Population dynamics of *Ascaridia galli* following single infection in young chickens

TANIA FERDUSHY[1]*, LUZ ADILIA LUNA-OLIVARES[1], PETER NEJSUM[1], ALLAN KNUD ROEPSTORFF[1], STIG MILAN THAMSBORG[1] *and* NIELS CHRISTIAN KYVSGAARD[2]

[1] *Section for Parasitology, Health and Development, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlægevej 100, DK-1870, Frederiksberg C, Copenhagen, Denmark*
[2] *Section for Production and Health, Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 2, DK-1870, Frederiksberg C, Copenhagen, Denmark*

(*Received 5 December 2012; revised 2 February and 16 March 2013; accepted 18 March 2013; first published online 14 May 2013*)

SUMMARY

The population dynamics of *Ascaridia galli* was studied in 70 ISA Brown layer pullets, 42 of them were each experimentally infected with 500 embryonated *A. galli* eggs and 28 chickens were kept as uninfected controls. Six chickens from the infected group and 4 from the control group were necropsied at 3, 7, 10, 14, 21, 28 and 42 days post-infection (d.p.i.). The mean worm recovery varied from 11–20% of the infection dose with the highest recovery at 3 d.p.i. and the lowest at 21 and 42 d.p.i. ($P<0{\cdot}05$). More larvae were recovered from the intestinal wall than from the content ($P<0{\cdot}0001$) and intestinal content larvae were longer than those from the wall (mean length 1·6 and 1 mm, respectively, $P<0{\cdot}0001$). Although larvae were growing over time, a population of small-sized larvae (length <1 mm) was recovered at all d.p.i. During the first week of infection most of the larvae were located in the anterior half of the jejunoileum but they moved posteriorly with the age of infection. Thus, a subpopulation of larvae mainly in the lumen grew with time while another subpopulation remained small and associated with the mucosa. During the infection both subpopulations moved to a more posterior localization in the gastrointestinal (GI) tract.

Key words: *Ascaridia galli*, parasite, population dynamics, worm recovery, agar-gel incubation, pepsin-HCl digestion.

INTRODUCTION

Ascaridia galli (Schrank, 1788), which is a common parasitic nematode of chickens, has a worldwide distribution (Kaufmann, 1996). This parasite has a direct life cycle and chickens become infected by ingestion of infective eggs containing the infective larval stage which has been referred to as either second-stage larvae (L2) (Moran and Mizelle, 1957; Herd and McNaught, 1975; Ramadan and Abou Znada, 1992) or third-stage larvae (L3) (Ackert, 1931; Araujo and Bressan, 1977). After ingestion, the eggs hatch in the duodenum and the infective larvae are released in the intestine (Ackert, 1923). Most of the larvae then undergo a so-called histotropic or mucosal phase, starting as early as 1 day post-infection (d.p.i.) (Tugwell and Ackert, 1952). The duration of this phase has been reported to vary from 2 to 7 weeks depending on the level of infection (Herd and McNaught, 1975) but whether some larvae stay in this phase for an extended period of time is largely unknown. The adult parasites reside in the lumen of the intestine (Ackert, 1923, 1931; Tugwell and Ackert, 1952). Generally the pre-patency period is reported as 5–8 weeks (Ackert, 1931; Kerr, 1955). Infection at any age is associated with substantial economic losses due to a decrease in growth rate and weight loss. Moreover, the larvae may damage the intestinal mucosa and a heavy infection with adult worms can obstruct the small intestine and cause death (Ackert and Herrick, 1928; Ramadan and Abou Znada, 1991; Phiri *et al*. 2007). *Ascaridia galli* may also play a role in transmission of other infections such as *Salmonella* in chickens (Chadfield *et al*. 2001) and not least cause aesthetic problems when worms are recovered in eggs by consumers, creating problems for the industry (Fioretti *et al*. 2005).

Recent European regulation for the protection and welfare of laying hens substituted the use of traditional (conventional unenriched cage) systems with enriched cage or floor husbandry systems with or without access to outdoor runs. Moreover, the attitudes of consumers towards animal welfare and organic food products have also increased the production in organic farming systems (1999/74/EC, Anonymous, 1999). These floor-housing systems together with the direct life cycle and the highly resistant nature of the eggs all favour the transmission of this parasite (Permin and Hansen, 1998; Permin *et al*. 1999; Kaufmann *et al*. 2011).

* Corresponding author. Tania Ferdushy, Section for Parasitology, Health and Development, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlægevej 100, DK-1870, Frederiksberg C, Copenhagen, Denmark. E-mail: tania_ferdushy@yahoo.com

doi:10.1017/S0031182013000401

Permin *et al*. (1999) reported that in Denmark *A*. *galli* prevalence in the free-range system was higher (63·8%) than in the deep-litter system (41·9%), the backyard system (37·5%) and the conventional cage system (5%). In Sweden the prevalence of *A*. *galli* in different housing systems in 2008 was 77·1% in free range and organic systems, 28·6–52·2% in different kinds of floor systems and 4·3% in cage systems (Jansson *et al*. 2010). Similarly, in Germany, a high prevalence (88%) of *A*. *galli* was reported in organic free-range systems (Kaufmann *et al*. 2011) and in Ethiopia, Abebe *et al*. (1997) found a higher prevalence of *A*. *galli* (71·6%) in free-range chickens followed by 49% in semi-intensive and 0% in cage systems.

Most of these studies carried out on the population biology of *A*. *galli* infection include only single-time necropsy at the time of patency and therefore do not provide information about the dynamics of the establishment of the infections (Permin *et al*. 1997; Gauly *et al*. 2005; Höglund and Jansson, 2011). Thus more information on the establishment of *A*. *galli* before patency period is needed in order to understand the population dynamic of the parasite and the host response to infection in the early phase of infection. Such information is crucial in order to implement effective control measures and to improve the overall performance and productivity of the bird. Therefore, this study was carried out to study the population dynamics of *A*. *galli* following single infection by serial necropsy of birds from 3 to 42 days post-infection.

MATERIALS AND METHODS

Experimental animals

Seventy ISA Brown chickens obtained from a commercial breeding and pullet-raising farm with no history of *A*. *galli* infection were used in this experiment. The chickens were 6 weeks of age and weighed 0·48 kg ± 0·06 kg (mean ± S.D.) at the start of the experiment. The chickens were randomly allocated into two groups consisting of 28 chickens in the control group and 42 in the infected group. They were housed in three pens separated from each other by at least 2 metres. All the control birds were placed in one pen and the birds of the infected group were housed in the other two pens. The birds were offered commercial pullet feed 3 times per day and had access to water and grit *ad libitum*.

Collection and preparation of the infection dose

Approximately 1 kg of fresh chicken feces was collected from a conventional indoor deep-litter layer farm with a known high prevalence of *A*. *galli* and the eggs were isolated by wet sieving according to Ferdushy *et al*. (2012). The collected eggs were then set for embryonation in 0·05 M H_2SO_4 (pH 1) in a culture flask at 22 °C kept in the dark for 6 weeks. Once a week, the eggs were aerated for 15 min and the rate of embryonation was checked. After embryonation the eggs were incubated at 5 °C until use (not more than 2 weeks after embryonation).

Experimental design

After a week of adaptation, chickens in both groups were allocated randomly into 7 necropsy dates (3, 7, 10, 14, 21, 28 and 42 d.p.i.) after stratification for body weight. Therefore, for each time-point there were 10 birds (4 controls and 6 infected). Birds from each group were marked by use of different coloured leg bands and individual wingtag numbers. On day 0, all the birds forming the infected group were inoculated with 500 embryonated *A*. *galli* eggs suspended in 1 mL of tap water using a plastic Pasteur pipette inserted via the oesophagus to the level of the crop. The vial with the inoculation dose was washed twice with 1 mL of water that was given to the birds by the same pipette, as per Ferdushy *et al*. (2012). The birds were not given the morning feed until after the infection had taken place. Body weight and fecal samples were taken from all birds of both control and infected groups 1 day before the inoculation and again at necropsy. Collected fecal samples were examined for the presence of helminth eggs by a modified McMaster method with a lower detection limit of 20 eggs per gram (EPG) of feces (Roepstorff and Nansen, 1998).

Necropsy and larval recovery from the intestinal content and wall

At each time-point (3, 7, 10, 14, 21, 28, 42 d.p.i.) respective numbers of infected and control chickens were killed by decapitation. The gastrointestinal tract was removed from the proventriculus to the cloaca, divided into two main sections; (i) duodenum (defined by the duodenal loop and referred to as section D) (Schummer *et al*. 1992) and (ii) jejunoileum (from entry of the bile duct to the origin of caeca as defined by Schummer *et al*. 1992) and divided into four equally sized subsections (J1, J2, J3, J4). Each intestinal section was opened separately in the longitudinal direction and washed by dipping the intestinal wall 10 times in 150 mL of 0·9% NaCl solution (38 °C). The washing water together with the intestinal contents was embedded in agar and incubated as described by Ferdushy *et al*. (2012). In short the samples were mixed with 150 mL of 2% agar solution (equivalent to 1% agar in the final solution), and immediately poured onto a humid agar cloth (45104S, Johnson's Universalduk, Johnson and Johnson AB, Sweden) placed on a tray and allowed to solidify for a few minutes at room temperature.

https://doi.org/10.1017/S0031182013000401 Published online by Cambridge University Press

Approximately 15 min elapsed between slaughter of the bird and setting the sample in agar-gel. The agar gels were incubated in warm physiological saline overnight at 38 °C. The following day larvae (no adult worms were found) were collected on a 15 μm sieve and stored in 70% alcohol until counting.

The rinsed sections of intestinal wall were further processed by artificial pepsin-HCl digestion (12 mL HCl (30%), 30 mL liquid pepsin (660 U per mL, Orthana Biofac A/S, Denmark) in 1 L of 42 °C tap water) as per Ferdushy *et al*. (2012). Briefly, the small intestinal wall was cut into small pieces of 0·5 cm and digested in 200 mL of digestion fluid under constant magnetic stirring of 250 rpm at 38 °C for 90 min or until full digestion of the tissue. Then the larvae were collected on a 15 μm sieve and stored in 70% alcohol until counting.

Data analysis and statistical methods

The statistical analyses were performed using SAS 9.1 and GraphPad Prism (version 5). Graphical presentations were made using GraphPad Prism (version 5) and Microsoft Excel 2007. The total number of larvae recovered at different d.p.i. were analysed in a Generalized Linear Model (PROC GENMOD procedure) specifying the negative binomial distribution in larval recovery and considering source (content and wall), section (D, J1, J2, J3, J4) and d.p.i. (3, 7, 10, 14, 21, 28, 42) as explanatory variables. Moreover, the interactions between d.p.i. and source, and d.p.i. and section, were also evaluated. The level of significance was considered as $P<0{\cdot}05$. Larval recovery over time was modelled by a simple exponential function with a constant rate of decay:

$$Y_t = Y_0 \times e^{-K \times t}$$

where Y_t is the number of larvae recovered at time t, Y_0 is the estimate of the number of larvae established at $t=0$, e is the exponential function with the rate of decay K.

RESULTS

Performances and prevalence

During the experimental period no clinical sign of infection was observed among the birds and also the fecal egg counts were negative for all the infected and control birds. No significant differences between groups were found with regard to weight gain ($P=0{\cdot}6$). The prevalence of infection was 100% at all d.p.i. and the mean (±S.D.) number of larvae recovered were (100 ± 17·4), (78 ± 22·6), (90 ± 10·2), (75 ± 34·5), (55 ± 13·8), (61 ± 27·2) and (53 ± 20·8) at 3, 7, 10, 14, 21, 28 and 42 d.p.i., respectively (Fig. 1). The recovery of larvae over time was modelled by an exponential function with a constant rate of decline ($K=0{\cdot}01832$ with 95% CI 0·009221 to 0·02742) (Fig. 1). All the control birds were found to be uninfected.

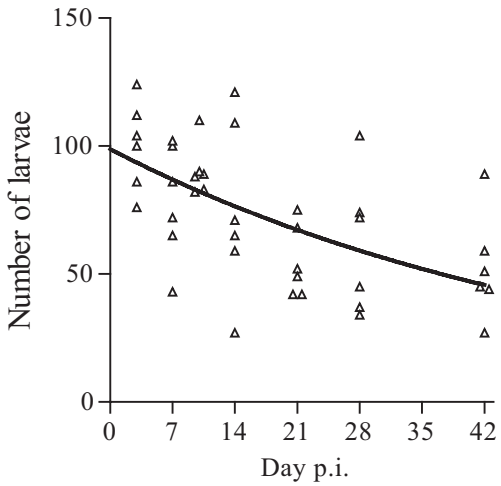

Fig. 1. Total number of larvae recovered at different days post-infection (d.p.i.) from chickens infected with 500 embryonated *Ascaridia galli* eggs. The triangles represent larval counts from individual chickens, where the curve represents an exponential function with a constant rate of decay and a half-life of 37·8 days. Some data points were overlapping. To present these points they have been separated manually. The curve was estimated from the original data.

Distribution of larvae between the intestinal content and wall

The mean percentage recovery of larvae between the intestinal content and intestinal wall after digestion differed significantly among the d.p.i. ($P<0{\cdot}0001$). Almost all (70–86%) worms were obtained from the intestinal wall in the youngest infection (3–14 d.p.i.), but this fraction was reduced at 21 and 28 d.p.i. (62 and 51% respectively) (Fig. 2). At the last sampling date at 42 d.p.i. there was again a higher proportion in the wall. Comparatively larger larvae were obtained from the intestinal content than from the intestinal wall. The mean approximate length of the larvae in the content increased until 28 d.p.i. whereafter a decrease was observed at 42 d.p.i. but the mean lengths of the larvae in the wall were almost similar throughout the experimental period (Fig. 2).

Distribution and size of larvae in different sections of the intestine

The number of larvae recovered from the intestinal sections differed significantly among the d.p.i. ($P<0{\cdot}0001$). Most of the larvae were located in section J2 at 3 and 7 d.p.i. From 10 d.p.i. onwards the larvae were located posteriorly being most abundant in section J3, and at 42 d.p.i. the highest number of larvae was again seen in section J2 (Fig. 3).

https://doi.org/10.1017/S0031182013000401 Published online by Cambridge University Press

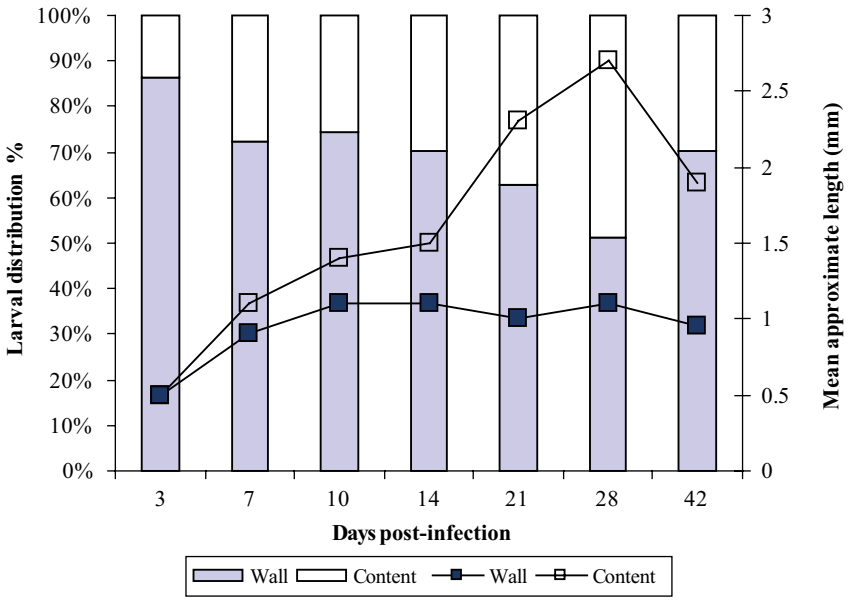

Fig. 2. Larval distribution between intestinal content and wall, and approximate length at different days post-infection (d.p.i.) of chickens infected with 500 embryonated *Ascaridia galli* eggs. Right y-axis: mean length (mm) of larvae obtained from the intestinal content and wall. Left y-axis: proportion of larvae in each of the two locations.

The size of the larvae also varied greatly according to the d.p.i. ($P<0{\cdot}0001$). All the larvae recovered at 3 d.p.i. were <1 mm. A large proportion of the larvae were growing. However, small larvae of a length below 1 mm were present at all necropsies (Fig. 4) and a mixture of different sizes of larvae was observed in different sections of the intestine at all d.p.i. except 3 d.p.i.

DISCUSSION

This study investigated the development and localization of *A. galli* larvae during the pre-patent period. Within the 6-week study period no worms reached a size close to the mature fertile stage (51–76 and 72–116 mm for male and female, respectively) (Ackert, 1931). Differential development of the larvae was observed from 7 d.p.i. onwards, and from 21 d.p.i. a bimodal size distribution was evident. Although the proportion of larvae found in the intestinal contents was increasing with time (except for the last necropsy), more larvae were consistently recovered from the intestinal wall after pepsin digestion than from the contents. The mean lengths of larvae recovered from the intestinal contents were larger than those of the intestinal wall, and the larvae isolated from the wall did not seem to grow in size with time but remained more or less 1 mm. Other authors have also found this kind of small-sized larvae at late stages of infection (around 7 weeks) and referred to them as 'static' or 'arrested larvae' (Moran and Mizelle, 1957; Herd and McNaught, 1975). According to Michel (1974) a bimodal size distribution after a single infection is indicative of arrested development and it is suggested to be related to three factors (or combinations hereof): (1) pre-determination induced by prior environmental exposure; (2) crowding effect/density dependency or (3) acquired immunity. Pre-determined arrested or inhibited development is a well-known phenomenon for the trichostrongylid nematodes in ruminants and is presumably induced in pre-parasitic L3 after exposure to seasonal changes in the environment (e.g. shorter photoperiod, decreasing temperature or humidity) (Eysker, 1997). Our study was carried out during the summer time in Denmark (June–July) and the lighting schedule followed the normal daylight. Thus, in this experiment it is difficult to explain the background for arrested development because of the environmental influence, but it cannot be ruled out that storage under refrigeration had induced this arrest, similar to trichostrongyles (Eysker, 1981). However, we have not experienced this after prolonged storage of other ascarids, e.g. *Ascaris suum*, and this remains hypothetical.

A reduced size of worms and resting stages due to crowding effect/density dependency for *A. galli* has been observed by Herd and McNaught (1975). They infected two groups of chickens either with 2000 eggs or with 50 eggs and in the high-dose group arrested larvae were found until 54 d.p.i. whereas in the low-dose group arrested larvae were observed only until 16 d.p.i. Although we used a lower inoculation dose compared with the higher dose (500 *vs* 2000) given by Herd and McNaught (1975) it can be speculated that the actual numbers of infective eggs in the two experiments were close as we used matured eggs collected from droppings which may have had a higher infectivity. This density-dependent

https://doi.org/10.1017/S0031182013000401 Published online by Cambridge University Press

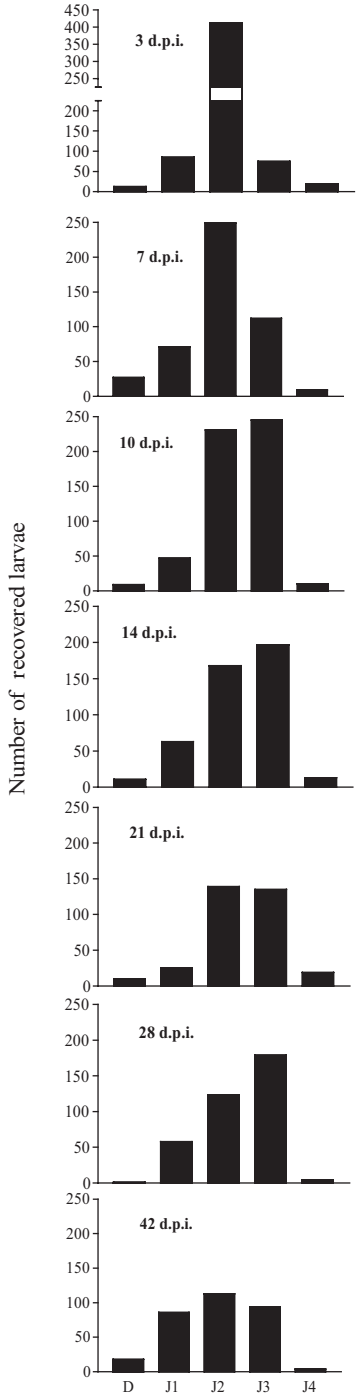

Fig. 3. Total number of larvae recovered from different sections of the intestinal wall at different days post-infection (d.p.i.) of chickens infected with 500 embryonated *scaridia galli* eggs. For definitions of sections D and J1–J4 see the Materials and Methods section. Six chickens were necropsied at each time-point.

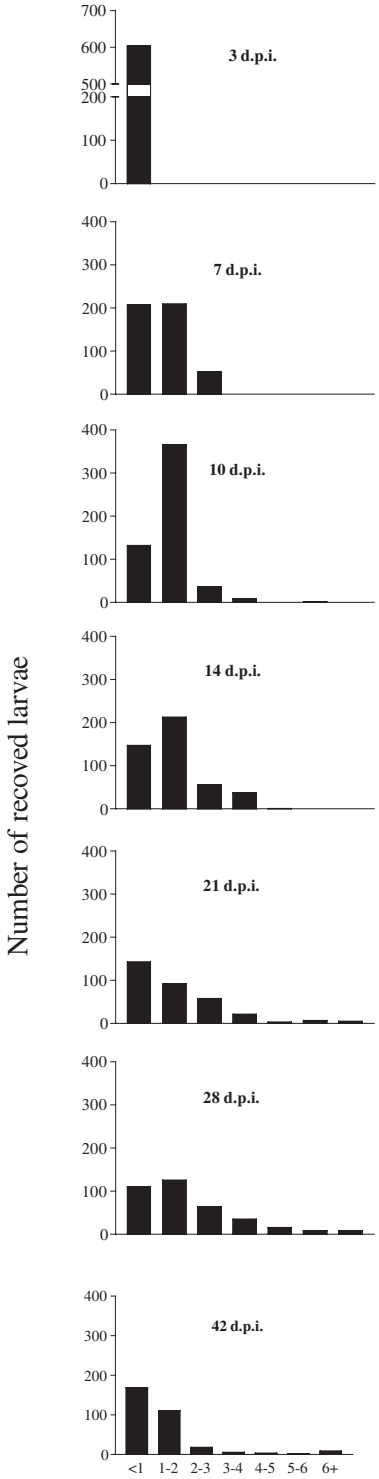

Fig. 4. Total larval recovery according to size (mm) from 6 chickens infected with 500 embryonated *Ascaridia galli* eggs and necropsied at different days post-infection (d.p.i.).

development was also evident for other parasites e.g. *Teladorsagia circumcincta* in sheep (Hong *et al*. 1986) and *Oesophagostomum dentatum* in pigs (Christensen *et al*. 1995).

The presence of smaller-sized larvae on all occasions may well be explained by acquisition of immunity. Comparable findings were also obtained by Idi *et al*. (2004) who infected 4-week-old layer chickens with 500 embryonated *A. galli* eggs and only larval stages were recovered up to 10 weeks post infection. Similarly, Gauly *et al*. (2005) infected four groups of chickens with *A. galli* eggs at the age of 6 weeks, 12 weeks, 18 weeks and 24 weeks and noticeably shorter and lighter worms were obtained from the 6-week-old group. Our chickens were

https://doi.org/10.1017/S0031182013000401 Published online by Cambridge University Press

7 weeks of age at the time of infection and the findings of Idi *et al*. (2004) and Gauly *et al*. (2005) indicate that the birds were immunocompetent at that age. Herd and McNaught (1975) also documented the effect of host immunity on *A. galli* growth and development as the proportion of arrested larvae was considerably lower in the birds treated with an immunosuppressive agent.

It remains speculative how the two subpopulations interact; the arrested population associated with the mucosa and the luminal population with larger and growing worms. The latter may be recruited from the first, but we do not know. We found no adult nematodes and can only assume that the luminal population will develop into adults with time as observed for *Ostertagia ostertagi* in both naturally and experimentally infected cattle (Michel *et al*. 1976*a*, 1976*b*).

We found that larvae were displaced more aborally with time. During the first week of infection most of the larvae were located in section J2 (i.e. the second quarter of the jejunoileum) similar to the findings of Ackert (1923, 1931), Herd and McNaught (1975) and Ferdushy *et al*. (2012). From 10 to 28 d.p.i. the larvae were located more posteriorly mainly in section J3 but at 42 d.p.i. they were again primarily found in section J2. Moran and Mizelle (1957) also obtained the majority (~ 64%) of the recovered larvae from the section corresponding to section J3 in our study from 9 to 25 d.p.i. This caudal translocation of larvae from 10–28 d.p.i. in our study might be related to expulsion of larvae. Similarly, Roepstorff *et al*. (1997) reported that in *A. suum*-infected pigs, larvae moved more caudally during the initial expulsion phase (14 to 21 d.p.i.) while they were again found in the oral part of small intestine from 28 d.p.i. and onwards.

The number of larvae was found to decline with time at a constant rate. The decrease in larval recovery over time may be influenced by the development of immunity and losses from other reasons e.g. expulsion or death of the larvae. *Ascaridia galli*-infected birds can develop specific IgG antibody and express a Th2 type response against *A. galli* antigens 14 days after infection (Degen *et al*. 2005; Marcos-Atxutegi *et al*. 2009). The innate immunity of the bird may be responsible for the relatively low initial establishment (20% of the infection dose) and the later development of acquired immunity may play a role in the gradual elimination of the worms during the pre-patent period.

Our results may differ from the previous findings of other researchers as the method we employed was much more sensitive for the recovery of small larvae from the chicken intestine (Ferdushy *et al*. 2012) and also because of the dissimilarities in other factors such as infection dose, breed, age and nutrition of the host (Permin *et al*. 1997; Schou *et al*. 2003; Gauly *et al*. 2005; Idi *et al*. 2007). During the experimental period no clinical signs were observed in control or infected birds, and both groups were gaining weight equally over time. This could be related to the absence of patent infection and to good management.

In conclusion it can be seen that the initial establishment of *A. galli* infection is much lower compared with other nematodes of monogastrics e.g. *A. suum* and *Trichuris suis* (Roepstorff *et al*. 1997; Kringel and Roepstorff, 2006) where more than 50% of the inoculation dose can be recovered as larvae within 2–3 weeks. Of the established *A. galli* larvae, half seem to be expelled during 3–42 d.p.i. These two observations might be related to host immunity. We observed a lack of patency but the presence of two subpopulations of *A. galli* in the chickens – a small luminal population of increasing length (size) and a larger population of inhibited larvae attached to the gut wall, only recoverable after digestion. How these two subpopulations interact will require detailed long-term studies in order to understand the parasite population dynamics and host–parasite relationship more clearly.

ACKNOWLEDGEMENTS

The skilful help of L. Christiansen is greatly appreciated. All the birds were treated according to Danish legislation (License: A. Roepstorff, 2010/561-1914, 13 October 2000).

REFERENCES

Abebe, W., Asfaw, T., Genete, B., Kassa, B. and Dorchies, P. (1997). Comparative studies of external parasites and gastro-intestinal helminths of chickens kept under different management system in and around Addis Ababa (Ethiopia). *Reuve de Medecine Veterinaire* **148**, 497–500.

Ackert, J. E. (1923). On the habitat of *Ascaridia perspicillum* (Rud). *Journal of Parasitology* **10**, 101–103.

Ackert, J. E. (1931). The morphology and life history of the fowl nematode *Ascaridia lineata* (Schneider). *Parasitology* **23**, 360–379.

Ackert, J. E. and Herrick, C. A. (1928). Effects of the nematode *Ascaridia lineata* (Schneider) on growing chickens. *Journal of Parasitology* **15**, 1–13.

Anonymous (1999). Council directive 1999/74/EC laying down minimum standards for the protection of laying hens. *Official Journal of the European Communities L* **203**, 53.

Araujo, P. and Bressan, C. R. (1977). Observations on the second moult of the larvae of *Ascaridia galli*. *Annales de Parasitologie Humaine et Comparee* **52**, 531–537.

Chadfield, M., Permin, A., Nansen, P. and Bisgaard, M. (2001). Investigation of the parasitic nematode *Ascaridia galli* (Schrank 1788) as potential vector for *Salmonella enterica* dissemination in poultry. *Parasitology Research* **87**, 317–325.

Christensen, C. M., Barnes, E. H., Nansen, P., Roepstorff, A. and Slotved, H-C. (1995). Experimental *Oesophagostomum dentatum* infection in the pig: worm populations resulting from single infections with the three doses of larvae. *International Journal for Parasitology* **25**, 1491–1498.

Degen, W. G. J., van Daal, N., Rothwell, L., Kaiser, P. and Schijns, V. E. J. C. (2005). Th1/Th2 polarization by viral and helminth infection in birds. *Veterinary Microbiology* **105**, 163–167.

Eysker, M. (1981). Experiments on inhibited development of *Haemonchus controtus* and *Ostertagia circumcincta* in sheep in the Netherlands. *Research in Veterinary Science* **30**, 62–65.

Eysker, M. (1997). Some aspects of inhibited development of trichostrongylids in ruminants. *Veterinary Parasitology* **72**, 265–283.

Ferdushy, T., Nejsum, P., Roepstorff, A., Thamsborg, S. M. and Kyvsgaard, N. C. (2012). *Ascaridia galli* in chickens: intestinal localization and comparison of methods to isolate the larvae within the first week of infection. *Parasitology Research* **111**, 2273–2279. doi: 10.1007/s00436-012-3079-3.

Fioretti, D. P., Veronesi, F., Diaferia, M., Franciosini, M. P. and Proietti, P. C. (2005). *Ascaridia galli*: a report of erratic migration. *Italian Journal of Animal Science* **4**, 310–312.

Gauly, M., Homann, T. and Erhardt, G. (2005). Age related differences of *Ascaridia galli* egg output and worm burden in chickens following a single dose infection. *Veterinary Parasitology* **128**, 141–148.

Herd, R. P. and McNaught, D. J. (1975). Arrested development and the histotrophic phase of *Ascaridia galli* in the chicken. *International Journal for Parasitology* **5**, 401–406.

Hong, C., Michel, J. F. and Lancaster, M. B. (1986). Populations of *Ostertagia circumcincta* in lambs following a single infection. *International Journal for Parasitology* **16**, 63–67.

Höglund, J. and Jansson, D. S. (2011). Infection dynamics of *Ascaridia galli* in non-caged hens. *Veterinary Parasitology* **180**, 267–273.

Idi, A., Permin, A. and Murrell, K. D. (2004). Host age only partially affects resistance to primary and secondary infections with *Ascaridia galli* (Schrank, 1788) in chickens. *Veterinary Parasitology* **122**, 221–231.

Idi, A., Permin, A., Jensen, S. K. and Murrell, K. D. (2007). Effect of a minor vitamin A deficiency on the course of infection with *Ascaridia galli* (Schrank, 1788) and the resistance of chickens. *Helminthologia* **44**, 3–9.

Jansson, D. S., Vågsholm, I., Nyman, A., Christensson, D., Göransson, M., Fossum, O. and Höglund, J. (2010). Ascarid infections in laying hens kept in different housing systems. *Avian Pathology* **39**, 525–532.

Kaufmann, J. (1996). *Parasitic Infection of Domestic Animals: A Diagnostic Manual*. Birkhäuser, Basel, Switzerland.

Kaufmann, F., Das, G., Sohnrey, B. and Gauly, M. (2011). Helminth infections in laying hens kept in organic free range systems in Germany. *Livestock Science* **141**, 182–187.

Kerr, K. B. (1955). Age of chickens and the rate of maturation of *Ascaridia galli*. *Journal of Parasitology* **3**, 233–235.

Kringel, H. and Roepstorff, A. (2006). *Trichuris suis* population dynamics following a primary experimental infection. *Veterinary Parasitology* **139**, 132–139.

Marcos-Atxutegi, C., Gandolfi, B., Arangüena, T., Sepúlveda, R., Arévalo, M. and Simón, F. (2009). Antibody and inflammatory responses in laying hens with experimental primary infections of *Ascaridia galli*. *Veterinary Parasitology* **161**, 69–75.

Michel, J. F. (1974). Arrested development of nematodes and some related phenomena. *Advances in Parasitology* **12**, 279–366.

Michel, J. F., Lancaster, M. B. and Hong, C. (1976*a*). Observations on the resumed development of arrested *Ostertagia ostertagi* in naturally infected yearling cattle. *Journal of Comparative Pathology* **86**, 73–80.

Michel, J. F., Lancaster, M. B. and Hong, C. (1976*b*). The arrested development of arrested *Ostertagia ostertagi* in experimentally infected calves. *Journal of Comparative Pathology* **86**, 615–619.

Moran, J. F., Jr. and Mizelle, J. D. (1957). Studies on *Ascaridia galli* (Schrank, 1758). *American Midland Naturalist* **58**, 170–181.

Permin, A. and Hansen, J. W. (1998). Epidemiology, diagnosis and control of poultry parasites. *FAO Animal Health Manual no. 4*. Food and Agricultural Organization of the United Nations, Rome, Italy.

Permin, A., Bojsen, M., Nansen, P., Bisgaard, M., Frandsen, F. and Pearman, M. (1997). *Ascaridia galli* populations in chicken following single infections with different dose level. *Parasitology Research* **83**, 614–617.

Permin, A., Bisgaard, M., Frandsen, F., Pearman, M., Kold, J. and Nansen, P. (1999). Prevalence of gastrointestinal helminths in different poultry production systems. *British Poultry Science* **40**, 439–443.

Phiri, I. K., Phiri, A. M., Ziela, M., Chota, A., Masuku, M. and Monrad, J. (2007). Prevalence and distribution of gastrointestinal helminths and their effects on weight gain in free-range chickens in central Zambia. *Tropical Animal Health and Production* **39**, 309–315.

Ramadan, H. H. and Abou Zanda, N. Y. (1991). Some pathological and biochemical studies on experimental ascaridiasis in chickens. *Food/Nahrung* **35**, 71–84.

Ramadan, H. H. and Abou Zanda, N. Y. (1992). Morphology and life history of *Ascaridia galli* in the domestic fowl that are raised in Jeddah. *Journal of King Abdulaziz University: Science* **4**, 87–99.

Roepstorff, A. and Nansen, P. (1998). Epidemiology, diagnosis and control of helminth parasites of swine. *FAO Animal Health Manual no. 3*. Food and Agricultural Organization of the United Nations, Rome, Italy.

Roepstorff, A., Eriksen, L., Slotved, H. C. and Nansen, P. (1997). Experimental *Ascaris suum* infection in the pig: worm population kinetics following single inoculations with three doses of infective eggs. *Parasitology* **115**, 443–452.

Schou, T., Permin, A., Roepstorff, A., Sørensen, P. and Kjær, J. (2003). Comparative genetic resistance to *Ascaridia galli* infections of 4 different commercial layer lines. *British Poultry Science* **44**, 182–185.

Schummer, A., Vollmerhaus, B., Sinowatz, F., Frewein, J. and Waibl, H. (1992). Anatomie der Vögel. In *Lehrbuch der Anatomie der Haustiere*, 2nd Edn (ed. Nickel, R., Schummer, A. and Seiferle, E.), pp. 203–204. Paul Parey, Berlin, Germany.

Tugwell, R. L. and Ackert, J. E. (1952). On the tissue phase of the life cycle of the fowl nematode *Ascaridia galli* (Schrank). *Journal of Parasitology* **38**, 277–288.