demonstrated that *A. galli* predisposed to infection and a subsequent carrier state of *P. multocida* and *E. coli* in chickens (Dahl *et al.*, 2002; Permin *et al.*, 2006). It is possible that this is related to a polarization of the immune response. The immune response of mammals is known to polarize into so-called type 1 (Th1) or type 2 (Th2) immune pathways depending on the type of pathogen encountered (Cox, 2001), and recently this has also been demonstrated for chickens (Degen *et al.*, 2005). Thus, a helminth infection might suppress the Th1 response and indirectly favour the establishment of bacterial infection and *vice versa*. However, it could also be speculated that lesions associated with the histotrophic phase of *A. galli* infection might facilitate intestinal colonization and persistence of subsequent bacterial infections. Infection with *Eimeria* spp. has been identified as a factor that



enhances the establishment and persistence of concurrent infection with *S. Typhimurium* in the intestinal tract of chickens (Stephens *et al.*, 1964; Stephens and Vestal, 1966; Arakawa *et al.*, 1981; Babe *et al.*, 1982) suggesting that *S. Typhimurium* persists in and penetrates the damaged mucosa of the intestine of the chickens infected with coccidia. For the same reason the histotrophic phase of *A. galli* may play an important role in developing a salmonella carrier state in chickens.

The mean worm burden demonstrated in all groups was relatively low with a mean establishment rate of less than 0.3% for all groups. This is somewhat lower than observed in comparable studies (Permin *et al.*, 1997a; Permin *et al.*, 1997b; Permin *et al.*, 1998b; Gauly *et al.*, 2001; Permin and Ranvig, 2001; Schou *et al.*, 2003; Gauly *et al.*, 2005; Permin *et al.*, 2006). It is possible that expulsion of worms had some impact on the mean worm burden as the proportion of chickens which had at least one positive FEC during the study period was significantly higher than the proportion harbouring female worms at slaughtering in all groups with the exception of Lohmann Brown chickens in group 4 where 13.3% (2/15) were found to harbour mature female worms at slaughtering, although all faecal samples were negative for parasite eggs. However, it is possible that the McMaster method used on the faecal samples was not sufficiently sensitive to detect some positive samples. The experiment should be repeated using more birds to support the results regarding the low number of *A. galli* positive birds and the low worm burden demonstrated.

The worm counts of *A. galli* (larvae + adults) were not significantly different between infected groups of Hellevad and Lohmann Brown chickens, however, the probability of establishing an *A. galli* infection was significantly higher for chickens first infected with *A. galli* and subsequently with *S. Enteritidis* compared to those infected in the reverse order. The observed difference might be due to lesions associated with early infection with *S. Enteritidis*. However, it is also possible that it is related to a polarization of the immune response directed by the sequence of infection. Thus, it is possible that a primary bacterial infection might suppress the Th2 response and indirectly favour

the establishment of a secondary helminth infection. In the studies involving *P. multocida* Dahl *et al.* (2002) showed that this bacterium had a significant impact on establishment of *A. galli*. Thus chickens first infected with *A. galli* and subsequently with *P. multocida* had a lower percentage of *A. galli* infected birds than those infected in the reverse order or those infected only with *A. galli*.

In addition to the impact of *S. Enteritidis* on the success of *A. galli* establishment it was shown that the probability for *A. galli* to establish infection was significantly higher in Hellevad chickens than Lohmann Brown chickens at the end of the experiment. This might indicate a difference in genetic resistance to *A. galli*, supporting earlier studies that found commercial chickens to be less susceptible to *A. galli* than more outbred chicken lines (Permin and Ranvig, 2001; Schou *et al.*, 2003) such as the Hellevad line. Based upon faecal shedding of *Salmonella*, no differences between the two lines were observed. A considerable amount of work has shown variations in resistance/susceptibility to colonization of intestines and caeca in outbred (Guillot *et al.*, 1995; Protais *et al.*, 1996; Duchet-Suchaux *et al.*, 1997) and inbred (Barrow *et al.*, 2004; Sadeyen *et al.*, 2004) lines of chickens infected with *S. Enteritidis*. Recently, heritability estimates for resistance to caecal colonization of *S. Enteritidis* have indicated genetic influences on carriage in the caeca (Beaumont *et al.*, 1999).

In conclusion, this appears to be the first experimental study demonstrating an interaction between *A. galli* and *S. Enteritidis* in chickens and suggests that under field conditions *A. galli* may increase the colonization rate and prolong the duration of faecal excretion of *S. Enteritidis* and increase the risk of persistence in the environment through infected *A. galli* eggs as previously reported (Chadfield *et al.*, 2001). Furthermore, this study indicated that some degree of genetic resistance against *A. galli* may exist in Lohmann Brown chickens compared to Hellevad chickens, while no difference with regard to faecal *Salmonella* excretion was observed. Control of *A. galli* in chickens may represent an important key to reduce the potential for spread of *Salmonella* infection.

Further research is needed to fully understand the mechanisms behind the interactions observed and genetic resistance to *A. galli* may improve the possibilities of preventing *S. Enteritidis* infections in humans.

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Table 1. Experimental design.

| Group number | Group name                                 | Group size                        | Age and infection(s)  |                       |
|--------------|--|-----------------------------------|-----------------------|-----------------------|
|              |  |                                   | 19 days               | 26 days               |
| 1            | Control                                    | 15 <sup>a</sup> + 15 <sup>b</sup> | None                  | None                  |
| 2            | <i>A. galli</i>                            | 15 <sup>a</sup> + 15 <sup>b</sup> | <i>A.galli</i>        | None                  |
| 3            | <i>S. Enteritidis</i>                      | 15 <sup>a</sup> + 15 <sup>b</sup> | <i>S. Enteritidis</i> | None                  |
| 4            | <i>S. Enteritidis</i> +<br><i>A. galli</i> | 14 <sup>a</sup> + 15 <sup>b</sup> | <i>S. Enteritidis</i> | <i>A. galli</i>       |
| 5            | <i>A. galli</i> + <i>S. Enteritidis</i>    | 14 <sup>a</sup> + 15 <sup>b</sup> | <i>A. galli</i>       | <i>S. Enteritidis</i> |

<sup>a</sup>Hellevad chickens  
<sup>b</sup>Lohman Brown chickens.

**Table 2.** Faecal excretion of *S. Enteritidis* following oral inoculation of Hellevad and Lohmann Brown chickens with *S. Enteritidis*

| Days p.i.<br>with <i>S.</i><br><i>Enteritidis</i> | Group 3 <sup>a</sup> : <i>S. Enteritidis</i> |      |               |      | Group 4 <sup>a</sup> : <i>S. Enteritidis</i> + <i>A. galli</i> |       |               |       | Group 5 <sup>a</sup> : <i>A. galli</i> + <i>S. Enteritidis</i> |        |               |        |
|---|--|------|---------------|------|--|-------|---------------|-------|--|--------|---------------|--------|
|   | Hellevad                                     |      | Lohmann Brown |      | Hellevad   |       | Lohmann Brown |       | Hellevad   |        | Lohmann Brown |        |
|   | Pos./15 <sup>b</sup>                         | %    | Pos./15       | %    | Pos./14  | %     | Pos./15       | %     | Pos./14  | %      | Pos./15       | %      |
| 7   | 1  | 6.7  | 0             | 0.0  | 5  | 35.7  | 3             | 20.0  | 7  | 50.0   | 10            | 66.7   |
| 14  | 1  | 6.7  | 1             | 6.7  | 5  | 35.7  | 9             | 60.0  | 13   | 92.9   | 14            | 93.3   |
| 21  | 0  | 0.0  | 1             | 6.7  | 14   | 100.0 | 15            | 100.0 | 13   | 92.9   | 10            | 66.7   |
| 28  | 6  | 40.0 | 4             | 26.7 | 14   | 100.0 | 15            | 100.0 | 10   | 71.4   | 8             | 53.3   |
| 35  | 1  | 6.7  | 0             | 0.0  | 13   | 92.9  | 15            | 100.0 | 4  | 28.6   | 5             | 33.3   |
| 42  | 2  | 13.3 | 0             | 0.0  | 11   | 78.6  | 10            | 66.7  | 6  | 42.9   | 4             | 26.7   |
| 49  | 1  | 6.7  | 0             | 0.0  | 9  | 64.3  | 10            | 66.7  | 5  | 35.8   | 1             | 6.7    |
| 56  | 0/13 <sup>c</sup>                            | 0.0  | 1             | 6.7  | 12   | 85.7  | 10            | 66.7  | 2  | 14.3   | 0             | 0.0    |
| 63  | 0/13 <sup>c</sup>                            | 0.0  | 0             | 0.0  | 5  | 35.7  | 4             | 26.7  | 1  | 7.1    | 0             | 0.0    |
| 70  | 0/13 <sup>c</sup>                            | 0.0  | 0             | 0.0  | 6  | 42.9  | 13            | 86.7  | (N.D.) <sup>d</sup>  | (N.D.) | (N.D.)        | (N.D.) |

<sup>a</sup>Chickens in group 3 and 4 were infected with *Salmonella* at 19 days of age; group 5 were infected 26 days of age.

<sup>b</sup>Number of chickens positive/number tested.

<sup>c</sup>13 chickens tested as two escaped on day 50 after *S. Enteritidis* inoculation.

<sup>d</sup>No data.

No significant difference in the number of positive cloacal swabs between the two lines in any of the groups ( $p=0.61$ ,  $p=0.73$ ,  $p=0.31$ ).

A significant difference in the number of positive cloacal swabs between group 3 and groups 4 and 5 for both lines over time ( $p<0.001$ ).

Significantly more positive cloacal swabs was demonstrated in group 4 compared with group 5 for both lines over time ( $p<0.001$ ).

**Table 3.** Parasitological parameters of Hellevad and Lohmann Brown chickens infected with 1000+/-50 embryonated *A. galli* eggs

| Parameter  | Group 2 <sup>a</sup> : <i>A. galli</i> |         |                 |         | Group 4 <sup>a</sup> : <i>S. Enteritidis</i><br>+ <i>A. galli</i> |        |                 |       | Group 5 <sup>a</sup> : <i>A. galli</i><br>+ <i>S. Enteritidis</i> |         |                 |        |
|--|--|---------|-----------------|---------|---|--------|-----------------|-------|---|---------|-----------------|--------|
|  | Hellevad                               |         | Lohman<br>Brown |         | Hellevad  |        | Lohman<br>Brown |       | Hellevad  |         | Lohman<br>Brown |        |
| Number of chickens                                       | 15                                     |         | 15              |         | 14  |        | 15              |       | 14  |         | 15              |        |
| Mean eggs/g in faeces<br>on days p.i.                    | Mean                                   | SD      | Mean            | SD      | Mean  | SD     | Mean            | SD    | Mean  | SD      | Mean            | SD     |
| 35   | 1.3                                    | (5.2)   | 8.0             | (31.0)  | 0.0   | (0.0)  | 0.0             | (0.0) | 17.1  | (64.1)  | 0.0             | (0.0)  |
| 42   | 21.3                                   | (77.3)  | 4.0             | (15.5)  | 0.0   | (0.0)  | 0.0             | (0.0) | 38.6  | (128.1) | 1.3             | (5.2)  |
| 49   | 17.3                                   | (56.5)  | 9.3             | (31.0)  | 4.3   | (11.6) | 0.0             | (0.0) | 15.7  | (36.1)  | 1.3             | (5.2)  |
| 56   | 13.3                                   | (51.6)  | 18.7            | (61.6)  | 17.1  | (25.8) | 0.0             | (0.0) | 48.6  | (133.1) | 18.7            | (32.5) |
| 63   | 34.7                                   | (118.2) | 49.3            | (123.7) | 22.9  | (66.0) | 0.0             | (0.0) | 87.1  | (123.9) | 37.3            | (61.8) |
| 70   | 38.7                                   | (144.3) | 20.0            | (52.9)  | (N.D.) <sup>b</sup>   |        | (N.D.)          |       | 107.1   | (114.0) | 30.7            | (61.3) |
| Percent positive faecal egg<br>counts (FEC) on days p.i. | %                                      |         | %               |         | %   |        | %               |       | %   |         | %               |        |
| 35   | 6.7                                    |         | 6.7             |         | 0.0   |        | 0.0             |       | 7.1   |         | 0.0             |        |
| 42   | 13.3                                   |         | 6.7             |         | 0.0   |        | 0.0             |       | 14.3  |         | 6.7             |        |
| 49   | 20.0                                   |         | 13.3            |         | 14.3  |        | 0.0             |       | 21.4  |         | 6.7             |        |
| 56   | 6.7                                    |         | 20.0            |         | 35.7  |        | 0.0             |       | 28.6  |         | 33.3            |        |
| 63   | 20.0                                   |         | 40.0            |         | 14.3  |        | 0.0             |       | 50.0  |         | 33.3            |        |
| 70   | 13.3                                   |         | 20.0            |         | (N.D.)  |        | (N.D.)          |       | 57.1  |         | 33.3            |        |

<sup>a</sup>The chickens in group 2 and 5 were *A. galli* infected at 19 days of age; group 4 were infected at 26 days of age.

<sup>b</sup>No data.

**Table 4.** Mean worm burden of *A. galli* (larvae + adult) in infected chickens at slaughter

|                              | Group 2         |                  | Group 4                                 |                  | Group 5                                 |                  |
|------------------------------|-----------------|------------------|---|------------------|---|------------------|
|                              | <i>A. galli</i> |                  | <i>S. Enteritidis</i> + <i>A. galli</i> |                  | <i>A. galli</i> + <i>S. Enteritidis</i> |                  |
|                              | Hellevad        | Lohmann<br>Brown | Hellevad                                | Lohmann<br>Brown | Hellevad                                | Lohmann<br>Brown |
| Number of chickens           | 15              | 15               | 14                                      | 15               | 14                                      | 15               |
| Number infected              | 8               | 5                | 6                                       | 4                | 12                                      | 7                |
| Mean worm burden (SD)        | 1.4 (2.5)       | 1.4 (2.1)        | 1.6 (2.5)                               | 0.5 (0.9)        | 2.7 (2.7)                               | 1.5 (2.1)        |
| Median (interquartile range) | 1 (0-2)         | 0 (0-4)          | 0 (0-3)                                 | 0 (0-1)          | 2 (0-2)                                 | 0 (0-1)          |
| Min – Max                    | 0 - 10          | 0 - 5            | 0 - 7                                   | 0 - 3            | 0 - 10                                  | 0 - 7            |