ANIMAL WELFARE IN ORGANIC EGG PRODUCTION - WITH EMPHASIS ON MORTALITY AND HELMINTH INFECTIONS

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PhD THESIS · SCIENCE AND TECHNOLOGY · 2015

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Preface

The present PhD thesis entitled "Animal welfare in organic egg production - with emphasis on mortality and helminth infections" is based on work carried out from October 2011 to January 2015 at the Department of Animal Science, Faculty of Science and Technology, Aarhus University, where I have been part of the section Epidemiology and Management.

The PhD project was part of an ERA-net project entitled "HealthyHens - Promoting good health and welfare in European organic laying hens" granted from the CoreOrganic 2 and a Danish project entitled "Færre døde høns" (English: Fewer dead hens) founded by "Fonden for økologisk landbrug" (English: Fund for Organic Farming). The PhD project was carried out with two thirds external financial support and one third financial support from the Graduate School of Science and Technology (GSST) at Aarhus University.

HealthyHens involved partners from eight countries: University of Kassel, Germany; Fondazione CRPA Studi e Ricerche onlus, Italy; ADAS UK Ltd., United Kingdom; Louis Bolk Institute, The Netherlands; University of Veterinary Medicine Vienna, Austria; Swedish University of Agricultural Sciences, Sweden; Institute for Agricultural and Fisheries Research, Belgium and Aarhus University, Denmark.

This PhD thesis includes data from 15 Danish farms with organic egg production, and the project resulted in three research papers:

Paper I, entitled "Helminth infection is associated with hen mortality in Danish organic egg production", about the association between helminth infection and mortality, has been submitted to Preventive Veterinary Medicine.

Paper II, entitled "Associations between helminth infection and clinical welfare indicators in organic layers", exploring if helminth infection can be predicted by clinical welfare indicators, has not been submitted.

Paper III, entitled "Danish organic egg producers' perceptions and experiences related to mortality and endoparasite infections", about the producers' experiences and perceptions with mortality and endoparasite infections, has been submitted to Livestock Science.

Acknowledgement

Table of content

Summary

The consumers' motivation to buy organic products includes animal welfare aspects, and even though the retail market share for organic eggs in Denmark is relative high, there are a number of welfare issues in the organic egg production compared to other production systems, like higher mortality and prevalence of helminth infections, that are not in agreement with the consumers' expectation.

The aim of this PhD study was to investigate animal welfare in organic egg production in Denmark, with emphasis on mortality and helminth infections. The specific hypothesis in the PhD study were *i)* helminth infections (*Ascaridia galli* and *Heterakis* sp.) increase the mortality rate at peak of lay (observational event study, Paper I), *ii)* helminth infections (*Ascaridia galli* and *Heterakis* sp.) diagnosed at end of lay can be predicted by at least one clinical welfare indicator diagnosed at peak of lay and end of lay (longitudinal study, Paper II). Moreover, *iii)* it is possible, based on qualitative interviews, to identify management strategies for maintaining low mortality and controlling endoparasite (mainly helminth) infections (qualitative interview study, Paper III).

Data were obtained from three studies conducted at 15 commercial Danish organic egg farms, and the results are presented in three scientific papers. In the first study (Paper I), weekly mortality rates were related to level of infection with *A. galli* and *Heterakis* sp. using survival analysis, and the study included 11 commercial organic egg farms, representing 15% of the Danish organic layers. An association was found between the *A. galli* and *Heterakis* sp. infection level and mortality rates. Low-infected farms (mean ≤200 eggs per gram of faeces (EPG)) had similar mortality rates in summer and winter; therefore, all low-infected farms despite of season were used as reference in the analysis. The mortality rate was twice as high for highly-infected farms (mean > 200 EPG) observed in summer compared to low-infected farms, whereas highly-infected farms observed in the winter did not have a significant different mortality rate compared to low-infected farms. Consequently, hypothesis *i)* was confirmed in relation to observations during summer, but not for observations during winter.

The second study (Paper II) included 214 individual hens from 12 farms assessed twice during the production period (at peak and end of lay) to investigate if helminth (*A. galli* and *Heterakis* sp.) infection can be predicted by clinical welfare indicators, like the condition of the plumage, keel bone, skin, feet and comb. A graphical model displayed that helminth infections (*A. galli* and *Heterakis* sp.) diagnosed at the end of lay only were associated with back feathering at the end of lay. Hens with a good plumage condition at the back at the end of lay had a higher incidence of

helminth infections than hens with a poor plumage condition at the back. Further, the study showed that the number of keel bone deformities increased and plumage conditions deteriorated between the two visits, whereas prevalence of bumble foot and pale combs decreased. Frequency of skin, feet and toe lesions did not differ significantly between the two visits. Therefore, hypothesis *ii)* was confirmed, as helminth infection could be predicted based on at least one clinical welfare indicator. However, in relation to daily management on commercials farms the relationship might not be useful.

The third study (Paper III) investigated seven organic egg producers' perception and experiences with maintaining a low mortality and controlling helminth infections using qualitative interviews. Four main causes of mortality were identified: predation, effect of weather, infectious diseases and smothering, and three themes important for maintaining low mortality were identified: pullet quality (uniformity and a good start-up at the egg production site), management and time (daily routines, time spent with the hens, and time for unforeseen matters), and feed (quality and quantity of feed and water). In relation to control of helminth infections, three practices were described by the producers: no testing of the infection level, regular testing of the infection level and irregular testing of the infection level. The two practices that included test of the infection level ended with deworming if it was considered necessary. The producers believed that management practices related to mortality had a positive effect on helminth infections and vice versa. Based on the results from the qualitative interviews hypothesis *iii)* was confirmed, as it was possible to identity management strategies for maintaining low mortality and controlling helminth infections.

The overall findings of the PhD study report that control of helminth infections could potentially reduce the mortality and improve the welfare of hens with an otherwise good welfare in relation to plumage condition, as these hens have the highest incidence of helminth infection. However, the fact that the producers lack knowledge of alternatives to deworming strategies, which potentially could improve the animal welfare in the organic egg production, calls for further research on how to prevent helminth infections.

Dansk sammendrag (Danish summary)

Forbrugernes motivation for at købe økologiske produkter inkluderer dyrevelfærdsaspekter, og selvom markedsandelen af økologiske æg i den danske detailhandel er relativt høj, er der dog en række velfærdsmæssige problemer i den økologiske ægproduktion sammenlignet med andre produktionssystemer, såsom højere dødelighed og prævalens af indvoldsorm, som ikke er i overensstemmelse med forbrugernes forventninger.

Formålet med denne ph.d.-afhandling var at undersøge dyrevelfærden i den økologiske ægproduktion i Danmark med vægt på dødelighed og forekomsten af indvoldsorm. De specifikke hypoteser i afhandlingen var *i)* infektioner med indvoldsorm (spolorm (*Ascaridia galli*) og blindtarmsorm (*Heterakis* sp.)) øger dødeligheden ved topydelse (observationelt hændelsesstudie, artikel I), *ii)* infektioner med indvoldsorm (spolorm/blindtarmsorm), diagnosticeret ved afslutningen af produktionsperioden, kan forudsiges med mindst en klinisk velfærdsindikator vurderet ved topydelse og afslutningen af produktionsperioden (forløbsstudie, artikel II) og *iii)* det er muligt, baseret på kvalitative interviews, at identificere en sammenhængende managementstrategi for opretholdelse af lav dødelighed og kontrol af indvoldsorm (kvalitativt interviewstudie, artikel III).

Der blev gennemført tre studier i 15 danske kommercielle bedrifter med økologisk ægproduktion, og resultaterne herfra er præsenteret i tre videnskabelige studier. Det første studie (artikel I) undersøgte om ugentlig dødelighed var relateret til niveauet af infektioner med indvoldsorm (spolorm/blindtarmsorm) ved hjælp af en overlevelsesanalyse. Studiet blev gennemført på 11 bedrifter med økologisk ægproduktion, herved var 15% af de danske, økologiske æglæggere repræsenteret. Der blev påvist en sammenhæng mellem dødelighed og niveauet af infektion med indvoldsorm (spolorm og blindtarmsorm). Lavtinficerede bedrifter (gennemsnit ≤ 200 æg per gram fæces (EPG)) havde den samme dødelighed om sommeren og om vinteren; alle lavtinficerede bedrifter blev derfor anvendt som reference i analyserne. Dødeligheden var dobbelt så høj hos de højtinficerede bedrifter (gennemsnit >200 EPG) om sommeren sammenlignet med de lavtinficerede bedrifter, hvorimod dødeligheden i højtinficerede bedrifter om vinteren ikke var signifikant forskellig fra dødeligheden i lavt-inficerede bedrifter. Dette bekræftede hypotese *i)* i forhold til sommerobservationerne, men ikke i forhold til vinterobservationerne.

Det andet studie (artikel II) inkluderede 214 individuelle æglæggere fra 12 flokke som var vurderet ved to tidspunkter i produktionsperioden (ved topydelse og afslutning af produktionsperioden) for at undersøge, om infektioner med indvoldsorm (spolorm og blindtarmsorm) kan forudsiges ved hjælp af kliniske velfærdsindikatorer, såsom tilstanden af

fjerdragt, brystben, huden, fødder og kammen. Den grafiske model viste, at antallet af indvoldsorm diagnosticeret ved afslutningen af produktionsperioden var relateret med fjerdragtens tilstand på ryggen ved afslutning af produktionsperioden. Høner med en god fjerdragt på ryggen ved afslutning af produktionsperioden havde en højere hyppighed af indvoldsormsinfektioner sammenlignet med høner med en dårlig tilstand af ryggens fjerdragt. Endvidere viste studiet en øget andel af brystbensskader og dårlig fjerdragt ved afslutning af produktionsperioden, i forhold til ved topydelse, hvorimod andelen af høner med fodbylder (bumble foot) og bleg kamfarve var reduceret. Andelen af høner med sår på huden, fødder eller tæer var ikke signifikant forskellig ved de to besøg. Dette bekræftede hypotese *ii),* da mindst en klinisk velfærdsindikator kunne forudsige forekomsten af indvoldsorm, men i forhold til anvendelse i praksis er den fundne sammenhæng ikke direkte anvendelig.

Det tredje studie (artikel III) undersøgte syv økologiske ægproducenters opfattelse af og erfaring med opretholdelse af en lav dødelighed samt kontrol af forekomsten af indvoldsorm ved hjælp af kvalitative interviews. Fire hovedårsager til dødelighed blev identificeret: prædation, påvirkninger fra vejret, smitsomme sygdomme og klumpning, ligesom tre temaer for opretholdelse af lav dødelighed blev fundet: kvaliteten af hønniker (ensartethed samt en god opstart efter flytning til ægproduktionsstalden), management og tid (daglige rutiner, tid brugt i stalden og tid til uforudsete ting) samt foderet (kvalitet og kvantitet af foder og vand). I relation til kontrol af indvoldsorm blev tre fremgangsmåder beskrevet af producenterne: ingen test af parasitniveauet, test af parasitniveauet med regelmæssig tidsinterval eller test af parasitniveau med uregelmæssig tidsinterval. De to fremgangsmåder, som involverede test af parasitniveauet, medførte ormebehandling, hvis det blev vurderet nødvendigt. Producenterne mener, at managementtiltag relateret til dødelighed også har en positiv effekt på indvoldsormsinfektioner og omvendt. Resultaterne fra de kvalitative interviews bekræftede hypotese *iii)*, da det var muligt at identificere managementstrategier for opretholdelse af lav dødelighed og kontrol af indvoldsorm.

De generelle resultater i denne afhandling viser, at kontrol af indvoldsorm potentielt kan reducere dødeligheden samt forbedre velfærden hos høner med en god fjerdragt, da disse høner har den højeste hyppighed af indvoldsorm. Da producenterne imidlertid mangler viden om alternativer til ormebehandling, som potentielt kunne forbedre dyrevelfærden i den økologiske produktion, er behovet for videre forskning, om hvordan indvoldsormsinfektioner forebygges, nødvendig.

List of included papers

Paper I

Hinrichsen, L.K., Labouriau, R., Engberg, R.M., Knierim, U., Sørensen, J.T. Helminth infection is associated with hen mortality in Danish organic egg production

- submitted to Preventive Veterinary Medicine

Paper II

Hinrichsen, L.K., Riber, A.B., Labouriau, R. Associations between helminth infection and clinical

welfare indicators in organic layers

- manuscript in preparation

Paper III

Hinrichsen, L.K., Vaarst, M., Sørensen, J.T. Danish organic egg producers' perceptions and experiences related to mortality and endoparasite infections

- submitted to Livestock Science

List of abbreviations/terms

A. galli	*Ascaridia galli*
A. galli/*Heterakis*	*Ascaridia galli* and *Heterakis* sp.
DTPHM	discrete-time proportional hazard model
E. coli	*Escherichia coli*
EPG	egg per gram
EU	European Union
FEC	faecal egg count
HR	hazard ratio
KM	Kaplan-Meier
sp.	species

1   General introduction

In the commercial egg production (from laying hens (*Gallus gallus domesticus*)) in the European Union (EU), four production system are allowed; enriched cage, barn, free-range and organic (Anonymous, 1991; Anonymous, 1999; Anonymous, 2008a). The organic production accounts for a low proportion of the egg production (Oliver et al., 2009); however, in some countries, the demand for organic eggs is increasing. In Denmark, the organic egg production has almost doubled in size during the past 15 years (Anonymous, 2014b), and the retail market share of organic eggs was 20.5% in 2013 (Anonymous, 2014e). The consumer's motivation for buying organic products is influenced by a concern for human health, environment and animal welfare (Hermansen, 2003; Jensen et al., 2008; Wier et al., 2008; Dawkins, 2012). For further development of the organic egg production, it is critical to be able to document a high level of animal welfare. Animal welfare can be seen from different perspectives or concepts; natural living, affective state or biological function (Fraser et al., 1997) and is multi-dimensional (Lay et al., 2011; Vannier et al., 2014). Assessing animal welfare includes several welfare indicators that can be resource-based, related to the physical environment and management, or animal-based (Main et al., 2003; Sørensen et al., 2013; Vannier et al., 2014). The welfare indicators can be related to one or more of the welfare concepts (Lund, 2006; Fraser, 2008). Animal-based welfare indicators measure the animals' response to the housing system, environment and management (Barnett & Hemsworth, 2009; Rousing et al., 2013), which enables assessments of animal welfare across different systems.

In the organic egg production, a number of animal health and welfare problems have been identified (Berg, 2001; Knierim, 2006; van de Weerd et al., 2009). High prevalence of helminth infection (over 60%) is reported in the organic egg production (Permin et al., 1999; Kaufmann & Gauly, 2009; Jansson et al., 2010; Kaufmann et al., 2011b; Sherwin et al., 2013), and helminth infection is regarded as a welfare measure (Welfare Quality, 2009). The dominating helminth species are *Ascaridia galli* and *Heterakis gallinarum* (Permin et al., 1999; Kaufmann & Gauly, 2009; Kaufmann et al., 2011b; Sherwin et al., 2013). *H. gallinarum* acts as an intermediate host for *Histomonas meleagridis* that causes blackhead disease (McDougald, 2005; Hess & McDougald, 2013), while *A. galli* infection, among others, can cause weight depression, blockage of the intestinal lumen, reduced feed intake, lower locomotion activity and weakens the hens (Ackert & Herrick, 1928; Reid & Carmon, 1958; Ikeme, 1971; Kilpinen et al., 2005; Gauly et al., 2007). *A. galli* can also affect the appearance of the plumage (ruffled feathers) and decrease the bone strength (Ackert & Herrick, 1928; Ikeme, 1971; Ramadan & Abou Znada, 1991). Therefore, helminth

infections could potentially be predicted by clinical welfare indicators, like the often used welfare indicators plumage condition, keel bone deformities and feet condition (Gunnarsson et al., 2000; Mollenhorst et al., 2005; Tauson et al., 2005; Hegelund et al., 2006b; Welfare Quality, 2009), where the individual hen's appearance is assessed. These welfare indicators might increase in prevalence with increasing age (Hegelund et al., 2006a; Richards et al., 2012). Further, variation in level of helminth infections between farms (Höglund & Jansson, 2011; Sherwin et al., 2013), indicates an effect of management, and the effect of different management strategies regarding helminth infection has been studied (Heckendorn et al., 2009; Maurer et al., 2009; Maurer et al., 2013).

Mortality is higher in organic systems compared to indoor systems (Häne et al., 2000; Anonymous, 2013) and is regarded as a welfare measure (Croxall & Elson, 2007; Whay et al., 2007; Welfare Quality, 2009). Reports from the Danish efficiency control program of the mortality from 2009 to 2013 showed that the average mortality ranged from 3.6 to 4.5% in enriched cage production, from 6.5 to 9.1% in barn production, from 8.6 to 10.6% in free-range production and from 6.3 to 10.2% in the organic production (Anonymous, 2012c; Anonymous, 2013; Anonymous, 2014e). Similar mortality rates are reported for organic systems in Switzerland, France and The Netherlands (Leenstra et al., 2012). However, the variation of mortality between farms is high (1.6 to over 60%) (Hegelund et al., 2006b; Stokholm et al., 2010), which indicates a potential for improvements and an effect of management.

Variation in mortality and helminth infection between farms indicates a potential effect of management for both welfare measures. However, knowledge about the producers' management strategies regarding control of helminth infections and maintaining a low mortality is lacking. The producers' perception and attitude towards these issues can affect daily practices (Vaarst & Sørensen, 2009; Horseman et al., 2014), and insight into the perceptions and experiences can be investigated through qualitative research methods (Kvale & Brinkmann, 2008).

Helminth infections might compromise the immune response of the host (Hørning et al., 2003; Degen et al., 2005; Pleidrup et al., 2014), and studies show that indications of bacterial infections following an *A. galli* infection become more severe (Dahl et al., 2002; Permin et al., 2006). A helminth infection might enhance the mortality through elevated severity of other infections. As the helminth infection fluctuates during the production period, with the maximum infection around peak of lay (Pennycott & Steel, 2001; Höglund & Jansson, 2011), it could be expected that the helminth infection might affect the mortality at this time. Previous studies reported no relationship between mortality and helminth infections (Häne et al., 2000; Sherwin et al., 2013); however, these

studies investigated the relationship based on helminth infection diagnosed in the last part of the production period.

1.1 Aim and hypotheses

The aim of this PhD study was to investigate animal welfare in organic egg production with emphasis on mortality and helminth infection.

The hypotheses for the thesis were:

Hypothesis 1: *Ascaridia galli*/*Heterakis* sp. infections increase the risk of hen mortality at peak of lay in organic egg production

Hypothesis 2: *Ascaridia galli*/*Heterakis* sp. infections diagnosed at the end of lay can be predicted by at least one clinical welfare indicator diagnosed at peak of lay and end of lay

Hypothesis 3: It is possible, based on qualitative interviews, to identify management strategies for maintaining low mortality and controlling endoparasite infections at organic egg farms

1.2 Outline of the thesis

Initially, a background chapter (Chapter 2) gives a presentation of organic egg production (Section 2.1), and animal welfare (Section 2.2). Thereafter, the main welfare measures, mortality and helminth infections, are presented in section 2.3 and section 2.4, followed by an introduction to the clinical welfare indicator included in this thesis (Section 2.5).

This leads to a presentation of the methodology, separated into a presentation of the three studies conducted (Chapter 3) and a chapter covering the results, divided in to a section per study and the related paper (Chapter 4). The first paper (Section 4.1) evaluates the association between helminth infection and mortality rate (hypothesis 1); the second paper (Section 4.2) evaluates whether helminth infection can be predicted by clinical welfare indicators (hypothesis 2); the third paper (Section 4.3) evaluates the producers' perception of mortality and endoparasite infections (hypothesis 3). Each paper presents the individual discussions and conclusions, which finally are discussed in relation to the overall aim and background in Chapter 5, followed by an overall conclusion of the thesis (Chapter 6) and a short discussion on research perspectives (Chapter 7).

2 State of art

2.1 Organic egg production

The egg production in Denmark is regulated by the EU (organic production: Anonymous (2008b) and protection of laying hens: Anonymous (1999)). In addition to the organic production system, three other production systems are allowed in EU. These are enriched cage, barn and free-range production (Anonymous, 1999; Anonymous, 2002; Anonymous, 2008a). Production figures for the production systems are presented in Table 1.

The regulation (Anonymous, 1999, 2008b) states that organic hens should be kept in floor or aviary system (i.e. single-tiered or multi-tiered) in flocks of maximum 3000 hens. The hens should be housed with an indoor stocking density of maximum six hens per m^2 with one third of the indoor being a solid littered area, have access to a free-range, and beak trimming is not allowed. There is no restriction on the genetic material, which can be used in organic systems. Further, the hens should be provided with roughage, and the diet should be composed of 95% organic protein (Anonymous, 2012b). In Denmark, the following forages are categorised as roughage: fresh greenery, silage, hay, root crops, foliage together with fruit and vegetable leftovers (Anonymous, 2014d).

Table 1: Differences between organic, free-range, barn and enriched cage production according to stocking density and free-range access (Anonymous, 1999; Anonymous, 2008a; Anonymous, 2008b) and production figures based on the Danish efficiency control data from 2012 (Anonymous, 2013).

	Organic white eggs	Organic brown eggs	Free-range	Barn	Cage
Stocking density, hens/ m^2	6	6	9	9	13a
Free-range area, m^2/hen	4	4	4	0	0
Length of production, days	336	336	336	364	392
Mortality, % placed	8.5	9.0	8.6	7.1	3.8
Egg performance, %	87.3	85.9	87.6	88.0	92.3
Egg, number per placed hen	282	277	282	311	356
Feed, kg feed per kg egg	2.35	3.37	2.32	2.32	1.96
Feed, gram per hen per day	127	126	124	126	112

a: each hen should have access to 750 cm^2 of cage (Anonymous, 1999)

The outdoor area should always count minimum 4 m^2 per hen per rotation (Anonymous, 2008b), and the Danish interpretation is that the egg producers can fulfil the requirements in four possible ways (Anonymous, 2012a):

- 6 m^2 per hen. In this case paddock rotations are not necessary. However, the free-range area should be covered with trees and bushes to give a forest or plantation-like appearance
- Gradual paddock rotation, were the hens always have access to 4 m^2 per hen
- 4 m^2 per hen, where the free-range area has a 60-day period during the growing season every year without hens
- 4 m^2 per hen, where the free-range area has a 120-day period during the growing season every second year without hens

Before the implementation of this act in July 2012, the Danish organic producer needed to have 8 m^2 per hen if there was no paddock rotation, and the free-range area had to have a forest or plantation-like appearance. This corresponds to the first option in the new act; except that the size requirement had decreased with the new act.

The Danish AgriFish Agency (Ministry of Food, Agriculture and Fisheries of Denmark) evaluates the welfare of the hens according to a set of criteria that must be fulfilled to have the welfare evaluated as satisfactory (Anonymous, 2014d):

- The mortality must not exceed 1.2% per month
- The proportion of hens that is severely feather pecked should not exceed 5% (defined as a hen with two or more nude areas of ≥5 cm in diameter)
- The proportion of hens that is moderately feather pecked changes with age. At 25 weeks of age, the proportion of moderately feather pecked hens should not exceed 10%, and this level increases with 1% per live week, i.e. at 65 weeks, the proportion should not exceed 50% (defined as a hen with one nude area of <5 cm in diameter)
- Presence of cannibalism

The Danish AgriFish Agency can, if the level of feather pecking exceeds the thresholds (both for severe or moderate plumage damage) or if cannibalism is present, order that an action plan is formulated to terminate the problem or prevent the problem to reoccur in the coming flocks (Anonymous, 2014d).

Organic egg production in EU is a growing sector; in 2004 0.4% of all layers were raised under organic conditions (Oliver et al., 2009) while this has increased to 3.8% of all layers in 2013 (Anonymous, 2014c). Denmark is one of the countries with the highest percentage of organic layers

compared to other EU countries, with almost 20% of the hens being organic (Anonymous, 2014c; Anonymous, 2014b) and with a Danish retail market share of organic eggs of 20.5% (Anonymous, 2014e). Of all egg-producing farms in Denmark, almost 40% are organic, which corresponds to 66 organic farms with egg production (Anonymous, 2013).

2.2 Animal welfare

Animal welfare is one of the consumers' motivation for buying organic products (Hermansen, 2003; Jensen et al., 2008; Wier et al., 2008; Dawkins, 2012). However, animal welfare can be defined in different ways. Broom (1996) defined animal welfare as the animal's attempt to cope with the environment, and Duncan (1996) stated that animal welfare depends on the animal's feelings. These views are also found in the three welfare concepts identified by Fraser et al. (1997):

- *i)* natural living; animals should freely be able to carry out normal behaviour, be free from constraints and live a natural life, e.g. outdoor access and having an intact beak
- *ii)* affective state (feeling and emotions); pain and suffering should be avoided and positive states should be emphasised
- *iii)* biological function; productivity and health of the animal are important

Broom (1996) and Duncan's (1996) definitions are in line with the concept of biological function and affective states, respectively. Another view of animal welfare is the five freedoms by FAWC (1979):

- *i)* freedom from thirst, hunger and malnutrition
- *ii)* freedom from discomfort
- *iii)* freedom from pain, injury and diseases
- *iv)* freedom to express normal behaviour
- *v)* freedom from fear and distress

The three welfare concepts and the five freedoms have similarities and, to some extent, cover the same aspects. Freedom from thirst, hunger and malnutrition is included in the concept of biological function, freedom from discomfort is included in the concept of affective state, freedom from pain, injury and diseases is included in the concept of biological function and affective state, freedom to express normal behaviour is included in the concept of natural living and affective state, and freedom from fear and distress is included in the concept of affective state.

Awareness of the different welfare definitions is important, as the chosen concept may influence the result of the welfare assessment, as good welfare in one concept does not ensure good welfare

when using another concept (Fraser, 2008). However, welfare indicators used to measure animal welfare can be related to more than one of the concepts (Lund, 2006; Fraser, 2008), and it is important to realise that the choice of indicators can be determined of one's own beliefs, i.e. a value-based approach (Fraser, 2008).

Animal welfare is multi-dimensional (Lay et al., 2011; Vannier et al., 2014), and a welfare assessment should therefore include several indicators to give a comprehensive view of the current situation. Welfare indicators can be resource-based, management-based or animal-based (Main et al., 2003; Vannier et al., 2014). The resource-based relate to the physical environment like floor type and length of perches; management-based relate to the treatment of the animals, like stocking density; and the animal-based relate to the condition of the animal like health and behaviour. Management-based indicator can be included among the resource-based indicator (Sørensen et al., 2013). Resource-based indicators are easier and faster to measure compared to animal-based indicators (Barnett and Hemsworth, 2009; Rousing et al., 2013). However, the animal-based indicators are more valid than recourse-based indicators, if the animal-based indicators are based on validated and reliable methods, because resource-based indicators are indirect measures of the welfare (Keeling, 2005). Further, when using the resource-based indicators, an assessment of the welfare risk and welfare potential is conducted, while animal-based indicators assess the animal's responses to the housing system and management (Main et al., 2003; Barnett and Hemsworth, 2009; Rousing et al., 2013; Sørensen et al., 2013). The animal-based indicators, which should be independent of the production system, can be measured in all types of housing systems and are easier compared across systems, compared to resource-based indicators (Rousing et al., 2013; Vannier et al., 2014). Animal-based welfare indicators are often measured at individual level, but expressed as proportion of a group or herd (Vannier et al., 2014). The present study focus on organic egg production, all farms have to fulfil the same set the requirements regarding the physical environment; therefore, primarily animal-based welfare indicators were included since they cover the individual animal's response to resources and management. In this thesis, animal welfare is investigated by the welfare indicators' mortality and helminth infection and several clinical welfare indicators, i.e. condition of plumage, keel bone, skin, feet and comb. All these animal-based welfare indicators/measures are mainly related to the concept of affective state. The following sections introduce these animal-based welfare indicators.

2.3   Mortality

EU requires that producers record the mortality in their herd (Anonymous, 1998). According to Hegelund (2007), the producers' recorded mortality is highly relevant as a welfare measure, and mortality is regarded as a welfare indicator in welfare assessments (Aerni et al., 2005; EFSA, 2005; Nicol et al., 2006; Croxall & Elson, 2007; Whay et al., 2007). A high mortality can be caused by disease, parasite infections, heat stress or outbreak of cannibalism (von Borell & Sørensen, 2004; EFSA, 2005), which all pose a threat to the welfare of the hens, primarily according to the concept of affective state (suffering) and biological function (health). However, a large variation between flocks indicates that there is potential for improvements (Hegelund et al., 2003; Knierim, 2006).

Mortality cannot stand alone in relation to an assessment of the welfare, as a low mortality is not necessarily an indicator of good welfare. Mortality is not only a welfare problem for the hens that die, but can also be a problem for the remaining hens. Death is often the end-point of a period of suffering (the affective state) and other welfare problems, which the living hens in the flock may experience without dying (Weeks & Nicol, 2006). This could be predators' attacks, where the remaining hens may experience stress and fear during and after the attack, or death due to cannibalism, where the problem is present before the first death occurs and continues after the first hens die.

Mortality in the Danish organic egg production has decreased from year 2000 to 2003 and has been stable since (Figure 1), and is on the same level as other alternative production systems but around twice as high as the mortality in cage production. This difference in mortality between cage and alternative production system has previously been reported (Häne et al., 2000; Rodenburg et al., 2008). The mortality in Danish organic egg production has been around 7-10% the last 10 years when looking at the entire laying period (Anonymous, 2012c; Anonymous, 2013; Anonymous, 2014e). For the production period until 60 weeks of age, mortalities are reported from 4.7 to 12% in systems with outdoor access and between 7.2 and 22.5% for the entire laying period (Table 2). Two Danish studies reported mortalities of more than 20% (Hegelund et al., 2006b; Stokholm et al., 2010). These studies were conducted around year 2000-2003, where mortality was higher than today (Figure 1). The mortality until 60 weeks of age was in most cases lower than the mortality for the entire laying period (Table 2). This can be due to an increased mortality as the hens get older (Sherwin et al., 2010). However, behind the average mortality presented in Figure 1 and Table 2, there is a large variation between flocks (Hegelund et al., 2006b), indicating a potential for improvements.
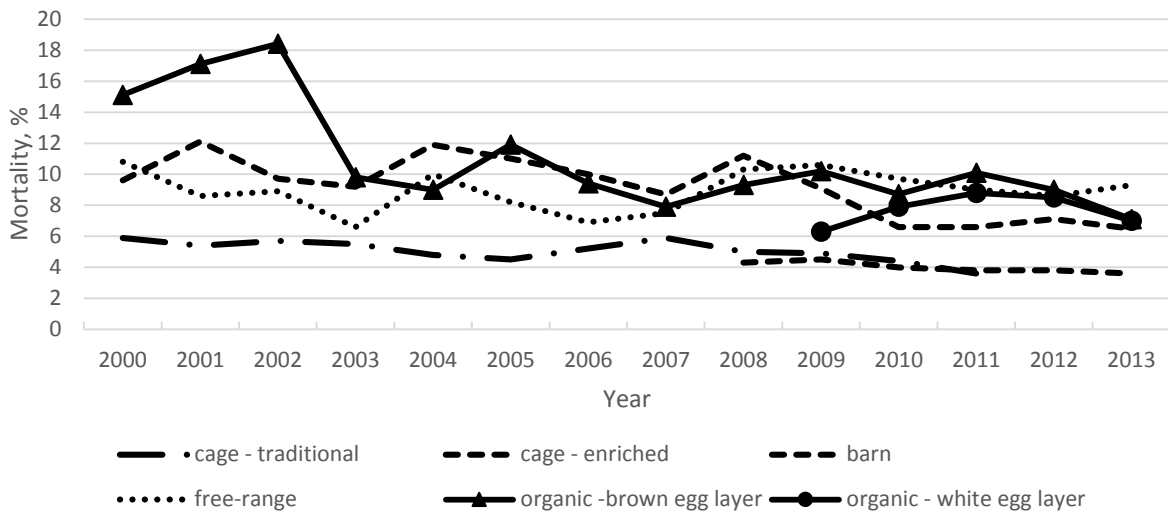
Figure 1: Mortality in Danish egg production since 2000 in the different production system based on the Danish efficiency control programme for the entire production period (Anonymous, 2012c; Anonymous, 2013; Anonymous, 2014e)

Table 2: Average mortality percentage under commercial settings in systems with outdoor access, based on producer registrations on farm or questionnaire until 60 weeks of age or for a complete laying period.

Mortality	Recorded for/at	System	Country	Reference
4.7%	60 weeks of age	organic	France	Leenstra et al. (2012)
4.9%	60 weeks of age	free-range	France	Leenstra et al. (2012)
5.9%	60 weeks of age	free-range	Switzerland	Leenstra et al. (2012)
6.6%	60 weeks of age	organic	Switzerland	Leenstra et al. (2012)
6.6%	60 weeks of age	free-range	Netherlands	Leenstra et al. (2012)
7.8%	60 weeks of age	organic	Netherlands	Bestman & Wagenaar (2014)
8%	60 weeks of age	free-range	Belgium, Germany, Netherlands	Rodenburg et al. (2008)
12.0%	60 weeks of age	organic	Netherlands	Leenstra et al. (2012)
7.2%	laying period	free-range	Austria	Sommer (2001)
8.0%	laying period	free-range	United Kingdom	Whay et al. (2007)
8.1%	laying period	free-range	United Kingdom	Moberly et al. (2004)
9%	laying period	organic	Netherlands	van der Meulen et al. (2007)
14%	laying period	free-range	Germany, Netherlands, United Kingdom	Croxall & Elson (2007)
20.8%	laying period	organic	Denmark	Stokholm et al. (2010)
22.5%	laying period	organic	Denmark	Hegelund et al. (2006b)

There are several causes for the higher mortality in the organic production. Predation has been reported by Danish producers to account for 0 to 14.2% in one study (Hegelund et al., 2006b), and from 0 to 3.7% in another study (Stokholm et al., 2012), while a British study reported 2% loss due to fox predation in free-range egg production (Moberly et al., 2004). Predation was the primary cause of mortality in around 12% of the rearing facilities with organic pullets (Sparks et al., 2008). Predation can both be ground predators, i.e. fox, and predator birds. The predation loss may be higher than actual reported, as when calculating the mortality based on the difference between the number of placed hens and slaughtered hens, the mortality was between 2 and 5% higher than the mortality based on the producer's recordings (Hegelund et al., 2006b; Stokholm et al., 2010). This indicates that the loss due to predation may be higher than what the producer reports, as there was no clear explanation of why the hens were missing in the producer's recordings. However, the missing hens could also be due to cannibalism or missing registration.

In a study of the cause of death in Danish organic egg production, *Escherichia coli* was the most common cause of mortality (Stokholm et al., 2010). *E. coli* was present in all flocks, and 40.5% of the dead hens submitted for post mortem examination had *E. coli* infections. Fossum et al. (2009) also reported *E. coli* infections as the dominating disease in cage, barn or free-range (incl. organic) production systems.

Some management practices affect the mortality. The choice of breed and genetic material may affect the mortality (Whay et al., 2007; Kaufmann et al., 2011a; Nordquist et al., 2011). Kaufmann et al. (2011a) reported a significant difference between mortality in two breeds raised in the same environment. Cannibalism is another common cause of mortality in systems with outdoor access (Fossum et al., 2009; Stokholm et al., 2010), but is also a common cause in non-organic systems (Tablante et al., 2000). An experimental study by Kjær & Sørensen (2002), with hens from 17 to 43 weeks of age and four breeds, found that the mortality from cannibalism was higher in one breed compared to the other three. ISA Brown had 17.5% mortality from cannibalism while New Hampshire, White Leghorn and cross between New Hampshire and White Leghorn showed mortality from cannibalism ranging from 0 to 2.4%. In this experiment, the overall mortality was between 3.9 and 21.3%. In a second experiment by Kjær & Sørensen (2002), the overall mortality for all breeds (ISA Brown, Lohmann LSL, and Danish landrace) was 2.4%. The variation in mortality from cannibalism, and mortality in general, can vary from one batch to another without any reason as seen in both experimental studies and on commercial farms (Kjær & Sørensen, 2002; Hegelund et al., 2006a).

Another management practice that affects mortality could be stocking density. In an experiment with single-tiered system without outdoor access, the morality depended on the stocking density, with higher mortality in flocks with seven or nine hens per m^2 compared to flock with 12 hens per m^2. However, without recording the cause of death, it is difficult to know how the stocking density affects mortality (Nicol et al., 2006). Keeping a good plumage cover can keep mortality at a low level, as the higher mortality the worse plumage damage (Whay et al., 2007; Bright et al., 2011). Nicol et al. (2006) reported that an increased mortality correlated to badly worn feathers, with small nude areas or worse. Plumage damage can occur due to feather pecking (see section 2.5.1 Plumage condition), and feather pecking is positively correlated with cannibalism (Cloutier et al., 2000) and can develop into vent pecking (Potzsch et al., 2001). There is an association between diseases and vent and feather pecking (Green et al., 2000; Potzsch et al., 2001) and between mortality and vent pecking (Potzsch et al., 2001). This indicates, as postulated by Nicol et al. (2006), that there are common risk factors between plumage damage and diseases that contribute to an increased mortality.

2.4 Helminth infection

The organic production with access to free-range and a littered area enhances the risk of endoparasite infections, as they are potential infection sources (Bray & Lancaster, 1992; Heckendorn et al., 2009; Maurer et al., 2009). There are several endoparasites of poultry (Permin & Hansen, 1998; McDougald, 2013); however, not all are equally important for the commercial poultry production. *Ascaridia galli* and *Heterakis gallinarum* are the most common helminths (Permin et al., 1999; Kaufmann & Gauly, 2009; Zeltner & Maurer, 2009; Kaufmann et al., 2011b; Sherwin et al., 2013). The life cycle of *A. galli* and *Heterakis* sp. is direct and embryonates outside the host in the environment (Table 3; life cycle illustrated in section 3.1.2 (Figure 3)). Diagnose of *A. galli* and *Heterakis* sp. is based on examination of faecal samples or direct identification of worms in the intestine (Permin & Hansen, 1998, see section 3.1.2 for details). The eggs of *A. galli* and *Heterakis* sp. (*A. galli/Heterakis*) have similar size and shape, therefore is it difficult to distinguish eggs of the two species in faecal samples (McDougald, 2013).

H. gallinarum is important, as it acts as an intermediate host for *Histomonas meleagridis* that causes blackhead disease (Permin & Hansen, 1998; McDougald, 2005; Hess & McDougald, 2013; McDougald, 2013). Histomonasis or blackhead disease causes ulceration and inflammation of the cecal walls and severe necrosis at a level that can result in death (Permin & Hansen, 1998; Hess &

Table 3: Description of different stages of the life cycles of *Ascaridia galli* and *Heterakis* sp. (Permin & Hansen, 1998).

	Ascaridia galli	*Heterakis* sp.
Life cycle	direct	direct
Mechanical transport host	earthworm	earthworm and houseflies
Embryonation[a]	3 weeks	2 weeks
Prepatant period[b]	5-8 weeks	24-30 days
Mature worm in	small intestine	caeca

a: under optimal condition (temperature and humidity)

b: interval between the hens are infected and eggs or larvae can be recovered

McDougald, 2013). A review by McDougald (2005) states that the mortality in chickens is 10 to 20% with a high morbidity; however, the outbreak can also pass wihout notice. A Danish study reported blackhead in 6 out of 15 organic layer flocks (Stokholm et al., 2010). A random sample of dead hens was submitted from the participating farms for post mortem exmination, and 2-23% of the hens from the blackhead positive flock were affected by blackhead. Also, increased mortality was observed in these flocks. The prevalence of blackhead was 6% of all hens submitted for post mortem exmination, which is higher than the reportings of Kaufmann-Bart & Hoop (2009) from Switzerland, who found that 0.4 and 1.1% of the dissected hens during a period of 12 years were diagonsed with blackhead. Morbidity and mortality due to blackhead are threats against the health and welfare of the animals.

There are several documented consequences of *A. galli* infections. *A. galli* can cause reduced body weight gain (Ackert & Herrick, 1928; Reid & Carmon, 1958; Ramadan & Abou Znada, 1991; Permin & Ranvig, 2001; Kilpinen et al., 2005; Schwarz et al., 2011) and diarrhoea (Ackert & Herrick, 1928; Ikeme, 1971; Ramadan & Abou Znada, 1991; Permin & Ranvig, 2001), and feed intake can either decrease (Ackert & Herrick, 1928; Ikeme, 1971) or increase (Gauly et al., 2007). Changes in plumage condition as ruffled feather (Ackert & Herrick, 1928; Ikeme, 1971; Ramadan & Abou Znada, 1991) and loss of brightness (Ramadan & Abou Znada, 1991) can occur due to an *A. galli* infection. A questionnaire study among 198 flocks in alternative production systems reported an association between feather pecking and intestinal worms (Green et al., 2000). The hens can further show traits like lower activity (Ikeme, 1971; Gauly et al., 2007), increased nesting (Gauly et al., 2007), weakness (Ackert & Herrick, 1928; Ikeme, 1971; Ramadan & Abou Znada,

1991) and blockage of the intestinal lumen, which can lead to death from starvation (Ikeme, 1971; Ramadan & Abou Znada, 1991). Ikeme (1971) describes that the *A. galli* infected chickens became emaciated, ate rarely, developed leg weakness and consequently died naturally or died due to cannibalism. Ackert & Herrick (1928) reported an increased mortality in the *A. galli* infected hens compared to non-infected hens with 15% and 2% mortality, respectively.

A. galli can also function as a vector for *Salmonella* (Chadfield et al., 2001). The egg production can be decreased during dual infections (Dahl et al., 2002). Studies of dual infections between *A. galli* and bacterial infections, including *E. coli*, *Pasteurella multocida* and *Salmonella*, showed that the dual infection seems to affect the hens more severely compared to the bacterial infection alone (Dahl et al., 2002; Eigaard et al., 2006; Permin et al., 2006). This can be due to an immune response to the *A. galli* infection (Cox, 2001; Hørning et al., 2003; Degen et al., 2005; Schwarz et al., 2011; Andersen, 2013), with a downward regulation of Th1 (T-helper type 1) immune responses and up-regulation of Th2 (T-helper type 2) immune response (Cox, 2001; Andersen, 2013). The Th2 immune response is suggested to be involved in the control of *A. galli* (Norup et al., 2013), while Stokholm et al. (2010) mention that the suppression of the Th1 immune response may favour establishment of a bacterial infection. It has also been reported that *A. galli* infections may influence the effect of vaccinations for Newcastle disease (Pleidrup et al., 2014).

The infection with *A. galli* fluctuates during the production period under both commercial and experimental settings (Höglund & Jansson, 2011; Norup et al., 2013), and between season, with higher infection burden during summer than winter (Kaufmann et al., 2011b; Sherwin et al., 2013). Under commercial settings, the first excretion of *A. galli* eggs was around six to eight weeks after placement (Maurer et al., 2009; Höglund & Jansson, 2011) and maximum infection is achieved 8 to 12 weeks later i.e. around peak of lay (Pennycott & Steel, 2001; Höglund & Jansson, 2011). However, this depends on the level of contamination in the system (inside and outside). This was confirmed under experimental condition, where Marcos-Atxutegi et al. (2009) found that the first excretion of *A. galli* eggs occurred six weeks after the hens were orally infected with an increase excretion of eggs until 12 weeks after infection, followed by a decrease until the end of the experiment 15 weeks after infection. Permin & Ranvig (2001) did not report *A. galli* egg excretion until four weeks after infection.

Parasitological findings can be presented in several ways: prevalence, which is the percentage of the sampled population with a given parasite species; abundance, which is the mean number of a given parasite in all hens examined (infected and non-infected hens); mean intensity, which is the mean number of a given parasite in infected hens (Margolis et al., 1982). The prevalence of *A. galli/Heterakis* infection in commercial free-range systems is high (Table 4; Appendix 4; Appendix 5), and the high prevalence of the infection indicates that most hens are subclinically infected (Kaufmann & Gauly, 2009; Kaufmann et al., 2011b). Kaufmann et al. (2011a) found a significant difference in prevalence and worm burden between breeds in the same environment, which may be caused by different levels of genetic resistance (Permin & Ranvig, 2001; Kaufmann et al., 2011a; Norup et al., 2013).

Table 4: Prevalence of *Ascaridia* sp. and *Heterakis* sp. in commercial organic or free-range system, in relation to age and evaluation method (post mortem worm count (PM) or faecal egg count (FEC))

Prevalence (%), species		System	Age	Method	Reference
63.8%	*A. galli*	free-range/organic	40	PM	Permin et al. (1999)
66.6%	*A. galli*	free-range		PM	Kaufmann & Gauly (2009)
88%	*A. galli*	Organic	54-72	PM	Kaufmann et al. (2011b)
84%	*Ascaridia*	free-range	65	FEC	Sherwin et al. (2013)
0%[a]	*A. galli/H. gallinarum*	Organic	50-60	FEC	Bestman & Wagenaar (2014)
77.1%	*A. galli/H. gallinarum*	free-range/organic	≥25	FEC	Jansson et al. (2010)
43%	*A. galli/H. gallinarum*	free-range	20	FEC	Pennycott & Steel (2001)
61%[b]	*A. galli/H. gallinarum*	Organic	50-60	FEC	Bestman & Wagenaar (2014)
62%	*A. galli/H. gallinarum*	free-range	33	FEC	Pennycott & Steel (2001)
79%	*A. galli/H. gallinarum*	free-range	46	FEC	Pennycott & Steel (2001)
81%	*A. galli/H. gallinarum*	free-range	59	FEC	Pennycott & Steel (2001)
83%	*A. galli/Heterakis*	Organic	50-60	FEC	Bestman & Wagenaar (2014)
89%	*Heterakis*	free-range	65	FEC	Sherwin et al. (2013)
72.5%	*H. gallinarum*	free-range/organic	40	FEC	Permin et al. (1999)
84%	*H. gallinarum*	free-range		FEC	Kaufmann & Gauly (2009)
98%	*H. gallinarum*	Organic	54-72	FEC	Kaufmann et al. (2011b)

a: dewormed as recommended (recommendation: Flubenol© every six weeks) (Bestman & Wagenaar, 2014)

b: dewormed less than recommended (recommendation: Flubenol© every six weeks) (Bestman & Wagenaar, 2014)

The morbidity caused by the *A. galli*/*Heterakis* infections might be the most important problem in relation to both health and welfare of the hens, compared to the mortality of few infected hens. Knierim (2006) states that as long as the endoparasite infection remains low, it is not necessarily a threat to animal welfare. However, the infection can also be seen as a risk factor for animal welfare despite of the infection level (Lund & Algers, 2003; Lund, 2006), and there is a risk that the consumer buys an egg with a parasite worm inside (Reid et al., 1973). When relating the three animal welfare concepts to a helminth infection with *A. galli*/*Heterakis*, the welfare of the hens could theoretically, be impaired in all three concepts.

A helminth infection, despite of the level, will affect the welfare of the hens seen from the concept of biological function, as subclinical infections might increase morbidity, and as stated earlier, increase the severity of other diseases. A hen could be subclinically infected and have an acceptable welfare in the concept of natural living, as infections is a part of the natural living condition for hens and will not limit the hen's possibility of performing natural behaviour. However, if the possibility of performing natural behaviour is reduced due to weakness and impaired locomotion behaviour, as stated above, the welfare will not be acceptable in the concept of natural living. The concept of affective state will potentially not be affected as long as the infection is subclinical. However, the welfare will be impaired when clinical signs are present or the infection level is high, as a high infection level with risk of obstruction of the intestine, due to the fact that a high worm level would cause pain and suffering to the hens.

2.5   Clinical welfare indicators

The clinical welfare indicators, which measure the individual hen's appearance at the time of the assessment, investigated in this thesis are condition of plumage, keel bone, feet, skin and comb. In previous studies, these have been concluded to be reliable, feasible and valid to be included in a welfare assessment (Tauson et al., 2005; Arnould et al., 2009).

2.5.1   Plumage condition

Plumage condition is often used as an important welfare indicator in poultry production (Bilcik & Keeling, 1999; Bartussek, 2001; Bestman & Wagenaar, 2003; Hegelund et al., 2003; Hermansen et al., 2005; Weeks et al., 2006; Whay et al., 2007; Welfare Quality, 2009; Savory & Hughes, 2010; Main et al., 2012; Anonymous, 2014d). Different methods can be used to assess the plumage condition (Tauson et al., 1984; Bilcik & Keeling, 1999; Bestman & Wagenaar, 2003; Tauson et al.,

2005; Mielenz et al., 2010); however, they all assess the damage to the plumage. Plumage damage can be missing and untidy feathers together with bare areas along the main shaft (rachis). The damage can be caused by abrasion against equipment (Menke et al., 2004; EFSA, 2005); however, the main cause of plumage damage is feather pecking (Bilcik & Keeling, 1999). Feather pecking is the process of pecking at feathers or pulling out feathers on another hen, causing plumage damage or total loss of feathers (McAdie & Keeling, 2000; Bestman & Wagenaar, 2003). The victim ignores gentle pecks and these gentle pecks do not appear to be related with plumage damage (Savory, 1995; Bilcik & Keeling, 1999; Lambton et al., 2010).

Feather pecking is not uniformly directed to the whole body (Figure 2). The tail, rump and back receive most pecks, and the rump and back are the areas to be denuded firstly together with the belly (Gunnarsson et al., 1995; Bilcik & Keeling, 1999). Feather pecking is usually the cause of plumage damage to the tail and rump (Tauson et al., 2005; Welfare Quality, 2009), while the plumage damage to other body parts is due to other causes, i.e. plumage damage to the neck, breast and belly is often due to abrasion (Kjær & Sørensen, 2002; Welfare Quality, 2009).

Feather pecking may reduce the welfare of both the victim and the performer (Berg, 2001; Bestman & Wagenaar, 2003). Wild birds do not perform feather pecking (Menke et al., 2004), therefore feather pecking may be seen as an abnormal behaviour. Feather pecking can be seen upon as a redirected foraging behaviour (Blokhuis, 1986; Huber-Eicher & Wechsler, 1998). Hens that perform feather pecking are genetically related, as results have shown that it is possible to breed for
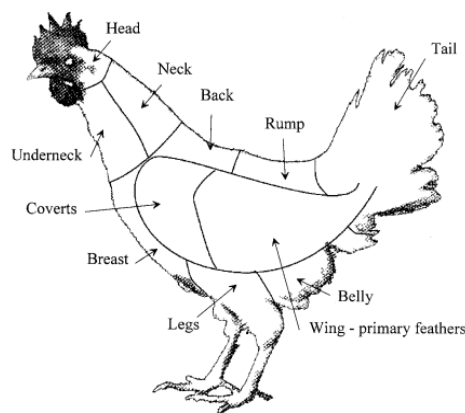
Figure 2: The body parts of a hen (Bilcik & Keeling, 1999)

hens with reduced feather pecking behaviour (Kjær & Sørensen, 1997; Jones et al., 2004; Kjær & Hocking, 2004), and there is an effect of genotype on feather pecking levels (Kjær & Sørensen, 2002).

The victims' welfare may be reduced, as the process of having feathers pulled out is painful (Gentle & Hunter, 1990), and nude areas increase the heat loss and the feed intake necessary to maintain body temperature (Leeson & Morrison, 1978; Tauson et al., 2006). The nude areas are postulated to make the hens more sensitive to cold weather (Hermansen et al., 2005; Hegelund et al., 2006a). Hens that are victim of feather pecking or damage due to abrasion are more susceptible to be further pecked (McAdie & Keeling, 2000). It has also been stated that feather pecking can spread between hens (McAdie & Keeling, 2000); however, other studies have not been able to confirm this (McAdie & Keeling, 2002). An association between stress and feather pecking is reported (El-Lethey et al., 2000), meaning that stress could increase the level of feather pecking.

Several studies have investigated the potential risk factors for plumage damage or specific feather pecking behaviour; below, some of the reported risk factors are stated:

- Risk factors associated with the rearing period: housing condition during the rearing period (Green et al., 2000; van de Weerd & Elson, 2006; Knierim et al., 2008; Bestman et al., 2009; Gilani et al., 2013) and age at placement (Bestman & Wagenaar, 2003)
- Risk factors associated with the production site; group size (Hughes & Duncan, 1972; Bilcik & Keeling, 1999), stocking density (Nicol et al., 2006; Zimmerman et al., 2006) and range use (Bestman & Wagenaar, 2003; Nicol et al., 2003; Mahboub et al., 2004; Lambton et al., 2010)
- Risk factors related to management and housing design; hybrids (Hughes & Duncan, 1972), farmers' experience (Niebuhr et al., 2005), design and placement of feeders (Green et al., 2000; Drake et al., 2010), distribution of daylight (Niebuhr et al., 2005) and light intensity (Hughes & Duncan, 1972; Drake et al., 2010).
- Environmental enrichment, like attractive range area (Bestman & Wagenaar, 2003) or strings (Jones et al., 2004), has also been reported as potential ways to decrease feather pecking.

This indicates that the feather pecking behaviour and the condition of the plumage, due to abrasion or feather pecking, are multi-factorial problems.

When assessing plumage condition, both feather pecking and abrasion are assessed, the victim is mainly assessed directly and the performer assessed indirectly. Plumage condition is a welfare indicator in relation to the three welfare concepts presented earlier (natural living, affective state

and biological function). The concept of biological function of the victim is affected because of physiological changes due to the featherless area, and the biological function of the performer is affected because of the non-coping with the environment, which leads to feather pecking. The concept of affective states is affected, as having feathers pulled out is a painful and negative experience. The concept of natural living is affected mainly because wild birds are not performing the behaviour, indicating that it is an abnormal behaviour.

Results from assessment of feather pecking or plumage damage on organic commercial farms (Table 5 and Appendix 4 for results from welfare assessments conducted during this PhD project) indicate that the level of severe plumage damage has decreased.

2.5.2 Keel bone deformities

In recent years, the attention on keel bone deformities as a welfare indicator has increased (EFSA, 2005; Whay et al., 2007; Clark et al., 2008; Kappeli et al., 2011). The keel bone is with its anatomical location exposed and especially prone to damage (Fleming et al., 2004; Kappeli et al., 2011). The deformities can be deviation and/or fractures. The prevalence of keel bone deformities is

Table 5: Percentage of flocks (or producers in case of questionnaire study) with feather pecking or plumage damage on organic commercial farms, related to year of the study and country.

Flocks with	Percentage	Year	Country	Reference
feather pecking	45%[a]	2000	Sweden	Berg (2001)
	30%[a]	2003	Netherlands	van der Meulen et al. (2007)
	19%	2002-2004	Austria	Niebuhr et al. (2005)
severe plumage damage	52%	1999-2001	Netherlands	Bestman & Wagenaar (2003)
	22%	2000-2003	Denmark	Hegelund et al. (2006b)
	8%	2008-2009	Netherlands	Bestman & Wagenaar (2014)
moderate plumage damage	19%	1999-2001	Netherlands	Bestman & Wagenaar (2003)
	39%	2001-2004	Denmark	Hermansen et al. (2005)
	24%	2008-2009	Netherlands	Bestman & Wagenaar (2014)
little or no plumage damage	29%	1999-2001	Netherlands	Bestman & Wagenaar (2003)
	34%	2000-2003	Denmark	Hegelund et al. (2006b)
	68%	2008-2009	Netherlands	Bestman & Wagenaar (2014)

a: questionnaire study (presented as percentage of producers with feather pecking hens)

Table 6: Percentage of flocks with keel bone deformities (deviation and fracture) or keel bone fractures on commercial farms, related to age of the hens and production system

Percentage, flocks with		Age	Production	Method	Reference
50-76%	fractures	end of lay	free-range	post mortem	Wilkins et al. (2004)
62-68%	fractures	end of lay	barn	post mortem	Wilkins et al. (2004)
52-65%	fractures	end of lay	barn	post mortem	Nicol et al. (2006)
82-97%	fractures	60 weeks	free-range	palpation[a]	Rodenburg et al. (2008)
25%	deformities	end of lay	barn, free-range	palpation[b]	Kappeli et al. (2011)
3-17%	deformities	70 weeks	cage	post mortem	Fleming et al. (2004)
21%	deformities	50-60 weeks	organic	palpation[a]	Bestman & Wagenaar (2014)
64-72%	deformities	65 weeks	free-range	palpation[a]	Richards et al. (2012)
59-65%	deformities	55 weeks	free-range	palpation[a]	Richards et al. (2012)
42-57%	deformities	45 weeks	free-range	palpation[a]	Richards et al. (2012)
24-27%	deformities	35 weeks	free-range	palpation[a]	Richards et al. (2012)
5-6%	deformities	25 weeks	free-range	palpation[a]	Richards et al. (2012)

a: palpation conducted on living hens

b: palpation conducted on both living and dead hens

highest in an alternative system (non-cage system) (Sandilands et al., 2009; Table 6), despite the fact that, exercise can increase the bone strength (Whitehead, 2004) and that there are more possibilities for moving in the alternative systems.

The keel bone can be deviated or twisted, both vertically and horizontally, having an s-shaped appearance, bumps or notches (Kappeli et al., 2011). Long-term pressure on the keel bone during roosting can result in a deviation (EFSA, 2005) and could potentially be linked to the roosting site, either perches or unintended places (Tauson, 2005). The design of the perches is a risk factor for development of deviations (Tauson & Abrahamsson, 1994). Bone weakening can further be a potential cause for deviations (Fleming et al., 2004; Bestman & Wagenaar, 2012). In case of severe deviations, the attachment of breast muscle to the keel bone can be affected, impairing the movement (Tauson et al., 2006), and become a welfare issue.

The cause of keel bone fractures can be collision with equipment in the hen house or other hens (Freire et al., 2003; Sandilands et al., 2009), which is more likely to occur for hens with weak bones (Fleming et al., 2004). The bone fractures are painful for the hens (Clark et al., 2008; Kappeli et al.,

2011), and during the following period with healing, it can result in constraints on the movement because of pain and discomfort or the actual physical appearance of the break (Richards et al., 2011).

Keel bone deformities can be a combination of deviation and fractures, as they can be present at the same time. Keel bone deformity is primarily a welfare indicator in relation to the concept of affective state and the concept of biological function, and a minor indicator in relation the natural living concept. The concept of affective state is affected, as keel bone deformities are painful and may impair movement due to severe deviation or fractures. The concept of biological function is affected as the health of the hens could be impaired due to limited access to feed and water caused by the impaired movement, and deviation due to roosting indicates coping problems. The concept of natural living could also be affected as the deformities potentially will constrain the hen from performing normal behaviour.

Results from assessment of keel bone deformities on commercial farms are presented in Table 6, and results from assessment of keel bone deformities conducted during this PhD project in Appendix 4.

2.5.3 Condition of skin, feet and comb

Damage to the skin may be a result of pecking behaviour or accidents resulting in scratches or larger wounds (Welfare Quality, 2009; Bestman & Wagenaar, 2014). As for plumage condition, pecking behaviour resulting in damage to the skin could reduce the welfare of the hens in relation to the concept of affective state. Also, the skin damage might potentially increase the risk of infections (Bestman & Wagenaar, 2014), which would impair the welfare in relation to the biological function. The back (incl. rump) and belly are the areas that are denuded firstly (Bilcik & Keeling, 1999), and thus will also be more exposed to skin damage.

The condition of the feet is often separated into damage on the footpad and the toes/claws (Gunnarsson et al., 1995; Welfare Quality, 2009). Toe damage includes wounds or incidences where a part of a toe or claw is missing. This may be caused by the design of the equipment leading to injuries of one or more toes or claws being torn off (Welfare Quality, 2009). In either case, the hens will experience pain and a reduced welfare in relation to the concept of affective state. The skin on the footpad should have a smooth appearance without lesions, thickening of the skin (hyperkeratosis) or swellings, called bumble foot (Welfare Quality, 2009). Bumble foot starts with minor swelling due to inflammation or lesions and can developed into a heavily swollen foot. The

abnormalities to the footpad might constrain the hen's movement and therefore affect the concept of natural living. The abnormalities are painful and will therefore affect the concept of affective state and impair the health of the hens, thus affecting the concept of biological function.

The condition of the comb can be evaluated in relation to different aspects, i.e. wounds, colour and other abnormalities. Comb wounds might be a result of aggressive pecks to the comb or be due to mating behaviour in flocks with cocks (Arnould et al., 2009), and dehydrated hens may have a comb that have blue coloration (Welfare Quality, 2009). The comb normally has an even red colour, and a pale comb colour can indicate that the hens are anaemic or have other health problems (Welfare Quality, 2009; Bestman & Wagenaar, 2014), and there is an association between free range use and a dark red comb (Whay et al., 2007; Bestman & Wagenaar, 2014). In the present study, the comb colour (pale or normal) was included as a welfare indicator, as a pale comb may reflect the health status of the hens (concept of biological function) and the paleness might be caused by suffering (concept of affective state). To some extent, the pale comb colour is related to the concept of natural living, as pale comb colour might be an indication of constraints in the hen's life, i.e. lack of outdoor access, whereas outdoor access potentially could result in a darker red comb.

3 Methodology

This PhD project was conducted in collaboration with the CORE organic II project HealthyHens (CORE organic HealthyHens project, 2014). HealthyHens aims to promote good health and welfare in organic laying hens and included an observational study of 111 flocks from eight participating countries: Austria, Belgium, Denmark, Germany, Italy, the Netherlands, Sweden and United Kingdom. The PhD project was conducted simultaneously with the HealthyHens project on the Danish farms.

The study included organic egg producers, and recruitment of participating farms was based on a set of exclusion criteria and voluntary participation. Each country was responsible for recruitment of participants in their country. The exclusion criteria were:

- flocks including different hybrids
- beak treated flocks (not possible in all countries, no Danish flocks were beak treated)
- farms with less than 500 hen places
- farms with own pullet rearing (not possible in all countries, no Danish participating farms had own pullet rearing)
- mobile housing systems with relocation more frequently than every two weeks (i.e. mobile housing could be included, but the houses should be located at the same place for minimum two weeks)

Recruitment of Danish flocks was initiated by sending an invitation to all organic egg producers in Jutland and Funen registered in the CHR register (Central Husbandry Register) in February 2012, which fulfilled the criteria of having more than 500 hen places and without pullet rearing. The invitation was thereby sent to 44 producers with a reply rate of 59%. In the invitation, the producers were informed that the project was related to animal health and welfare, with a focus on mortality and endoparasites among other subjects in the HealthyHens project. The aim was 15 participating flocks, and based on the replies, 11 participating flocks were achieved. The remaining four flocks were recruited by approaching the producers that did not respond to the initial invitation by phone. Once 15 flocks were achieved, the recruitment process was finalised.

The 15 Danish producers were geographically spread all over Jutland. With 66 organic egg producing farms in Denmark (Anonymous, 2013), slightly more than one fifth of the Danish producers were represented (22.7%). The farms differed in size (Table 7), and the average number of hens on all farms was 11,990 (median 10,500). The majority of the farms had a single-tiered

Table 7: Distribution of farm sizes, housing system, veranda and hybrid

	Categories	Number of farms	Percentage of farms
Size of farm	≤ 3000	1	6.7%
	3001-9000	6	40.0%
	9001-15000	6	40.0%
	>15000	2	13.3%
Housing system	Single-tiered	11	73.3%
	Multi-tiered	4	26.7%
Covered veranda	Yes	10	66.7%
	No	5	33.3%
Hybrids	Lohmann LSL	6	40.0%
	Lohmann LB-lite	4	26.7%
	Hisex White	5	33.3%

system and a veranda, and three different hybrids were represented on the farms (Table 7; Appendix 1). At the beginning of the study, the producers had been organic egg producers for a minimum of 5 years and a maximum of 17 years (median 13 years).

The three hypotheses (section 1.1) were investigated in three studies (Table 8). As seen in Table 8, not all 15 farms were included in all three studies, and none of the producers dropped out of the study after recruitment. Of the 15 farms, one was omitted in all three studies, while 14 farms were included in minimum one study (see Appendix 1). Reasons for omitting farms from each study are explained later for each study. The observational study (mortality/helminth study, named Study I) and the longitudinal study (helminth/clinical welfare indicator study, named Study II) were conducted simultaneously on the farms, while the qualitative study (management study, named Study III) was conducted after finalising the data collection for the mortality and individual hen studies (Table 8). The methods of each of the three studies are described in more detail in the papers (Chapter 4: Results). In the sections below, some fundamental methodological information and consideration are given for each study.

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Table 8: Overview of the data material for the three studies

	Study I	Study II	Study III
Hypothesis no.	Hypothesis 1	Hypothesis 2	Hypothesis 3
Included in	Paper I	Paper II	paper III
Study design	Observational, event	Longitudinal	Qualitative interview
No. of farm included[a]	11 farms	12 farms	7 farms
Registration at peak of lay	Faecal samples (20 samples per farm)	Clinical assessment and tagged hen with leg brands (100 hens per farm)	
Registration at end of lay		Clinical assessment of 20 tagged hens	
Registration after slaughtering	Weekly mortality data	Faecal samples (tagged hens)	Qualitative interview

a: description of included farms are presented in Appendix 1

3.1 Observational event study (Study I)

The aim of Study I was to investigate the effect of an *A. galli*/*Heterakis* infection on the mortality at peak of lay in organic egg production in Denmark. To address the situation currently present on Danish farms, an observational event study was performed. In an observational study, there are no interventions and no control of events (Ersbøll et al., 2004), which makes it possible to investigate the actual situation on the farms. Therefore, the results are also directly usable on farms (Ersbøll & Toft, 2004; Dawkins, 2012). This is not always the case with experimental studies, where an intervention with the applied treatments is included, as the uniform condition present during the experiment might not be present on farms in praxis (Ersbøll & Toft, 2004; Dawkins, 2012). Despite the advantage of an observational study being able to investigate the actual situation on farm, the lack of control during the study, and the potentially higher variation between animals, may be a disadvantage (Ersbøll & Toft, 2004; Ersbøll et al., 2004).

Study I included determination of the *A. galli*/*Heterakis* infection level and collection of mortality data and investigation of the association between *A. galli*/*Heterakis* infection and mortality using a survival analysis model. Methodological considerations and choices are presented below, together with the theory of survival analysis.

3.1.1 Mortality recording

Mortality data can be collected in different ways, but is in general based on the producer's own recordings. Database recording can be easy to collect and use, if the information is complete. Thomsen et al. (2006) used the Danish Cattle Database as a basis for their records of number of dead cows, and this database is almost 100% complete, as the farmers are required to report to the database. A similar database and requirement do not exist for poultry production. The poultry sector has a management tool, called the Danish efficiency control, which is based on voluntary registrations. Due to the voluntary participation, the efficiency control database does not include all farms. In 2012, 34% of the organic eggs delivered at the egg packing centres were registered in the efficiency control database (Anonymous, 2013); indicating that this source is not reliable and potentially would not include data from the participating farms. However, results from efficiency control can be used as a reference of the mortality in Denmark.

The producers are required by EU regulations to record the daily number of dead hens (Anonymous, 1998), and this recording has previously been used (Hegelund et al., 2006b; Stokholm et al., 2010; Leenstra et al., 2012). In order to determine the overall mortality from placement to slaughtering, the number of placed and sold hens can be used (Hegelund et al., 2006b; Stokholm et al., 2010). A comparison of the two methods showed that between 2 and 5% of the hens were missing in the producers daily registrations when compared to the difference between placed and slaughtered (Hegelund et al., 2006b; Stokholm et al., 2010). This means that the overall mortality was higher when using the difference between placed and slaughtered compared to the daily registrations, which could be a result of predation, cannibalism and lack of registration. In Study I, we focused on the time around peak of lay, and therefore it was impossible to use the difference between placed and slaughtered hens, as this holds little or no information about the specific period. The producer's daily recordings of number of dead hens were then summarised into weekly mortalities. From 11 farms, the mortality data were of a quality that weekly mortality could be generated; the other four farms were completely excluded from Study I.

3.1.2 *A. galli* and *Heterakis* sp. infection

Examination of the level of helminth infections can be conducted using different methods (Permin & Hansen, 1998), where different stages in the life cycle of the helminth infections are examined (Figure 3). The level of infection can be examined based on faecal samples counting the number of excreted helminth eggs, named Faecal Egg Count (FEC) (Figure 3), which is used in the literature
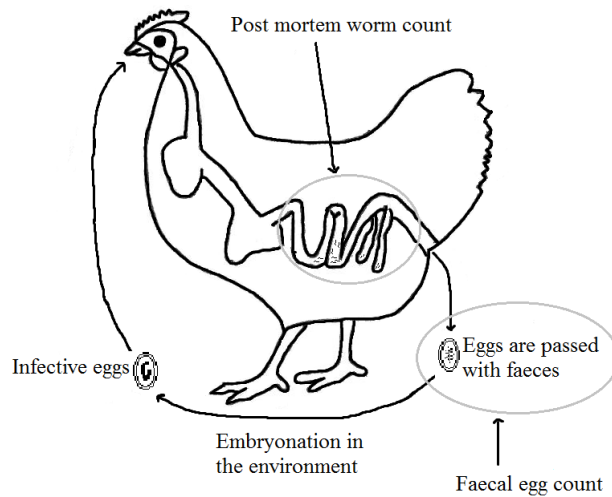
Figure 3: Life cycle of *Ascaridia galli* and *Heterakis* sp. with an illustration of the stages at which the two methods (post mortem worm count and faecal egg count) are used for determination of the infection level

(Pennycott & Steel, 2001; Jansson et al., 2010; Sherwin et al., 2013; Bestman & Wagenaar, 2014). Another method is the post mortem worm count (Figure 3), where the number of worms in the gastrointestinal tract is counted. This is a commonly used method (Permin et al., 1999; Heckendorn et al., 2009; Maurer et al., 2009; Kaufmann et al., 2011b). The FEC method is non-invasive, while the worm count method is invasive, as the hens have to be slaughtered before examination can occur.

The FEC is an indirect measure of the parasite burden, as the method counts the number of parasite eggs passed with faeces. Using the FEC, there is a possibility of false positive or false negative results. False positives are hens that excrete parasite eggs without being infected, as helminth eggs can be ingested and pass the intestines without the establishment of an infection (Permin & Hansen, 1998; Heckendorn et al., 2009; Maurer et al., 2013). False negatives are hens that are diagnosed with no eggs in faeces, but are infected (Torgerson et al., 2012). This may be observed when hens are infected with immature worms (not producing eggs) or adult worms of the same sex (Permin & Hansen, 1998). Another case could be that the sensitivity level is too high to detect the actual infection level (Permin & Hansen, 1998).

A post mortem worm count gives a direct measure of the number of worms in the intestine, and the sex of the worms can be obtained. The advantages of this method are that the number of immature and adult worms can be directly counted, and that the method is more sensitive than FEC

(Permin et al., 1997; Gauly et al., 2001). The main disadvantage is the need for slaughtering the hens before examination. For on-farm studies, slaughter of the hens in order to count the worms is a very important factor, as the producers might need to be economically compensated for the slaughtered hens, or they may not be willing to participate in the project, especially if the hens are young at the time of sampling. Both FEC and post mortem wound count require work hours and laboratory equipment (microscope, stereoscope, McMaster slide, sieves etc.) for examination of the faecal samples or hens. With the mentioned limitation of the two methods in mind, the FEC method was chosen mainly due the non-invasive nature of the method. The purpose of examining the infection level was to categorise the farms as either low or high level infection (see Paper I for more details).

On each farm, 20 individual droppings were collected from multiple random sites from the littered area in one compartment. The droppings were intestinal droppings, which are covered with white uric acid and are light brown (Fuller, 2004). The droppings were stored at 5°C until individually examined using the simple McMaster technique, which gives the number of eggs per gram of faeces (EPG) (Permin & Hansen, 1998). The method has a sensitivity of 50 eggs per gram of faeces, i.e. a dilution factor of 50 and a detection limit of 50 eggs per gram of faeces. Based on all samples from one farm, a farm mean EPG was calculated and used as indicator for the farms' infection pressure (low-infected farm ≤ 200 EPG; highly-infected farm>200 EPG).

3.1.3 Theory of survival analysis

The weekly mortality and FEC results were analysed using a survival analysis model. Survival analysis is the analysis of the time until an event occurs, and the following description is based on the book by Kleinbaum & Klein (2012). The event often referred to as a failure can be death (as in the case of this study), disease incidence or recovery among others. The time of the event, referred to as the survival time, is measured in different units (years, months, weeks, days) depending on the study. Survival analysis can be used to estimate, interpret and compare hazard functions and analyse the relationship between the explanatory variables and the survival time.

The hazard function gives the conditional probability (rate) for an event to occur in a specific time unit (weeks in this case), given that the subject has not experienced the event before the specific time. More precisely, if T is a random variable representing the time measured in weeks (w), the probability (P) that the event occurs at certain week, is the hazard function (h) given by

$$h(w) = P(T = w | T \geq w)$$

A hazard function is defined for each group/treatment, in this case low- or highly-infected with *A. galli/Heterakis*. The ratio between hazard functions is called the hazard ratio (HR), and is used to determine the relationship between hazard functions. The aim of analysing the difference between hazard functions is to analyse if the HR is significantly different from 1. The HR has an interpretation similar to the odds ratio used in logistic regression. If the HR is 1, the interpretation is that there is no effect of treatments/groups, i.e. no difference between the hazard functions. If the HR is significantly different from 1, and the HR is 5, the interpretation is that the hazard is five times higher than the baseline hazard function. The baseline hazard function is the hazard function, which is taken as the reference hazard function.

The probability of surviving in a given time unit can be presented graphically by using Kaplan-Meier (KM) survival curves. An example of a KM survival curve based on data from Paper I is given in Figure 4.
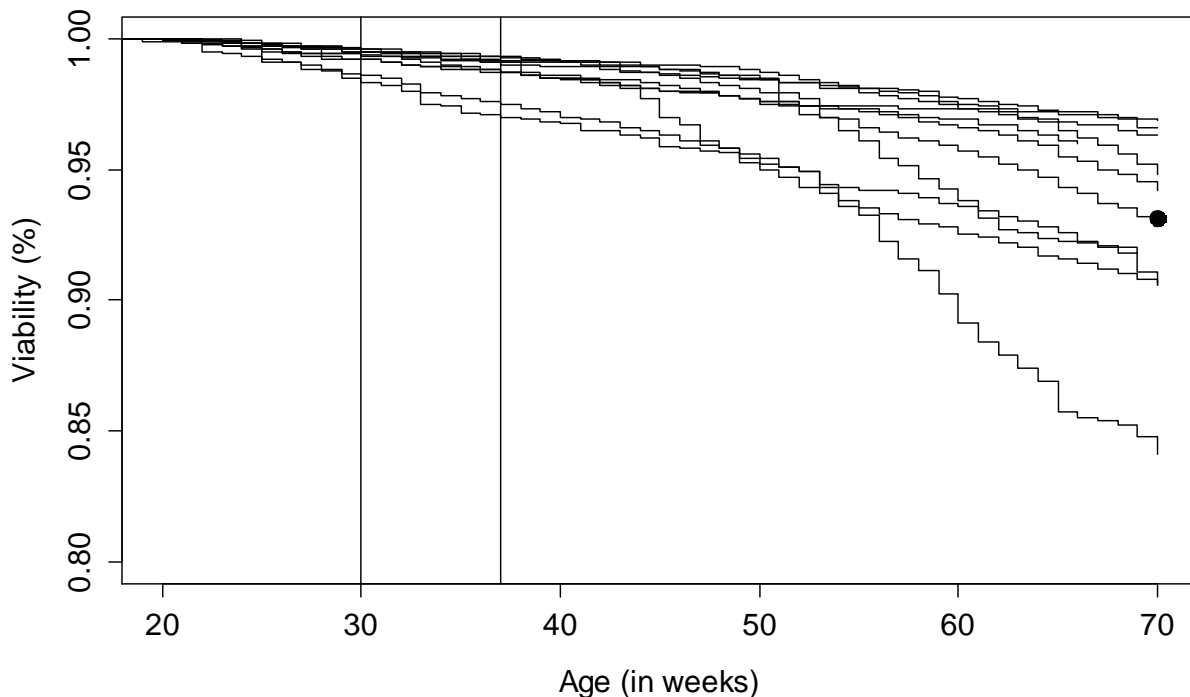
Figure 4: Kaplan-Meier estimate of the survival curves from the 11 Danish farms analysed in paper I from 18 to 70 weeks of age. Each line represents data from one farm, the vertical line represents the interval (30-37 weeks of age) studied in Paper I, and the dot represents the mean viability for the production period until week 70 (i.e. a mean mortality of 6.8% for all 11 Danish farms (range: 3.1-15.9%)).

Survival analysis can handle data where the exact survival time is unknown, which is the process called censuring, and censoring may occur if:

i)   the event does not occur before the study ends (right censoring)

ii)   subjects are lost or withdrawn during the study period (right censoring)

iii)   subjects experience the event before the study period begins (left censoring)

iv)   subjects experience the event in an interval between to observation points in a study with several observation points (interval censoring).

Right censoring is the case where the true survival time is equal to or higher than the time studied. Left censoring is the case where the true survival time is less than or equal to the actual observed survival time, i.e. in case of some diseases, the true survival time will be to the point of exposure, however, the observed survival time may be later (when clinical signs or test results reveal the disease). With the use of censuring in survival analysis, it is possible to use the information up to the point of the censorship, without having to exclude these data points from the analysis.

In addition to censoring, truncation may also be included. Truncation is when the design of the study includes a systematic selection. For instance, data from individuals are only included in the study if they fulfil some predefined requirements. This may be that the subjects should have survived until a certain age.

The survival analysis may also include stratification and competing risk. Stratification is the process where the hazard functions are stratified by a variable, say season (summer and winter), meaning that there are different baseline hazard functions for the two seasons. Competing risks are the case where there are at least two possible ways that a subject can fail (experience the event); however, it is only possible for one failure to occur. This could be two different death causes; natural death or death due to farmer's decisions.

The classical survival analysis method, Cox proportional hazard model, expresses the hazard at time t for a subject, given at set of explanatory variables, X (which is used to predict the subject hazard). More precisely, the hazard at time t is expressed by the baseline hazard function $h_0(t)$ and the exponential (*exp*) to linear sum of $\beta_i X_i$, where *i* is from 1 to *p*.

$$h(t, X) = h_0(t) exp(\sum_{i=1}^{p} \beta_i X_i), where\ X = (X_1, ..., X_p)$$

β is the coefficient for each explanatory variable, *X*. The baseline functions are unspecified and estimated from the data. The Cox proportional hazard model assumes that the different hazards are proportional. This requires that the HR is constant over time, i.e. that the hazard of one

subject/group is proportional to any other subjects/groups. The time until an event occurs is measured as continuous time in the Cox proportional hazard model; however, it is possible to analyse the time for an event as a discrete time with a discrete-time version of the Cox proportional hazard model, i.e. a discrete-time proportional hazard model (DTPHM).

3.1.3.1   Discrete-time proportional hazard model (DTPHM)

Paper I, based on Study I, included measurements of time categorised as discrete, as there was only one observation per week in eight weeks, where all the hens' status according to the event (failure=death) were registered for each hen. Hence, the survival analysis was conducted using a DTPHM. Survival analysis was chosen as the data set contained both truncation and right censoring. It included truncation, as only hens that had survived until the start of week 30 were included, and right censoring, as all hens that survived during the study period were right censored at the end of the study, as the actual time of the event occurred after the study period.

For the statistical analysis in Paper I, a full DTPHM describing the effect of the *A. galli*/*Heterakis* infection (high or low), season (summer or winter) and a random effect of farm, including stratification by season, was defined. The random effect of farm took the possible dependencies between observations of hens from the farm into account. The full DTPHM was tested against a saturated model, in order to test for homogeneity, and whether the three-way interaction between week, season and the *A. galli*/*Heterakis* infection should be included in the model. The conclusions were that no evidence for rejecting homogeneity of the data was found, and there was no evidence that the three-way interaction should be included in the model. The fact that homogeneity has not been rejected means that the hazard functions of all hens in the same group (defined by the explanatory variables) are equal.

The full DTPHM was tested for whether it could be reduced to a DTPHM without stratification by season. The likelihood ratio test revealed a not statistical-significant p-value (>0.05), providing evidence that the DTPHM without stratification was the best choice of model in this case. It was further tested whether the DTPHM without stratification could be reduced to a DTPHM without effect modification (interaction). The likelihood ratio test presented a significant p-value (<0.001) indicating that the DTPHM with effect modification (but without stratification) was the best choice of model to describe the data in Paper I. Hereafter, and in Paper I, the model with effect modification (between *A. galli*/*Heterakis* infection and season), and with a random effect of farm and without stratification, is called the DTPHM.

As the variables *A. galli*/*Heterakis* infection and season are binary, four groups were defined. The variable *A. galli*/*Heterakis* infection was either low-infected or high-infected, and the variable season was defined as summer or winter based on the time when the hens were 30-37 weeks of age (see Paper I for more details). This gives four hazard functions, however, in the DTPHM there were no statistically significant differences between the hazard function for low-infected farms in the summer and the hazard function for high-infected farms in the winter (p-value>0.05). This means that it was possible to describe the data with three hazard functions. One hazard function for hens in low-infected farms (summer and winter), that was used as the baseline hazard function (i.e. the reference), one hazard function for hens in high-infected farms in the summer and one hazard function for the hens in high-infected farms in the winter. In Paper I, the hazard function used is described together with the procedure of the HR testing.

3.2 Longitudinal study (Study II)

The purpose of Study II was to investigate whether clinical welfare indicators, such as condition of plumage, keel bone, skin, feet and comb can predict helminth infections (*A. galli*/*Heterakis*) in individual hens. In order to address this, a longitudinal study was performed, where individual hens needed to be identified. A longitudinal study is an observational study with repeated observation of the same individuals during a predefined period (Ersbøll et al., 2004). Identification of individual hens in large flocks is problematic, and Freire et al. (2003) state that this could be a reason for why studies of individual hens' welfare in large flocks are rare. The individual tagging should be suitable for on-farm use, and it should be relatively easy to recognise the tagged hens. The choice of identification method in Study II was brightly coloured numbered plastic leg brands (Figure 5).

At the peak of lay visit, 100 hens per flock were tagged with a leg brand and clinically examined as described in section 3.2.2. The 100 hens were selected as random as possible, i.e. hens were captured from multiple sites in the compartment. At the end of lay visit, tagged hens underwent a second clinical examination, thereafter killed by neck dislocation and stored at -18°C for later examination (see section 3.2.1). The producer or a farm employee located and captured the tagged hens in order to minimise the disturbance. The process of capturing tagged hens was finalised when 20 tagged hens were captured, or when no tagged hens were located for approximately 10 minutes. A farm was included in the analysis if at least 10 tagged hens were examined twice. Consequently, 12 flocks and 214 hens were included in the study. Of the 1,200 tagged hens, 50 hens (flock range:

Figure 5: Pictures of hens with leg brands; left panel: a newly placed leg brand at peak of lay; mid and right panel: hens with a leg brand placed around 25 weeks earlier.

0-11 hens) died between the two visits, these hens were only clinically exanimated once and therefore not included in this study (Study II/Paper II).

3.2.1 *A. galli* and *Heterakis* sp. infection

After the 214 tagged hens were killed on the farms, the hens were stored frozen at -18°C for later examination. After the hens were thawed, a faecal sample was collected from the cloaca and analysed using the concentration McMaster method (Permin & Hansen, 1998) with a sensitivity of 20 eggs per gram of faeces. The hens were categorised as non-infected if the result of the analysis was ≤100 EPG and infected if the result was >100 EPG, based on the suggestion from Heckendorn et al. (2009). Non-infected and infected hens were present on all farms.

The 214 hens and the 50 fallen tagged hens underwent a post mortem examination by a veterinarian; data are not included in Paper II, however, main findings are presented in Appendix 7, which shows that the 214 hens mainly were infected with *A. galli* as only three had a *Heterakis* sp. infection.

3.2.2 Clinical welfare assessment

The clinical welfare assessment protocol was developed in the project HealthyHens (CORE organic HealthyHens project, 2014), and included examination of the plumage, keel bone, skin, feet and

comb. The protocol is described in Appendix 2. Two Danish observers participated in the training session and inter-observer reliability testing related to the protocol (Appendix 3). The same trained observer conducted all assessments, and at all peak of lay visits, the other trained observer recorded the results. During the end of lay visit, the recorder was not always trained in the protocol.

For the plumage condition assessment, four body parts were assessed; the neck, the back, the tail and the belly (see Figure 2.1 in Appendix 2). The method used was a modified version of the method described in Tauson et al. (2005), where written explanations of the difference between the scores were included.

The protocol divided keel bone deformities into keel bone deviation and keel bone fractures (Appendix 2). However, in Paper II, the two indicators were combined to an overall indicator for keel bone deformities. Assessment of the keel bone was done by palpation, performed by running two fingers down each side of the keel bone (Wilkins et al., 2004; Kappeli et al., 2011). The palpation methods are not sensitive enough to detect minor fractures (Richards et al., 2011), however, there is good agreement between palpation methods and methods where the hens are dissected. Wilkins et al. (2011) reported a 91% agreement between palpation and dissection methods.

For the statistical analysis, all the clinical welfare indicators were transformed into binary variables representing good or poor welfare, which is further explained in Paper II.

3.2.3   Statistical analysis

Parasitological data are often presented as prevalence or mean (worm or egg count). Prevalence data can be analysed as a binomial distribution, while count data will follow a Poisson or negative binomial distribution. In case of FEC, the raw data are, before accounting for the dilution factor, Poisson distributed (Torgerson et al., 2012). Normally, the first step of a statistical analysis is to check how the data actually are distributed. The effect of explanatory variables (clinical welfare indicators) on the response variable (helminth infection) could have been investigated under a generalised linear model using the correct distribution. However, the statistical analysis of the data from Study II was analysed using a graphical model (Whittaker, 1990; Lauritzen, 1996). Graphical models present the result systematically in a graph or network. Directed graphical models use the casual structure of the variables to guide the construction of the graph, meaning that information about the casual structure is needed. Undirected graphical models are suitable in cases where little or no information of the casual structure is known, as in Study II.

In graphical models, the variables included are represented as points (vertices) and lines (edge) that connect the points. A line (edge) will connect two variables (two points) if the conditional correlation between them, given the other variables, is significantly different from zero (Abreu & Labouriau, 2010; Kristensen et al., 2010, see figure 6 for an example). If two points are not connected with a line (variable *A* and *C* in figure 6), the two variables are not significantly correlated when the other variables are included. In graphical models, two variables will only be connected by a line in the graph if, and only if, they carry new information that is not already contained in the other variables in the model (Whittaker, 1990; Abreu & Labouriau, 2010; Kristensen et al., 2010). However, two variables that are indirectly connected might be correlated (variable *A* and *C* in figure 6), but this correlation is spurious, meaning that the correlation is completely explained by another variable in the model. This is further elucidated with an example (Figure 6). In this graphical model, variable *A* is directly associated with variable *B*, whereas variable *A* and variable *C* are indirectly connected through variable *B*. This means that when variable *B* is given, variable *A* will not contain any information about variable *C*, which is not already contained in variable *B* and vice versa. In other words, variables *A* and *C* are conditionally non-correlated as long as variable *B* is given, i.e. the direct association between variables *A* and *C* is spurious as it disappears when controlling for (conditioning) variable *B*. The same is applied for the connection between variables *A* and *D*, and variables *C* and *D*.

All clinical welfare indicators, with at least 5% of the hens diagnosed with poor welfare condition with respect of the given indicators (see Paper II), are included in an undirected graphical model, as no casual structure between the variables is known in Paper II. The included variables were keel bone deformities at peak and end of lay, back feathering at peak and end of lay, body (neck, belly and tail) feathering at peak and end of lay, *A. galli/Heterakis* infection at end of lay, hen's age in weeks at end of lay and housing system. Two variables were included as descriptive
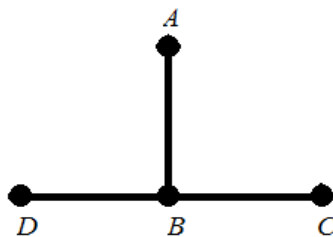
Figure 6: Example of a graphical model with four variables (*A-D*)

variables; age in weeks at end of lay due to the large age range (62 to 77 weeks) and housing system due to the apparent difference between single-tiered and multi-tiered systems. The graphical model allows variables to perform a circular connection, and the length of the lines contains no information as the graphs are unweighted.

Fisher's exact test was used to analyse the level of significance between the directly associated indicators in the graphical model, between not directly associated indicators, and for investigating whether the animal-based welfare indicators differed at the two time points. The Fisher's exact test is applicable when having low frequencies in r x c contingency tables (Blæsild & Granfeldt, 2002), which is the case for some of the clinical welfare indicators in Paper II.

3.3   Qualitative interview study (Study III)

The aim of Study III was to identify management strategies suitable for controlling mortality and endoparasite infections at organic egg farms. To address this, qualitative research methodology is appropriate, as the qualitative interview is a method which attempts to understand and explore the interviewed persons' view and experiences (Kvale & Brinkmann, 2008). The qualitative interviews were conducted in order to explore and understand the management practices on the farms by encouraging the producers to tell their stories and experiences in their own words. Each new interview will add new perspectives or experiences to the subject or support statements by others. The interviewer's task is to follow up and explore what the interviewee says and to keep the interview within the theme as outlined in Vaarst et al. (2007) and Vaarst & Sørensen (2009). The qualitative interview methods are beneficial when exploring farm practices as the answers should fit into predefined categories, as they would, if quantitative methods were used as a questionnaire. Therefore, the qualitative interview method explores the producer's perception and practices, which can be used to identify different strategies, practices or perceptions. The qualitative result can and should not be generalised beyond the in-depth understanding within the field of study, as the aim is not to present a representative sample of opinion or quantify the experience (Kvale & Brinkmann, 2008). This is in contrast to the questionnaire study, where it is possible to test the prevalence of certain beliefs and practices.

The method used was a semi-structured qualitative research face-to-face interview conducted according to a specific interview guide (Appendix 6) including selected themes and open-ended questions (Kvale & Brinkmann, 2008). Seven interviews were conducted and tape-recorded with a length from 22 to 50 minutes. At the seventh interview, the interviews were quite repetitious and,

consequently, it was concluded that the point of saturation was reached. The point of saturation is the point where further interviews are expected to give only little or no new knowledge. All interviewed producers were informed that the interview and quotes from the interview would be published in an anonymised form. At the beginning of the interview, the producers were informed that the aim was to hear their stories, and in order to get the producers familiar with the process, the first theme was their egg production in general, with a special focus on the batch included in the observational studies. This approach was chosen in order to have an open conversation about the fact that the interviewer had been on the farm before as an observer during the other part of the project. Sometimes, the producer referred to the interviewer's knowledge about the production, in such cases, the producer was asked to explain it further in order to ensure a common understanding.

3.3.1   Analytical approach

The first step of the analysis process was to transcribe the interview, which is the process of converting the oral speech into written text. This was done using a combination of verbatim (word by word) and meaning condensates. Creating meaning condensates is the process in which the meaning of each statement is formulated short and precise while maintaining the understanding and language of the interviewee (Kvale & Brinkmann, 2008). After the transcribing was finalised, all interviews were divided into three parts: mortality, endoparasite infections and the connection between the two. After that, the text was coded with keywords identified in the interviews and defined by a given definition. The process of coding the interviews is an important part of the modified grounded theory approach, which is a process of developing a theory without having or testing a certain theory (Kvale & Brinkmann, 2008). A part of the analysis process is to group codes with similar contents into themes or concepts, which can be collected into a model of understanding that connects the facts from the different interviews.

The results of the findings from the interviews are often supported by quotes. After translation to English, the quotes included in Paper III were checked against the original spoken Danish to ensure that the contexts and meanings were maintained during the translation process. To ensure confidentiality, the quotes were anonymised, and characteristics of the specific farms were not presented in the paper.

4 Results

This chapter includes the results of the thesis presented as three papers that investigate the three hypotheses in this PhD project. Below is a short summary of the main findings.

Paper I: **Helminth infection is associated with hen mortality in Danish organic egg production**
The aim of this paper, based on data from Study I, was to investigate the association between *A. galli*/*Heterakis* infections and hen mortality at peak of lay. Using survival analysis, an association between helminth infections and hen mortality was apparent.

Paper II: **Associations between helminth infection and clinical welfare indicators in organic layers**
The aim of this paper, based on data from Study II, was to investigate whether clinical welfare indicators could predict *A. galli*/*Heterakis* infections. Further, the paper included an investigation of the association between different clinical welfare indicators at two ages (peak and end of lay) and an investigation of the development with increasing age in prevalence of the clinical welfare indicators. A graphical model showed that back feathering at end of lay could predict *A. galli*/*Heterakis* infections, with a higher incidence of *A. galli*/*Heterakis* infections in hens with a good back feathering.

Paper III: **Danish organic egg producers' perceptions and experiences related to mortality and endoparasite infections**
The aim of this paper, based on data from Study III, was to investigate and understand a group of producer's practices and experiences relating to mortality and endoparasite infection using a qualitative interview. Three themes related to maintaining a low mortality, and three strategies for controlling endoparasite infections were identified.

4.1   Paper I – Association between helminth infection and mortality

Submitted to Preventive Veterinary Medicine – short communication - in December 2014

Helminth infection is associated with hen mortality in Danish organic egg production

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Abstract

Organic egg production provides access to outdoor area, low stocking density and access to forage promoting natural behaviour. However, organic hens have a relatively high mortality and a high level of helminth infection. The level of helminth infection depends on the season and maximum helminth infection occurs around peak of lay. The aim of this study was to investigate whether two highly prevalent helminth infections (*Ascaridia galli* and *Heterakis* sp.) are associated with an increased mortality rate for hens at the peak of lay.

An observational study with 11 farms representing over 15 % of the Danish organic hens was conducted between 2012 and 2013, with weekly mortality recordings and grouping of the farms into low-infected with *Ascaridia galli* and *Heterakis* sp. (0 to 200 eggs per gram faeces (EPG)) or highly-infected (over 200 EPG).

Survival analysis was performed using a discrete-time proportional hazard model with 791,371 observations. The difference between the hazard functions for low-infected farms and highly-infected farms in either summer (August to September) or winter (January to March) were analysed. No statistically significant differences were found between the mortality rate in winter and summer in low-infected farms. However, the mortality rate was doubled for hens from highly-infected farms observed in the summer season compared to hens from low-infected farms (winter and summer). Whereas highly-infected farms observed in the winter did not have a significantly different mortality rate compared to low-infected farms (summer and winter). The results suggest that the mortality in organic egg production can be reduced by measures to control *Ascaridia galli* and *Heterakis* sp. infections.

Introduction

In organic egg production systems, hens are kept in floor or aviary systems in flocks with at maximum 3000 hens per flock. The hens have access to an outdoor area, an indoor area with a littered floor area, perches, nests and roughage (Anonymous, 2008). These production conditions promote natural behaviour and potentially increase animal welfare. On the other hand, the access to litter (Maurer et al., 2009) and outdoor area (Heckendorn et al., 2009) poses an increased risk of endoparasite infections. Compared to other egg production systems, the prevalence of helminth infection is higher in organic production (Permin et al., 1999), and infections with *Ascaridia galli* and *Heterakis* sp. play by far the dominant role in organic egg production (Permin et al., 1999; Kaufmann et al., 2011; Sherwin et al., 2013). The *A. galli* infection can cause weight depression, diarrhoea, weakness and in severe cases blockage of the intestinal lumen (Ramadan and Abou Znada, 1991; Kilpinen et al., 2005). Further, *Heterakis gallinarum* acts as an intermediate host for *Histomonas meleagridis* that causes blackhead disease (Permin and Hansen, 1998). The infection with *A. galli* and *Heterakis* sp. may therefore have consequences both regarding production economy and the hens' welfare.

Furthermore, the mortality is reported to be higher in organic production compared to cage production. In Denmark, the average mortality for hens in enriched caged and organic hens in 2012 was 3.8 % and 8.5 %, respectively (Anonymous, 2013). The major causes of mortality in organic egg production are infectious diseases and cannibalism (Stokholm et al., 2010). It has been suggested that helminth infections may compromise the immune response in the host (Degen et al., 2005; Schwarz et al, 2011; Pleidrup et al., 2014) and studies indicate that infections following an *A. galli* infection become more severe (Dahl et al., 2002; Permin et al., 2006). An *A. galli* infection may therefore enhance mortality in organic egg production by increasing the prevalence and severity of other infections.

The first excretion of *A. galli* eggs in faeces appears six to eight weeks after placement of the hens (Maurer et al., 2009; Höglund and Jansson, 2011), depending on the contamination of the indoor and free-range area. The maximum infection is typically reached 8 to 12 weeks later (Höglund and Jansson, 2011). With a placement age of 17-18 weeks, the first excretion will occur around 23-26 weeks of age and the expected maximum infection around 31 to 36 weeks of age. Our study period was chosen to be at peak of lay and the expected maximum infection investigating the period from 30 to 37 weeks of age.

The aim of this study was to investigate a possible association between *A. galli* and *Heterakis* sp. infection and mortality in Danish organic egg production. The hypothesis was that *A. galli* and *Heterakis* sp. infections increase the risk of hen mortality between 30 and 37 weeks of age.

Material and methods

An observational study was conducted during the period from August 2012 to March 2013, involving 11 Danish commercial organic laying hen farms that were recruited based on voluntary participation during spring 2012. The farms had either a single-tiered or a multi-tiered (aviary) system and the hybrids used were either Lohmann LSL, Lohmann LB-lite or Hisex White.

The producers recorded mortality on a daily basis, and mortality was subsequently converted to weekly viabilities. The viabilities were recorded at house level with a house size range of 3,000 to 21,000 hens and one only house per farm were included. Faecal samples were collected on the farms once when the hens were between 30 to 37 weeks old, with a mean age of 34 weeks. Two seasons were defined: summer, for visits conducted in August to September 2012 and winter, for visits conducted in January to March 2013.

On each farm, faecal samples were collected from one flock of at maximum 3000 hens. From each flock, 20 individual freshly deposited droppings were collected from multiple sites in the stable. The individual samples were analysed using a simple McMaster method (Permin and Hansen, 1998). Due to the similar size and appearance of *A. galli* and *Heterakis sp.*, eggs, they were not counted separately, i.e. given one count per sample of the number of *A. galli*/*Heterakis* eggs per gram faeces (EPG) is given. Mean farm EPGs were calculated based on the individual samples. Based on the farm mean EPGs, subsequently each farm was categorised as either low-infected (mean farm EPG 0 to 200) or highly-infected (mean farm EPG over 200). None of the farms deworming during the study period. The number of hens from low-infected or high-infected farms in the two seasons is presented in Table 1.

Table 1: Number of hens grouped in the two season, summer and winter, and the two infection levels, low or high (number of farms).

Infection\season	Summer	Winter	Total
Low infection (0 to 200 EPG)[a]	5980 (1)	26661 (2)	32641
High infection(over 200 EPG)	29355 (4)	37202 (4)	66557
Total	35335	63863	99198

a: the low-infected farms were put in the same group for the analysis

Statistical analysis

The deaths of hens that occurred in the time-period from 30 to 37 weeks of age (both extremes included) were studied using a discrete-time proportional hazard model (DTPHM, see Kalbfleisch and Prentice, 2002 and Maia et al., 2014a and 2014b for technical details). This is a discrete-time version of the classic Cox proportional hazard model. Here the use of survival analysis techniques is necessary since the data contain right-censored observations. The deaths that occurred in the study period were described by the hazard function, which associates to each week, say w, the conditional probability (denoted P) of a hen to die in the week w given that the hen had not died before the week w (for $w = 30,\ldots, 37$). More precisely, if T is a random variable representing the time measured in weeks to death of a hen, the hazard function h at the week w is given, for $w = 30,\ldots, 37$, by

$$h(w) = P(T = w | T \geq w) \ (1)$$

The hazard function is interpreted as describing the rate at which the deaths occurred at each week.

The DTPHM used here describes the hazard function of three groups of hens: 1) hens in low-infected farms (summer and winter combined, as there were no statistically significant difference between the hazard functions from low-infected farms (p-value > 0.05)); 2) hens in high-infected farms observed in the summer season; 3) hens in high-infected farms observed in the winter season. The first group of hens (hens in low-infected farms) was taken as a reference; their hazard function, termed the baseline hazard function, was estimated (see Fig. 1) and compared with the hazard functions of the two other groups. According to the DTPHM, the hazard function of a hen in group 2 and group 3 is proportional to the baseline hazard function, with proportionality parameters β and γ, respectively. More precisely, the hazard function in the study period is given, for $w = 30,\ldots, 37$, by

$$h(w) = \begin{cases} b(w), \text{ if the hens is in a low-infected farm (summer or winter)} \\ \beta \ b(w), \text{ if the hens is in a highly-infected farm in the summer} \\ \gamma \ b(w), \text{ if the hens is in a highly-infected farm in the winter,} \end{cases} \ (2)$$

where $b(w)$ is the baseline hazard function evaluated at the wth week. The parameters β and γ were interpreted as hazard ratios, since they describe the ratio between hazard functions.

Additionally, the model incorporated a normally distributed random component taking the same value for each farm and in this way possible dependencies between the observations of hens from the same farm were taken into account. The inference was performed defining a suitable generalized linear mixed model as specified in Maia et al (2014a; 2014b). Hypotheses tests on fixed

Table 2: Mean and weekly mortality for the study period (30-37 weeks of age) and prevalence of *Ascaridia galli* and *Heterakis* sp. infection and mean eggs per gram faeces (EPG) in the study population and for the two groups (low- and highly-infected) for from 30 to 37 weeks of age.

	Mean/prevalence	Range (min. – max. on farms)
Mean mortality	0.6 %	0.2-1.7 %
Mean weekly mortality	0.077 %	0.029-0.21 %
Prevalence of *A. galli* and *Heterakis* sp.	58.3 %	0-90 %
EPG, mean *A. galli* and *Heterakis* sp. (±SD)	670±752 EPG	0-2370 EPG
EPG, mean low-infected farms (EPG<200)	68 EPG	0-136 EPG
EPG, mean high-infected farms (EPG>200)	895 EPG	360-2370 EPG

effects were performed by applying parametric bootstrap tests on the likelihood ratio statistics (100,000 bootstrap samples). All the models were fitted using the software DMU version 6.0, release 5.1 (Madsen et al., 2010).

Results

The dataset used for the analysis in the period from 30 to 37 weeks of age included 99,198 hens (Table 1) and had 791,371 observations. The mortality for the period from 30 to 37 weeks of age and the weekly mortality is presented in Table 2, together with the prevalences of *A. galli* and *Heterakis* sp. and mean EPGs of *A. galli* and *Heterakis* sp. for the study population and mean EPG for low- or highly infected farms, respectively.

The hazard function of the hens from highly--infected farms observed in the summer was almost doubled as compared to the baseline (hens from low-infected farms); i.e. the hazard ratio β was estimated as 1.99, (95% CI 1.31-3.02, p-value= 0.0014). During the winter season, no differences between the hazard function of hens from highly-infected farms and the baseline were found; i.e. the hazard ratio γ was estimated as 0.93, (95% CI 0.73-1.14, p-value: 0.456). Figure 1 displays the estimates of the hazard baseline and the hazard functions of the hens from high-infected farms in the winter and the summer seasons.
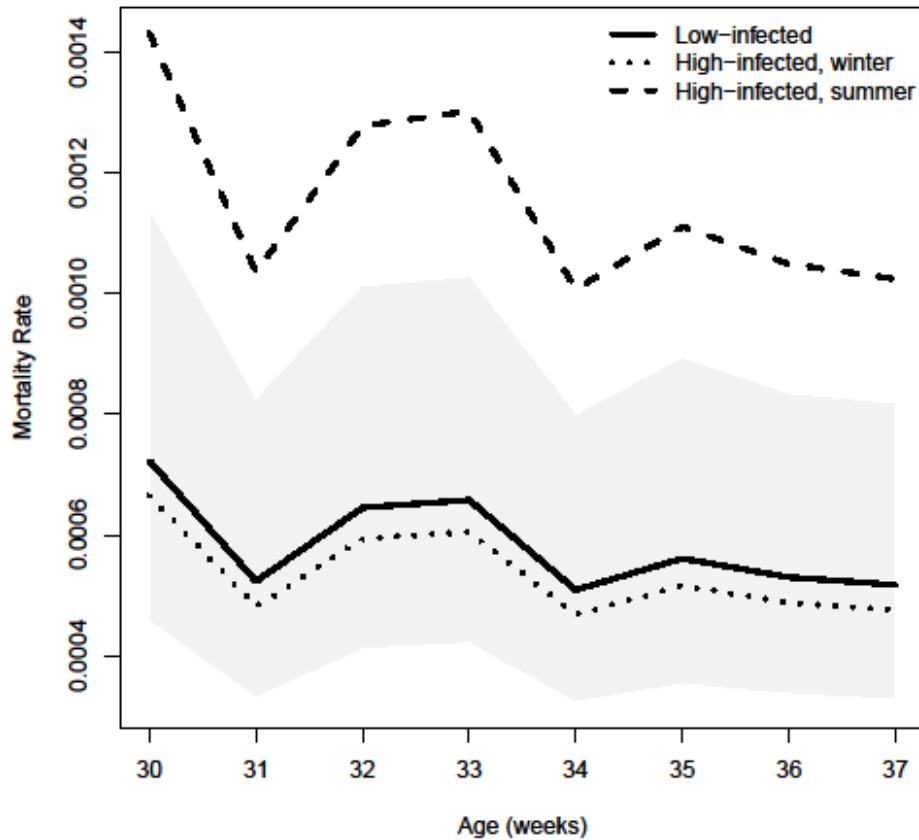
Fig. 1: Time development of the mortality rate (i.e. conditional probability of death of an animal at a given week given that the animal survived until this week) of hens in low-infected farms (continuous line, i.e. the baseline hazard function), in high-infected farms observed in the winter season (dotted lines) and in high-infected farms observed in the summer season (interrupted lines). The gray region is a 95% pointwise confidence interval for the baseline hazard function.

Discussion

The present study involved 15.4% of the 642,565 organic hens in Denmark in 2013 (Anonymous, 2014) and 19% of the Danishs farm with organic egg production registered in the Danish central husbrandry register at the time of recruitment. The prevalence and mean EPGs of *A. galli* and *Heterakis* sp. (Table 2) were in agreement with previous results (Pennycott and Steel, 2001; Heckendorn et al., 2009) or lower (Permin et al., 1999; Sherwin et al., 2013). It was not possible to determine if the maximum infection had been reached, however all flocks achieved their peak egg production during the study period. The present study reports an association between *A. galli* and *Heterakis sp.* infection and hen mortality in the period from 30 to 37 weeks of age. Previous studies

did not find evidence for an association between faecal egg counts and cumulated mortality for the entire production period in commercial free-range units (Sherwin et al., 2013). The level of helminth infection fluctuates during the production period (Höglund and Jansson, 2011). Our study focusses on the time around peak of lay, where mortality and helminth infected were recorded simultaneously, whereas Sherwin et al. (2013) investigated the cumulated mortality representing the entire production period and helminth infection at depopulation.

In the present study, the farms were categorised as either low- or highly-infected based on mean farm EPGs, and used as a binary explanatory variable in the survival analysis model. However, the sample variation within farms was high. The models of survival analysis used here ensure that the entire information on mortality is used efficiently, as the weekly event status of the hens as death or alive are used. This increases the statistical power substantially. However, it has to be kept in mind that the mortality rate is evaluated in relation to the farms infections pressure, not in relation to the hens' individual infection level. There are need for studies that are more comprehensive before our results can be generalised.

Infection with *A. galli* are found to impair the immune response induced by vaccinations (Pleidrup et al., 2014) and increase the severity of bacterial infections (Dahl et al., 2002; Permin et al., 2006). This can be related to the immune response caused by the *A. galli* infection, where the T-helper type 1 (Th1) responses are suppressed and Th2 responses are up-regulated (Degen et al., 2005; Schwarz et al., 2011). The suppression of Th1 may cause the increased severity of the bacterial infections, which may consequently lead to a higher mortality. Stokholm et al. (2011) found that the most common cause of mortality among Danish organic hens were bacterial infection with *Escherichia coli*. This indicates that the association between helminth infection and mortality potential are conditional on the immune response.

The mortality rate was twice as high for hens in farms with high levels of *A. galli* and *Heterakis sp.* infection during the summer season, whereas no difference was observed in the winter season. A possible explanation for the different relations between helminth and mortality during summer and winter for hens from highly-infected farms is that weather conditions during the winter visits included frost and snow cover. This might have kept the hens indoors for a prolonged period and might have decreased the number of bacterial pathogens, decreasing consequently the negative impact of high levels of helminth infection.

In conclusion, the present study presents indications for an association between *A. galli* and *Heterakis* sp. infection and increased rate of mortality in organic hens at peak of lay. During the

summer season, the mortality rate was twice as high on farms with a high worm infection level compared to farms with low infection levels, whereas during the winter season, the level of worm infection did not play a significant role. Preventive and therapeutic measures to control helminth infection, in particular during the summer might contribute to a reduced the mortality among organic hens.

Acknowledgement

References

Anonymous, 2008. Commission regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. Official Journal of the European Union L 250, 1

Anonymous, 2013. Det danske fjerkræraad årsberetning 2012 (In Danish: "Danish poultry council annual report 2012").

Anonymous, 2014. Laying hens by way of keeping. https://circabc.europa.eu (accessed July 2014).

Dahl, C., Permin, A., Christensen, J.P., Bisgaard, M., Muhairwa, A.P., Petersen, K.M.D., Poulsen, J.S.D., Jensen, A.L., 2002. The effect of concurrent infections with *Pasteurella multocida* and *Ascaridia galli* on free range chickens. Vet. Microbiol. 86, 313-324.

Degen, W. G. J., Daal, N. v., Rothwell, L., Kaiser, P., & Schijns, V. E. J. C. 2005. Th1/th2 polarization by viral and helminth infection in birds. Vet. Microbiol. 105, 163-167.

Heckendorn, F., Häring, D.A., Amsler, Z., Maurer, V., 2009. Do stocking rate and a simple run management practice influence the infection of laying hens with gastrointestinal helminths? Vet. Parasitol. 159, 60-68.

Höglund, J., Jansson, D.S., 2011. Infection dynamics of *Ascaridia galli* in non-caged laying hens. Vet. Parasitol. 180, 267-273.

Kalbfleisch, J. D., Prentice, R. L., 2002. The Statistical Analyses of Failure Time Data. 2nd ed. Wiley Series in Probability and Statistics, John Wiley & Sons, Inc., Hoboken, New Jersey.

Kaufmann, F., Das, G., Sohnrey, B., Gauly, M., 2011. Helminth infections in laying hens kept in organic free range systems in Germany. Livest. Sci. 141, 182-187.

Kilpinen, O., Roepstorff, A., Permin, A., Nørgaard-Nielsen, G., Lawson, L.G., Simonsen, H.B., 2005. Influence of *Dermanyssus gallinae* and *Ascaridia galli* infections on behaviour and health of laying hens (*Gallus gallus domesticus*). Br. Poult. Sci. 46, 26-34.

Madsen, P., G. Su, R. Labouriau, and O. F. Christensen. 2010. DMU - Package for analyzing multivariate mixed models. Proc. 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, paper 732.

Maia, R.P., Ask, B.,Madsen, P., Pedersen, P. , Labouriau, R., 2014a. Genetic determination of mortality rate in Danish dairy cows: a multivariate competing risk analysis based on the number of survived lactations. J. Dairy Sci. 97, 1753–1761.

Maia, R.P., Madsen, P., Labouriau, R., 2014b. Multivariate survival mixed models for genetic analysis of longevity traits. J. Appl. Stat. 41, 1286-1306.

Maurer, V., Amsler, Z., Perler, E., Heckendorn, F., 2009. Poultry litter as a source of gastrointestinal helminth infections. Vet. Parasitol. 161, 255-260.

Pennycott, T.W., Steel, F., 2001. Parasitic worms in commercial free-range poultry flocks in England and Wales. Vet. Rec., 149, 428.

Permin, A., Bisgaard, M., Frandsen, F., Pearman, M., Kold, J., Nansen, P., 1999. Prevalence of gastrointestinal helminths in different poultry production systems. Br. Poult. Sci. 40, 439-443.

Permin, A., Christensen, J., Bisgaard, M., 2006. Consequences of concurrent *Ascaridia galli* and *Escherichia coli* infections in chickens. Acta Vet. Scand. 47, 43-54.

Permin, A., Hansen, J.W., 1998. Epidemiology. Diagnosis and control of poultry parasites. FAO Animal Health Manual.

Pleidrup, J., Dalgaard, T. S., Norup, L.R., Permin, A., Schou, T. W., Skovgaard, K., Vadekær, D. F., Jungersen, G., Sørensen, P., Juul-Madsen, H.R., 2014. *Ascaridia galli* infection influences the development of both humoral and cell-mediated immunity after Newcastle Disease vaccination in chickens. Vaccine 32, 383-392.

Ramadan, H.H., Abou Znada, N.Y., 1991. Some pathological and biochemical studies on experimental ascaridiasis in chickens. Nahrung 35, 71-84.

Schwarz, A., Gauly, M., Abel, H., Daş, G., Humburg, J., Rohn, K., Breves, G., Rautenschlein, S. 2011. Immunopathogenesis of *Ascaridia galli* infection in layer chicken. Dev. Comp. Immuno. 35, 774-784.

Sherwin, C.M., Nasr, M.A.F., Gale, E., Petek, M., Stafford, K., Turp, M., Coles, G.C., 2013. Prevalence of nematode infection and faecal egg counts in free-range laying hens: Relations to housing and husbandry. Br. Poult. Sci. 54, 12-23.

Stokholm, N.M., Permin, A., Bisgaard, M., Christensen, J.P., 2010. Causes of mortality in commercial organic layers in Denmark. Avian Dis. 54, 1241-1250.

- 55 -

4.2   Paper II – Helminth infection and clinical welfare indicators

Manuscript in preparation

Associations between helminth infection and clinical welfare indicators in organic layers

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ABSTRACT

The retail market share of organic egg in Denmark is high, and the consumers expect high animal welfare standards in the organic production. Documentation of animal welfare is important, however, knowledge about the association between animal-based welfare indicators is limited. The aims of this study were threefold. First, to investigate the association between clinical welfare indicators at peak and end of lay with helminth (*A. galli* and *Heterakis* sp.) infection at end of lay. Second, to investigate the associations between different clinical welfare indicators at two ages (peak and end of lay), and third to examine the development with age in the prevalence of the chosen welfare indicators.

An observational study with twelve organic egg farms was conducted in 2012 and 2013 with 214 hens assessed individually at peak and end of lay.

A graphical model was used to analyze the associations between clinical welfare indicators (keel bone deformities and back feathering and body feathering at both peak and end of lay), helminth infection at end of lay, housing system, and age at end of lay. Helminth infection was only directly associated with back feathering at end of lay (P=0.011), with an increased incidence of helminth infection in hens with good back feathering. Between the two visits, the prevalence of hens with keel bone deformities increased and the plumage condition deteriorated (P<0.001), whereas the number of hens with bumble foot (P=0.037) and pale combs (P=0.020) decreased. No significant differences were found for other feet problems and skin damage. Thus, back feathering at end of lay may provide unique information about a possible helminth infection, but in daily on-farm management not a useful indicator.

Keyword: organic, layer, helminth, animal welfare, graphical model

INTRODUCTION

Organic egg production in Denmark has increased by a factor 1.4 the last decade. In 2013, 18% of the eggs delivered at the packing facilities in Denmark was organic eggs (Anonymous, 2014a) and the retail market share for organic eggs was 20.5 % (Anonymous, 2014b). The consumers' motivations for buying organic products are human health, environmental concerns, and animal welfare (Hermansen, 2003; Wier, et al., 2008). Thus, the consumers expect that the animal welfare is better in the organic production than in conventional production (Hermansen, 2003). Therefore, it is important to be able to document the level of animal welfare, which can be done by conducting an animal welfare assessment. Welfare assessment can be performed using methods like the welfare quality protocol (Welfare Quality, 2009) and the animal needs index (Bartussek, 2001) or by assessing one or a few welfare indicators, depending on the purpose of the assessment. The welfare indicators used may be resource-based or animal-based. Resource-based indicators are indirect indicators of the welfare of hens, collected by examining the physical environment, whereas animal-based welfare indicators are direct measures, examining the hen's response to the environment (Barnett & Hemsworth, 2009). Several animal-based welfare indicators have been found to be valid, reliable, and feasible for on-farm assessment (Arnould, et al., 2009). Among these are wounds, plumage condition, feet condition, and bone damage (incl. keel bone damage), which all indicate welfare problems that potentially may be present in the organic egg production.

Another welfare problem that has received more attention by the poultry sector and producers level in recent years in Denmark is helminth infections (Hinrichsen et al., unpublished results). In the organic system, hens are kept in floor (single-tiered) or aviary (multi-tiered) systems, where one-third of the indoor area has to be solid and littered, with a maximum stocking density of six hens per square meter and access to an outdoor area of four square meter per hen is required (Anonymous, 2008). Access to litter and an outdoor area are both potential sources of helminth infections (Heckendorn, et al., 2009; Maurer, et al., 2009). Compared to other systems, the prevalence of helminth infections is high in organic production (Permin, et al., 1999), and the most dominating helminth species are *Ascaridia galli* and *Heterakis* sp. (Permin, et al., 1999; Sherwin, et al., 2013).

Helminth infections may affect the plumage condition. Ackert and Herrick (1928) and Ramadan and Abou Znada (1991) reported that infected broilers showed ruffled feathers. Plumage damage may also be a result of feather pecking and abrasion against the equipment (Bilcik and Keeling, 1999; Welfare Quality, 2009), and denuded areas are more prone to skin damage. Helminth

infections have been reported to decrease the locomotory activity (Gauly, et al., 2007), and as exercise increases the bone strength (Whitehead, 2004), less locomotory activity may result in increased prevalence of bone damage in infected hens compared to non-infected hens. This could potentially be observed in the prevalence of keel bone deformities, as the keel bone is anatomically located such that it is exposed and prone to damage (Fleming, et al., 2004; Kappeli, et al., 2011). Clinical welfare problems often depend on the age of the hens. For example, the prevalence of keel bone deformities and plumage damage increases with increased age of the hens (Nicol, et al., 2006; Drake, et al., 2010; Richards, et al., 2012). Furthermore, helminth infection fluctuates during the production (Höglund and Jansson, 2011), and the prevalence of helminth infections increase with increasing age of the hens (Pennycott and Steel, 2001).

The associations between the welfare problems related to helminth infection, plumage condition, keel bone deformities, skin, comb color, and feet is limited and age of the hens are likely to affect the level of the welfare problems. Therefore, a study collecting data on helminth infection at end of lay and clinical welfare indicators at two ages (peak and end of lay) was conducted. The aims of this study were threefold. First, the aim was to investigate the association between clinical welfare indicators at peak and end of lay with helminth (*A. galli* and *Heterakis* sp.) infection at end of lay. The second aim was to investigate the associations between different clinical welfare indicators at two ages (peak and end of lay). Finally, the third aim was to examine the development with age in the prevalence of the chosen welfare indicators. The hypothesis of this study was that helminth infection (*A. galli* and *Heterakis* sp.) diagnosed at end of lay could be predicted by clinical welfare indicators at two ages (peak of lay and end of lay).

MATERIALS AND METHODS

A longitudinal study including 12 Danish commercial organic egg farms was conducted from August 2012 to December 2013. Each farm participated with one flock, one farm placed 2140 hens in the flock and the eleven other farms placed 3000 hens. Each flock was visited twice; first visit between 30 and 38 weeks of age, named peak of lay, and again in the week before slaughter (from 62 to 77 weeks of age), named end of lay. The housing system, hybrid, age at the visits, and number of included hens per farm are presented in Table 1. During the peak of lay visit 100 randomly selected hens were tagged with bright colored numbered plastic leg bands and clinically examined, as described below. At the end of lay visit tagged hens underwent a second clinical examination

Table 1: Description of the twelve commercial organic layer flocks according to housing system, presence of veranda, hybrid, age at the two visits, and number of hens per flock included in the present study of the 100 tagged hens per flock. In total, 214 hens were included.

Housing system	Veranda	Hybrid	Age at peak of lay visit	Age at end of lay visit	No. hens included
Multi-tiered	yes	Hisex White	36	75	18
Multi-tiered	yes	Hisex White	38	73	19
Multi-tiered	yes	Lohmann LSL	36	77	20
Single-tiered	no	Hisex White	34	66	16
Single-tiered	no	Lohmann LB-lite	30	69[a]	20
Single-tiered	no	Lohmann LB-lite	32	75	17
Single-tiered	no	Lohmann LB-lite	36	72	11
Single-tiered	no	Lohmann LSL	34	72	21
Single-tiered	yes	Hisex White	31	66	17
Single-tiered	yes	Lohmann LSL	34	62[a]	15
Single-tiered	yes	Lohmann LSL	34	74	20
Single-tiered	yes	Lohmann LSL	34	74	20

a: Hens in this flock were slaughtered later than one week after the visit.

thereafter killed by neck dislocation and stored at -18°C until later examination. The percentage of tagged hens relocated at the second visit were between 11 and 21% (Table 1), resulting in a total of 214 hens. Each tagged hen was given a unique identification number based on the numbered band and farm of origin.

The clinical examination included scoring of plumage, keel bone, skin, feet, and comb color of each hen, and it was performed by the same trained observers. The scoring of plumage was conducted on four body parts: back (incl. rump), tail, belly (incl. vent), and neck following the procedure described by Tauson et al. (2005). Scoring of the keel bone was done by scoring the level of deviation on a three points scale modified from Gunnarsson et al. (1995). No deviation and deviations smaller than 0.5 cm were given the best score, deviation over 1 cm the worst score, and deviation between 0.5 and 1 cm an intermediate score. Fractures to the keel bone were recorded as present or not, by detecting callus material or breaks in the bone. The keel bone deformities were examined by palpation, performed by running two fingers down each side of the keel bone. Damage to the skin was assessed as presence of wound or not on the back (incl. rump) and belly (incl. vent).

The feet were assessed using five indicators of potential welfare problems: foot pad lesions, dorsal swellings (bumble foot), hyperkeratosis on the foot pads, toe wounds, and missing toes/claws as present or not. Comb color was assessed as pale or red (normal).

At the post mortem examination, fecal samples were collected from the cloaca after thawing and a fecal egg count of the two helminth species *Ascaridia galli* and *Heterakis* sp. was obtained using the concentrate McMaster technique (Permin and Hansen, 1998), with a sensitivity of 20 eggs per gram faces (EPG). *A. galli* and *Heterakis* sp. eggs have similar appearance and size (McDougald, 2013) and were therefore not separated, and named *A. galli*/*Heterakis* in this study. Hens from five of the twelve farms were dewormed between 11 and 40 weeks before the end of lay visit. The deworming reduces the parasite egg output in the feces, but they reappear after two to four weeks (Höglund and Jansson, 2011), therefore the deworming were not taken into account.

Data management

Back feathering, i.e. plumage condition at the back, was assessed as poor if the score were ≤ 2 and good if the score were ≥ 3 based on the definition by Tauson et al. (2005). Body feathering, i.e. the summed plumage scores for tail, neck, and belly, was assessed according to the median score for the peak of lay and the end of lay visits, respectively. Hens with body feathering scores below the median score were categorized as having poor body feathering and those with body feathering scores above or equal to the median score as having good body feathering. For the peak of lay visit the median score was 12 (equal to the best score for all three body parts), implying that a score of 12 defined good body feathering at peak of lay. At end of lay, the median score was nine, implying that a score ≥ 9 defined good body feathering at end of lay. At end of lay, the median score were equal to the criteria for poor plumage used by Tauson et al. (2005).

Based on the scoring of keel bone fractures and deviations each hen was categorized as having no keel bone deformities, i.e. the hen had no deviations or fractures, or as having keel bone deformities, i.e. the hen had fractures and/or deviations. The examination of feet and skin problems were assessed as present or not, while comb color was assessed as pale or red.

Regarding the fecal egg count, the suggestion by Heckendorn et al. (2009) to set individual samples with ≤ 100 EPG as zero to avoid false positive was applied. False positive hens are hens that have ingested helminth eggs that are excreted again after passing through the gut without establishing an infection. Based on the result of the fecal egg count individual hens were categorized as non-infected (i.e. EPG ≤ 100) or infected (EPG > 100).

Statistical Analysis

The differences between prevalence of the clinical welfare problems and *A. galli*/*Heterakis* infection at the peak of lay and the end of lay visits were analyzed using Fisher exact tests with 1,000,000 Monte Carlo samples. The associations between clinical welfare indicators with at least a 5% prevalence at peak of lay and end of lay (keel bone deformities, back feathering, body feathering, see Table 2), occurrence of *A. galli*/*Heterakis* infection, age at end of lay, and housing system were studied using graphical models (Whittaker, 1990; Lauritzen, 1996; Labouriau and Amorim, 2008a,b).

In graphical models, the variables in play are represented by vertices in a graph, i.e. a collection of vertices (points) and edges connecting the vertices (lines). Two vertices (i.e. two variables) are connected by an edge (line) when the conditional correlation between them, given all the other variables, is different from zero. The absence of an edge joining two variables indicates that the two variables are not significantly correlated given the other variables. According to the theory of graphical models (Whittaker, 1990; Jørgensen and Labouriau, 2012), two variables are directly connected in the graph if, and only if, they carry new information on each other that is not already contained in the other variables included in the model. If two variables are only connected indirectly, i.e. two variables that are included in the graphical model but not directly connected, the variables may be correlated, but this correlation is spurious, in the sense that it could be completely explained by correlations with the other variables. The graphical model with minimum BIC (Bayesian Information Criterion) was selected for the key welfare indicators using the R package gRapHD (Abreu et al., 2009), which yields the graphical model that best represents the data (Haughton, 1988). Confirmatory analyses were performed using Fisher exact tests with 1,000,000 Monte Carlo samples.

RESULTS

Of the originally 1,200 tagged hens, 17.8 % were assessed both at peak of lay and at end of lay. Table 2 displays the prevalence of the clinical examinations of welfare indicators and *A. galli*/*Heterakis* at peak of lay and at end of lay. Significantly higher prevalences of the welfare indicators related to plumage and keel bone were found at end of lay compared to peak of lay ($P < 0.001$, Table 2). On the other hand, dorsal swellings (bumble foot) and pale combs significantly decreased between the two visits.

Table 2: Prevalence of clinical welfare indicators among the 214 examined hens at peak of lay and end of lay (number of hens in brackets), prevalence of *A. galli*/*Heterakis* infection, and the level of significance (Fisher's exact test) of the difference between the two visits.

	Peak of lay	End of lay	Fisher's exact test
Back feathering, poor[1]	7.9% (17)	23.4% (50)	<0.001
Body feathering (neck, tail, belly), poor[2]	25.7% (55)	43.5% (93)	<0.001
Keel bone fractures, present	3.7% (8)	18.7% (40)	<0.001
Keel bone deviation, present	8.9% (19)	26.6% (57)	<0.001
Keel bone deformities, present[3]	10.7% (23)	29.0% (62)	<0.001
Foot pad lesions, present	0.5% (1)	0.5% (1)	ns
Dorsal swellings (bumble foot), present	3.7% (8)	0.5% (1)	0.037
Foot pad, hyperkeratosis, present	0.0% (0)	0.5% (1)	ns
Toe wounds, present	0.5% (1)	0.0% (0)	ns
Missing toes, present	1.4% (3)	1.9% (4)	ns
Skin wounds, present	3.3% (7)	0.9% (2)	ns
Comb color, pale	4.2% (9)	0.5% (1)	0.020
A. galli and *Heterakis* sp., > 100 EPG	-	56.1% (120)	-

1: Poor plumage condition at the back were equal to a score ≤ 2

2: Poor plumage condition at peak of lay: score ≤ 11 and at end of lay: score ≤ 8

3: Total prevalence of keel bone deviation and/or fractures.

Figure 1 shows the minimal BIC graphical model representing the association between clinical welfare indicators (keel bone deformities, back feathering and body feathering), housing condition, age, and *A. galli/Heterakis* infection. The only variable found to be directly connected to *A. galli/Heterakis* infection was back feathering at end of lay ($P = 0.011$, see Figure 1). The direction of this association was that 61.6% of hens with good plumage condition on the back were infected with *A. galli/Heterakis*, and 38.0% of the hens with poor plumage condition on the back were infected with *A. galli/Heterakis*. Investigation of the indirectly connection by ignoring the other variables in the graphical model, none of the variables directly connected to back feathering at end of lay (i.e. back feathering at peak of lay, body feathering at peak and end of lay and housing system) were significant correlated with *A. galli/Heterakis* ($P > 0.05$).

Furthermore, the graphical model represented in Figure 1 shows that back feathering at end of lay was directly connected to back feathering at peak of lay, body feathering at peak of lay, body
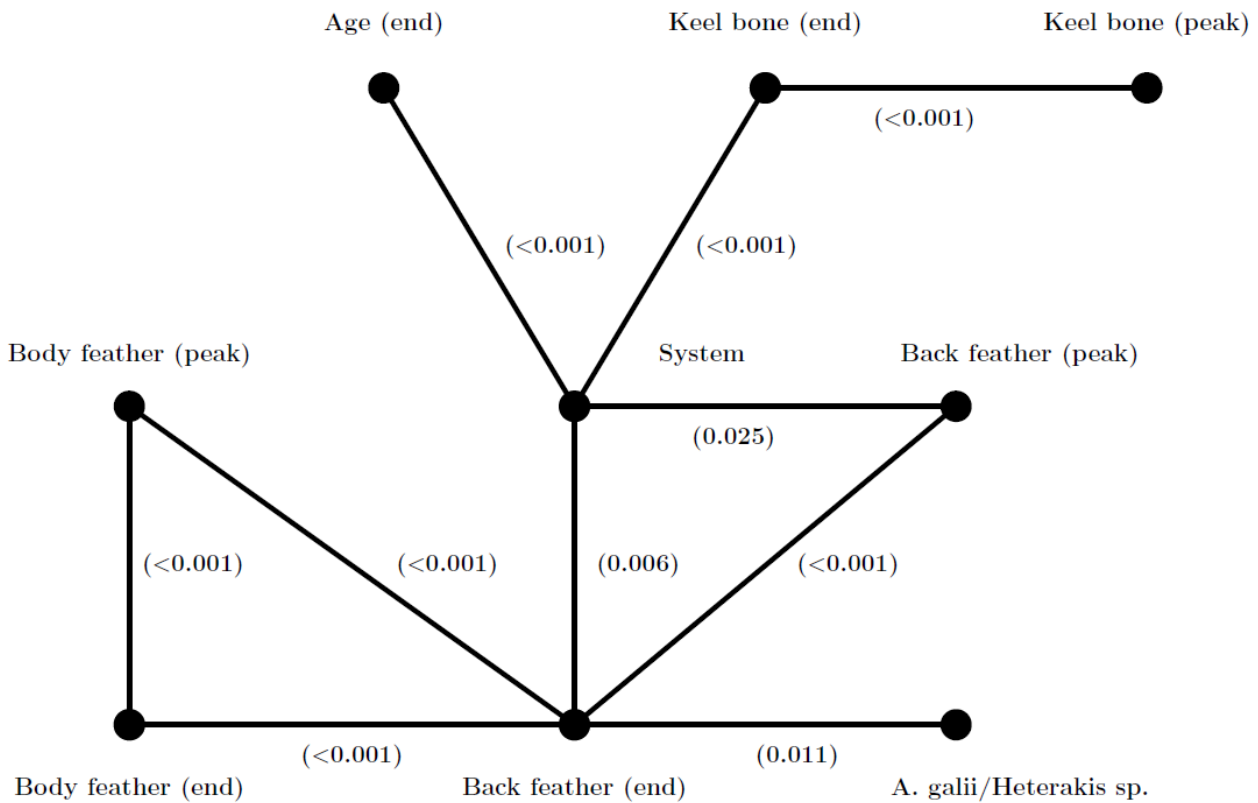
Figure 1: Graphical model showing significant relationships between back feathering (Back feather), body feathering (Body feather), and keel bone deformities (Keel bone) at peak of lay (peak) and end of lay (end), incidence of *A. galli* and *Heterakis* sp. infection at end of lay (A. galli/Heterakis sp.), age at end of lay and housing system (System). The level of significance for each conditional correlation (edge (line) between two vertices (points representing a variable)) is presented in brackets below or beside the line.

feathering at end of lay and housing system; the other variables were not directly connected with back feathering at end of lay. Housing system was directly connected to age, keel bone deformities at end of lay, and back feathering at peak lay and at end. The direction of the associations were that hens from multi-tiered system had a higher prevalence of keel bone deformities when hens from single-tiered system (52.6 % and 20.4%, respectively), and hens from multi-tiered systems had lower prevalence of poor plumage condition on the back compared to single-tiered systems both at peak of lay (0 % and 10.8 %, respectively) and end of lay (15.8 % and 26.1 %, respectively). Further, the end of lay visit was conducted at a higher age in multi-tiered systems compared to single-tiered systems (mean 75 and 70 weeks of age, respectively). Age at end of lay was only associated with housing system.

The clinical welfare indicators (back feathering, body feathering, and keel bone deformities) assessed at end of lay were all directly associated with the same clinical welfare indicator at peak of lay. Further, in the graphical model housing system separates keel bone deformities (at peak of lay and at end of lay) from the other welfare indicators (back feathering and body feathering) and helminth infection (*A. galli/Heterakis*).

DISCUSSION

Identification of individual hens in large commercial flocks poses a problem (Freire, et al., 2003), which was also experienced in this study. Only 18% of the tagged hens were re-caught and thereby assessed twice. The identification mark used may be of great importance for the success of re-catching, as Gunnarsson et al. (1995) found 90% of hens marked with wing-tags at later occasions in large flocks.

Abrasion, wear, and feather pecking can cause damage to the plumage. Plumage damage to the back is usually related to feather pecking (Tauson et al., 2005; Welfare Quality, 2009) and is one of the first areas to become denuded (Bilcik and Keeling, 1999). The belly is also one of the first areas to become denuded (Bilcik and Keeling, 1999), however this is often due to abrasion and is often seen in highly productive hens (Kjaer and Sørensen, 2002; Welfare Quality, 2009). Abrasion can also cause plumage damage on the neck and tail feathers (Bilcik and Keeling, 1999; Welfare Quality, 2009). This was the motive for separating the plumage condition into back feathering, i.e. the plumage score for the back, and body feathering, i.e. a summed plumage score for tail, neck, and belly.

Association between helminth infection and welfare indicators

The graphical model provided evidence for a direct association between incidence of *A. galli/Heterakis* infection and back feathering at end of lay (Figure 1), with increased incidence of *A. galli/Heterakis* infection in hens with a good plumage condition on the back. Previous studies have shown a positive relation between outdoor use and good plumage condition; the more hens outside, the better plumage condition (Lambton, et al., 2010; Bestman and Wagenaar, 2014). The causation of this positive correlation is unclear, but there may be two possible explanations: 1) the actual use of outdoor area decreases the feather damage and 2) mainly hens with good feather condition use the outdoor range. The result of the present study showed that hens with good back feathering had a higher incidence of *A. galli/Heterakis* infection, suggesting that these hens spent more time in areas

with a high risk of attracting an *A. galli*/*Heterakis* infection, i.e. the outdoor area and the littered area (Heckendorn, et al., 2009; Maurer, et al., 2009). However, further investigation of the association between back feathering and incidence of *A. galli*/*Heterakis* infection is required to separate cause and effect.

The association between *A. galli*/*Heterakis* infection and back feathering existed even when controlling for all the other measured variables (body feathering at peak of lay and at end of lay, keel bone deformities at peak of lay and at end of lay, age at end of lay, and housing system). This suggests that the other variables in the model do not carry any information about *A. galli*/*Heterakis* infection that is not already contained in the variable back feathering. This suggestion were confirmed, as none of the indirectly connection, i.e. between *A. galli*/*Heterakis* infection and either body feathering at peak of lay, body feathering at end of lay, or housing system, were significant. Therefore, in the present study, back feathering at end of lay is the only variable that provide any information on the incidence of *A. galli*/*Heterakis* infection.

All clinical welfare indicators at end of lay (back feathering, body feathering, and keel bone deformities) were directly associated with the same clinical welfare indicator at peak of lay (Figure 1). Therefore, the incidence of a welfare problem at peak of lay provided information about the incidence at end of lay. This suggests that a certain welfare problem may be related to the same casual mechanism independent of time, but also that a welfare problem may be accumulated over time and thereby the prevalence of the problem may increase with increasing age (see Table 2). Previous studies have reported an increased prevalence of welfare problems with increasing age of the hens. This is the case for keel bone deformities (Fleming, et al., 2004; Richards, et al., 2012) and plumage condition (Nicol, et al., 2006; Drake, et al., 2010). Damage to the plumage of pullets has been reported as a predictor for plumage condition at a later stage (Drake, et al., 2010). The present study provides evidence that the plumage condition at peak of lay can be a predictor for plumage condition at end of lay. According to our results, back feathering at end of lay can be predicted by both body and back feathering at peak of lay, as both are associated with back feathering at end of lay in the graphical model (Figure 1).

Housing system and age at end of lay were associated, with a higher end of lay age in multi-tiered systems compared to single-tiered systems. This does not provide evidence that the age at depopulation are significant different in the two systems, as two farms single-tiered were visited earlier then the last week before depopulation. Nevertheless, the end of lay visit were conducted at a younger age in single-tiered system. This may influence the prevalence of hens with a poor plumage

condition and with keel bone deformities, as the prevalence of these problems increase with increasing hen age (Table 2 and Fleming et al., 2004; Nicol et al., 2006; Drake et al., 2010; Richards et al., 2012).

An association between housing system (single-tiered or multi-tiered) and keel bone deformities was found in the present study (Figure 1), with increased incidence of keel bone deformities in multi-tiered system. This can be related to the increased age at end of lay among hens in multi-tiered system. In contrast, Bestman and Wagenaar (2014) found no relationship between keel bone deformities and housing system (single-tiered or multi-tiered). The graphical model indicated that keel bone deformities are related to a casual path where the other clinical welfare indicators (*A. galli*/*Heterakis* infection and body and back feathering) are not included, as housing system separates keel bone deformities from the other variables. The associations between keel bone deformities and the other clinical welfare indicators would, if present, be spurious, as any potential associations would disappear when the variable housing system is taken into account.

Back feathering, both at peak and end of lay, and housing system were associated, with an increased deterioration of the plumage condition on the back in single-tiered systems compared to multi-tiered systems. Therefore, this association may not be related to the age of the hens at end of lay, as the youngest hens (hens in single-tiered system) had the worst plumage condition on the back. In contrast to the findings in the present study, Häne et al. (2000) found no difference in the plumage condition between multi-tiered systems and single-tiered systems (with permanent access to littered area) for hens between 50 to 70 weeks of age.

Prevalence of clinical welfare indicators

The prevalence of hens with pale combs and bumble feet decreased between peak of lay and end of lay, however, the number of hens categorized with pale combs and bumble feet was low at both peak of lay and end of lay (Table 2). Whereas, there was a significant increase in the prevalence of hens with poor plumage condition on the body and back and keel bone deformities between the visits which is in agreement with previous studies (Drake et al., 2010; Richards et al., 2012). Bestman and Wagenaar (2014) reported that 32% of the investigate commercial organic flocks (H&N Silver Nick, Hy-line Silver, Hy-line Brown, and Lohmann LB-lite) were in poor plumage condition at 50-60 weeks of age, which is lower than the 43.5% of hens in the present study that were categorized as having a poor body feathering. In the same study (Bestman and Wagenaar, 2014), 21% of the hens were based on the palpation methods of alive hens assessed as having keel

bone deformities, a level that is similar to the prevalence of keel bone deformities in the present study. Others studies have found a much higher percentage of keel bone fractures than in the present study (18.7 % at end of lay, Table 2). Nicol, et al. (2006) found 52-65 % of Shaver hens with keel bone fractures in commercial flocks in single-tier system, and Wilkins et al. (2011) found keel bone fractures in 45-86 % of the hens in commercial free-range and organic systems (primarily Hy-line Brown and Lohmann Tradition). In those studies the keel bone assessment were conducted on dissected hens at end of lay. A study comparing assessment of keel bone deformities using the palpation method with results found during subsequent dissections concluded that palpation method in 91% of the cases complied with the dissection method (Wilkins et al., 2011).

The prevalence of *A. galli*/*Heterakis* infection was in accordance with previous reports (Heckendorn, et al., 2009). Other studies reports the percentages of flock being infected (Pennycott and Steel, 2001; Sherwin, et al., 2013; Bestman and Wagenaar, 2014), and they all reports higher percentage of flock with helminth infections when the percentage of hens with infection in the present study, however prevalence of flocks and hens are not directly comparable.

In conclusion, helminth infection (*A. galli*/*Heterakis*) was only directly associated with back feathering at end of lay, with an increased prevalence of helminth infection in hens with good plumage condition on the back. Back and body feathering were the only clinical welfare indicators that were associated, however, the prevalence keel done deformities increased and the plumage condition deteriorated significantly with increasing age. In the present study, back feathering at end of lay provided unique information about helminth, as none of the other included welfare indicators were associated with helminth infection, and back feathering at end of lay can be used as a predictor of helminth infection. However, the information back feathering at end of lay provides is not a useful indicator in daily on-farm management practices.

REFERENCES

Abreu G. C. G., D. Edwards, and R. Labouriau, 2010. High-dimensional graphical model search with the gRapHD R package. J. Stat. Softw. 37:1–18.

Ackert, J. E., and C. A. Herrick. 1928. Effects of the nematode *Ascaridia lineata* (Schneider) on growing chickens. Jour. Parasitol. 15:1-13.

Anonymous. 2008. Commission regulation (EC) No 889/2008 of 5 September 2008, laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. Official Journal of the European Union L 250:1-84.

Anonymous. 2014a. Statistics Denmark, http://www.statistikbanken.dk/statbank5a/default.asp?w=1920. Accessed September 2014.

Anonymous. 2014b. Tabelbilag til formændenes beretninger ved årsmødet den 4. februar 2014. Dansk Erhvervsfjerkræ 43:114-123. (In Danish: "Appendix for the presidents report at the annual meeting 4th of February 2014").

Arnould, C., A. Butterworth, and U. Knierim. 2009. Standardisation of clinical scoring in poultry. Pages 7-30 in Assessment of animal welfare measures for layers and broilers. Welfare quality report no. 9. B. Forkman, and L. Keeling (eds.)

Barnett, J. L. and P.H. Hemsworth. 2009. Welfare monitoring schemes: using research to safeguard welfare of animals on the farm. J. Appl. Anim. Welf. Sci. 12:114-131

Bartussek, H. 2001. Animal needs index for laying hens ANI 35-L/2001 − laying hens. Bundesantalt für Alpenländishe Landwirtschaft, Gumpenstein, Austria, 30 pages.

Bestman, M., and J. P. Wagenaar. 2014. Health and welfare in Dutch organic laying hens. Animals 4:374-390

Bilcik, B., and L. J. Keeling. 1999. Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. Br. Poult. Sci. 40:444-451.

Drake, K. A., C. A. Donnelly, and M. S. Dawkins. 2010. Influence of rearing and lay risk factors on propensity for feather damage in laying hens. Bri. Poult. Sci. 51:725-733.

Fleming, R. H., H. A. McCormack, L. McTeir, and C. C. Whitehead. 2004. Incidence, pathology and prevention of keel bone deformities in the laying hen. Bri. Poult. Sci. 45:320-330.

Freire, R., L. J. Wilkins, F. Short, and C. J. Nicol. 2003. Behaviour and welfare of individual laying hens in a non-cage system. Bri. Poult. Sci. 44:22-29.

Gauly, M., C. Duss, and G. Erhardt. 2007. Influence of *Ascaridia galli* infections and anthelmintic treatments on the behaviour and social ranks of laying hens (*Gallus gallus domesticus*). Vet. Parasitol. 146:271-280.

Gunnarsson, S., K. Odèn, B. Algers, J. Svedberg, and L. Keeling. 1995. Poultry Health and Behaviour in a Tiered System for Loose Housed Layers. Report 35. Swedish University of Agricultural Sciences, Faculty of Veterinary Medicine, Department of Animal Hygiene.

Haughton, D.M.A., 1988. On the choice of a model to fit data from an exponential family. Ann. Stat. 16, 342-355

Heckendorn, F., D. A. Häring, Z. Amsler, and V. Maurer. 2009. Do stocking rate and a simple run management practice influence the infection of laying hens with gastrointestinal helminths? Vet. Parasitol. 159:60-68.

Hermansen, J. E. 2003. Organic livestock production systems and appropriate development in relation to public expectations. Livest. Sci. 80:3-15.

Häne, M., B. Huber-Eicher, and E. Frohlich. 2000. Survey of laying hen husbandry in Switzerland. World Poult. Sci. J. 56:21-31.

Höglund, J., and D. S. Jansson. 2011. Infection dynamics of *Ascaridia galli* in non-caged laying hens. Vet. Parasitol. 180:267-273.

Jørgensen, B., and R. Labouriau. 2012. Exponential Families and Theoretical Inference. (2 ed.) Rio de Janeiro, Brazil: Springer. (Monografías de Matemática; No. Zbl 0968.62500, Vol. 52). http://www.impa.br/opencms/pt/biblioteca/mono/Mon_52.pdf

Kappeli, S., S. G. Gebhardt-Henrich, E. Frohlich, A. Pfulg, and M. H. Stoffel. 2011. Prevalence of keel bone deformities in Swiss laying hens. Bri. Poult. Sci. 52:531-536.

Kjaer, J. B., and P. Sørensen. 2002. Feather pecking and cannibalism in free-range laying hens as affected by genotype, dietary level of methionine plus cystine, light intensity during rearing and age at first access to the range area. Appl. Anim. Beha. Sci. 76:21-39.

Labouriau, R. and A. Amorim, 2008a. Comment on 'An Association Between the Kinship and Fertility of Human Couples'. Science, 322

Labouriau, R., and A. Amorim, 2008b. Human fertility increases with marital radius. Genetics 178, 601-603

Lambton, S. L., T. G. Knowles, C. Yorke, and C. J. Nicol. 2010. The risk factors affecting the development of gentle and severe feather pecking in loose housed laying hens. Appl. Anim. Beha. Sci. 123:32-42.

Lauritzen, S.L. 1996. Graphical Models. Oxford University Press, New York.

Mahboub, H. D. H., J. Müller, and E. Von Borell. 2004. Outdoor use, tonic immobility, heterophil/lymphocyte ratio and feather condition in free-range laying hens of different genotype. Bri. Poult. Sci. 45:738-744.

Maurer, V., Z. Amsler, E. Perler, and F. Heckendorn. 2009. Poultry litter as a source of gastrointestinal helminth infections. Vet. Parasitol. 161:255-260.

McDougald, L. R. 2013. Internal Parasites. Pages 1117-1146 in Diseases of poultry. D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez, and V. L. Nair eds. Wiley-Blackwell.

Nicol, C. J., S. N. Brown, E. Glen, S. J. Pope, F. J. Short, P. D. Warriss, P. H. Zimmerman, and L. J. Wilkins. 2006. Effects of stocking density, flock size and management on the welfare of laying hens in single-tier aviaries. Bri. Poult. Sci. 47:135-146.

Pennycott, T. W., and F. Steel. 2001. Parasitic worms in commercial free-range poultry flocks in England and Wales. Vet. Rec. 149:428.

Permin, A., and J. W. Hansen. 1998. Epidemiology. Diagnosis and control of Poultry parasites. FAO Animal Health Manual.

Permin, A., M. Bisgaard, F. Frandsen, M. Pearman, J. Kold, and P. Nansen. 1999. Prevalence of gastrointestinal helminths in different poultry production systems. Bri. Poult. Sci. 40:439-443.

Ramadan, H. H., and N. Y. Abou Znada. 1991. Some pathological and biochemical studies on experimental ascaridiasis in chickens. Nahrung 35:71-84.

Richards, G. J., L. J. Wilkins, T. G. Knowles, F. Booth, M. J. Toscano, C. J. Nicol, and S. N. Brown. 2012. Pop hole use by hens with different keel fracture status monitored throughout the laying period. Vet. Rec. 170:494.

Sherwin, C. M., M. A. F. Nasr, E. Gale, M. Petek, K. Stafford, M. Turp, and G. C. Coles. 2013. Prevalence of nematode infection and faecal egg counts in free-range laying hens: relations to housing and husbandry. Bri. Poult. Sci. 54:12-23.

Tauson, R., J. Kjaer, G. Maria, R. Cepero, and K. Holm. 2005. Applied scoring of integument and health in laying hens. Anim. Sci. Pap. Rep. 23:153-159.

Welfare Quality. 2009. Welfare Quality assessment protocol for poultry. Welfare Quality Consortium, Lelystad, Netherlands:119.

Whitehead, C. C. 2004. Overview of bone biology in the egg-laying hen. Poult. Sci. 83:193-199.

Whittaker, J. 1990. Graphical Models in Applied Multivariate Statistics. John Wiley & Sons, Chichester, UK

Wier, M., K. O. Jensen, L. M. Andersen, and K. Millock. 2008. The character of demand in mature organic food markets: Great Britain and Denmark compared. Food Policy 33:406-421.

Wilkins, L. J., J. L. McKinstry, N. C. Avery, T. G. Knowles, S. N. Brown, J. Tarlton, and C. J. Nicol. 2011. Influence of housing system and design on bone strength and keel bone fractures in laying hens. Vet. Rec. 169:414.

- 73 -

4.3   Paper III – Management related to mortality and helminth infections

Danish organic egg producers' perceptions and experiences related to mortality and endoparasite infections

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Abstract

Qualitative interviews were conducted to investigate Danish organic egg producers' experiences and perceptions related to management of mortality and endoparasite infections. Seven organic egg producers with floor housing were included in the study.

The producers identified four main causes for hen mortality. Predator attacks mainly by fox, effect of weather with increased number of dead hens around weather changes, infectious diseases dominated by *Escherichia coli* and smothering. The producers described different practices related to maintaining a low mortality. These practices were grouped into three themes: *Pullet quality*, *Management and time*, and *Feed*. Theme *Pullet quality* included pullets' uniformity at placement and the first days at the production site. Theme *Management and time* included the daily routines, time spent with the hens and having time for unforeseen thing. Theme *Feed* included the supply of feed and water of suitable quality and quantity. It became apparent that there was limited knowledge about endoparasite infections among the producers, which may be due to endoparasite infections being a recently emerging focus area. Despite this, a model of understanding of the strategies for controlling endoparasite infections was suggested, identifying three strategies: *no*, *regular* or *irregular testing*, with *regular* and *irregular testing* being test and treat strategies, whereas *no testing* is the strategy without testing or use of anthelmitics. The producers just recently realised a relationship between mortality and the endoparasite burden. In conclusion, Danish egg producers appear to have three areas of focus in relation to mortality control and believe in a test-and-treat strategy in relation to controlling endoparasite infections.

Keywords: organic; egg producer; mortality; endoparasite; qualitative semi-structured research interview

1. Introduction

The organic egg production has increased steadily over the past 15 years, and the consumers' motivations for buying organic products are human health, environmental and animal welfare (Hermansen, 2003; Wier et al., 2008; Dawkins, 2012). Organic egg production in Denmark has a relatively high retail market share (20.5% in 2013; Anonymous, 2014b), and the organic egg production has almost doubled since 1998 (Anonymous, 2014a). Presently, approximately 66 farms produce about 10.6 million kg organic eggs to the packing facilities (Anonymous, 2013), corresponding to over 600,000 hens. The consumers expect that the animal welfare is better in the organic production compared to other production concepts (Hermansen, 2003). However, both mortality and endoparasite infections pose a threat for the hen's welfare. Compared to other production systems, mortality and the prevalence of endoparasite infections are higher among organic layers (Permin et al., 1999; Jansson et al., 2010, Anonymous, 2013).

Mortality became an area of focus in Denmark in the research community at the beginning of the 2000s, with observational studies identifying level and causes of mortality based on autopsies in the organic production (Hegelund et al., 2006; Stokholm et al., 2010). The common causes for mortality in the organic system have been reported as *Escherichia coli* and cannibalism (Fossum et al., 2009; Stokholm et al., 2012).

The focus on endoparasite infections in egg production seems to have increased over the past decade. Permin et al. (1999) conducted a literature review in the late 1990s and identified only few studies in the previous decades, which focused on the prevalence and the significance of parasite infections in commercial poultry production systems. Since then, several studies on endoparasites have been conducted (Heckendorn et al., 2009; Jansson et al., 2010; Kaufmann et al., 2011; Maurer et al., 2013). These studies have used observational or experimental studies for identifying the prevalence of and risk factors for endoparasite infections.

Endoparasite burden and mortality differ between farms indicating a major effect of management (Hegelund, 2007). However, no investigations on how organic egg producers actually perceive and handle management problems in their herds have been conducted. Such investigations could contribute to the understanding of ways to reduce the impact of endoparasite infections and mortality. Qualitative research methods have been suitable for providing an understanding of farmers' perception and behaviour in relation to health and welfare problems such as lameness (Horseman et al., 2014) and calf mortality (Vaarst and Sørensen, 2009) in dairy production.

The aim of this study was to investigate organic egg producers' practices relating to mortality and endoparasite infections and to explore how the experiences and perceptions of organic egg producers potentially influence the practices related to reducing mortality and endoparasite infections. This article aims at answering the research question: How do experiences and perceptions of Danish organic egg producers relate to their health management practices, with specific focus on reducing mortality and avoiding endoparasite infections.

2. Materials and methods

A study based on semi-structured qualitative research interviews (Kvale and Brinkmann, 2008) was planned and conducted as a part of a research project investigating the health and welfare in organic egg production. Fifteen organic Danish egg-producing farms were recruited as study herds in the project, based on voluntary participation (observational studies were conducted from August 2012 to December 2013). Seven of these producers were interviewed during a three-week period in February-March 2014. The seven producers were selected among the fifteen study herds in the following way: Three farms were excluded due to travel distance, and one farm was excluded because of shift of farm manager. Eleven farms remained for potential interviews. The farms were listed in a random order, and agreements to be interviewed were made by phone, starting from number one on the list. After three calls during one day without getting hold of the producer, the next producer on the list was contacted. All interviews were conducted at the kitchen table at each individual farm by the first author, who had been involved in the entire research project and thereby had visited the farms prior to the interviews. The first interview was conducted as a test interview, and this producer had confirmed that he was willing to be interviewed again, if necessary, in case the interview guide was changed as a result of the outcome of the test interview. It turned out that this was not necessary. At the seventh interview, it seemed that no new information came up, and the interviews started to be quite repetitious. Consequently, the first author concluded that a point of saturation was reached.

2.1. Participating producers and farms

The participating producers had been organic egg producers for at least ten years, and the educational backgrounds were diverse: agricultural education, office clerk, master degree in economics and one farm with a combination of nurse and dairyman, as the husband and wife, where both were working with the egg production. The size of the seven participating farms ranged from

3,000 to 12,000 hens, with the hens divided into flocks of maximum 3,000 hens as according to the EU regulation (Anonymous, 1991). Three different breeds were present on the farms: Hisex White, Lohmann LB-Lite and Lohmann LSL. All included farms had a floor housing system, either a single level system (littered floor area and slats; six producers) or a deep litter system (whole floor area littered, no raised areas beside perches; one producer).

2.2. The qualitative research interview method

The semi-structured qualitative research interview is a research method for exploring and describing experiences, beliefs and practices related to a given field or theme from the interviewees' perspective (Kvale and Brinkmann, 2008; Vaarst and Sørensen, 2009). A semi-structured interview is conducted according to a specified interview guide, which includes selected themes and open-ended questions (Kvale and Brinkmann, 2008). During the interview, the interviewed person is encouraged to tell his or her story in their own words with freedom and time to do this. The interviewer follows up when needed, explores statements and keeps the interview on track. A qualitative interview study explores the range of a given area and is context specific and does not quantify in any way. The results will therefore provide insight and in-depth understanding within the field, but not be generalizable beyond the context. It is, for example, not possible to conclude that a certain percentage of producers have a certain belief or opinion. Each interview will add new information to the study, support the information given by others or present a new angle to the area. The interview guide used in this study was structured into four parts: introduction to the interview where the producers were familiarised with the interview process. This was followed by a part about mortality and a part about endoparasite infections, where the producers were asked about endoparasite infections in general. However, the producers' answers mainly concerned *Ascaridia galli* infections. Finally, a short part explored the connections between mortality and endoparasites, ending with the producer's background. For each part, and especially the two parts about mortality and endoparasites, a list of open-ended questions guided the interview, where the producer was asked to tell about his or her experience with high or low incidence of mortality and endoparasite infections, including case stories, management practices on the farm, use of other actors and how it affected the hens. The finalising part of the interview was more explorative including storytelling. The interviewer followed up on the open-ended questions with relevant context specific questions, which gave a deeper and more detailed understanding of the producer's perception of the situation.

2.3. Data analysis

The seven interviews were recorded and transcribed in a combination of verbatim transcription and meaning condensates (Kvale and Brinkmann, 2008) in the software Transana 2.50 (Transana 2.50 [Computer software], 2012). Creating meaning condensates is a process in which the meaning of each interviewee statement is formulated short and precise, but remains with the understanding and language of the interviewee (Kvale and Brinkmann, 2008).

The three parts of the interview, mortality, endoparasite infections and connection between mortality and endoparasite infections, were identified and analysed separately using a modified grounded theory approach, which is a strategy for development of a theory without a predetermined theoretical framework (Kvale and Brinkmann, 2008). The entire interview was checked and coded in a process giving the meaning condensates one or more keywords. In this process, the keywords were developed so that each keyword was assigned a specific definition and used uniformly across all interviews. The keywords were mainly related to either the themes of mortality or endoparasite infections. Only one keyword described the connection between mortality and endoparasites, and one described the use of other actors.

After coding all interviews, each interview was checked to ensure consistency and to identify potential disagreements or disconnections. One particular keyword that related to what the producers found important to ensure low mortality was subdivided into more sub-keywords for further analysis. Based on the keywords related to managing endoparasite infections, a model of understanding was formed explaining the endoparasite management practices expressed by the producer.

3. Results

The results of the analysis of the seven interviews were divided into three sections: Mortality, Endoparasite infections and Connection between mortality and endoparasite infections. The structure of each section is as follows: Mortality; firstly, the producers' view on mortality; secondly, the four identified main causes of mortality: *Predators*, *Effect of weather*, *Infectious diseases* and *Smothering* and thirdly, the three identified themes related to management strategies for maintaining low mortality (Fig. 1). Endoparasite infection: firstly, the producers' view on endoparasite infections and secondly, an explanation of the model of understanding identifying three strategies for controlling endoparasite infections (Fig. 2): *no testing*, *regular testing* and

irregular testing. Connection between mortality and endoparasite infections: the producers' view of the connection followed by the use of other actors.

3.1. Mortality

The statement *"It agonises me every time I drag a handful of hens out of the stable (Producer A)"* illustrates the feelings related to mortality.

The producers clearly expressed awareness of their surroundings' view on the higher mortality in the organic egg production system, but also that it is important to recognise the difference between the different production systems with regard to challenges. One of the producers explained: "*It is of great importance to our image. When mortality stories are in the media, people will have a meaning about it no matter whether there is a high or a low mortality. (…) we are doing our best every day (…) but there will always be some unforeseen things we cannot control (Producer B)"*. Another continues: *"We have focus on mortality, and there have been some success stories. It has been possible to reduce the mortality considerably, compared to some years ago where mortality was much higher than today. But we should not rest on our laurels, because we want to decrease it even further (Producer C)"*.

Four main courses of mortality were identified in this interview study: *predators*, *effects of weather*, *infectious diseases* and *smothering*. The producers tried to manage and meet the challenges specifically related to each of these four main causes. Apart from that, they revealed various strategies to keep a low mortality that will be presented in section Management strategies for maintaining low mortality.

3.1.1. Mortality cause 1: Predators

Predation loss was addressed in several ways. Methods like good fencing, including fence along the ground and electric wire, and continuously checking the fence and free-range area were emphasised. Producer B mentions: *"You can observe if there are changes, if suddenly there are 20 dead hens in one place killed by the fox. In the summer, I am mowing the grass around the fence to look for any activity"*. However, sometimes good fencing seems not to be enough: *"I had a fox inside the henhouse, ending up with 15-20 dead hens plus the ones it left with (…) in this case, snow piled up around the fence in such a way that the fox simply could walk over the fence (Producer C)"*. Further strategies to reduce fox predation, which they mentioned, include traps and reduction of the number of foxes through shooting. A combination of these two strategies reduced the

problem for Producer D: *"When I realised how many hens I lost [to predators], then it was evident that I had to do something (...) I got electric wire along the fence and bought a fox trap and started to catch foxes. During three years, I caught ten foxes per year, thereby reducing the population"*. Mink (*Neovison vison*) and stoat (*Mustela ermine*) are more problematic and difficult to avoid, as Producer C mentions: *"I can't keep them out. It is unusual that it happens, but the mink and the stoat cost several hens. I had one flock (of 3,000 hens) where I lost around 200 hens to a stoat over a period of three months. It was a solitary case"*.

   The presence of predator birds is a problem in some areas, and they were seen upon as responsible for a variable number of killings. Producer B expresses problems with northern goshawk (*Accipiter gentilis*): *"Earlier, when I had white-feathered hens, I was plagued by the northern goshawk. The loss decreased after I started having brown-feathered hens instead of white-feathered. We were highly plagued by the northern goshawk, and I have tried to contact the public authority (to get permission to kill the northern goshawk) but there was nothing I could to do about the problem"*. Producer C mentions problems with predator birds: *"My impression it that the predator birds do not kill numerous hens, but their presence stresses the hens in the free-range area. I haven't done anything preventive yet, but there are different sirens that can intimidate the predator birds"*.

3.1.2. Mortality cause 2: Effect of weather

The farmers address weather-related problems by applying a range of different management practices. Producer E describes a strategy of activating the hens as much as possible by providing different feed sources, especially during wintertime when it snows, as the popholes are then kept closed: *"We try to distract the hens by giving them different materials inside. Often we have - because we also have cattle - some wholegrain silage, which may be a little better for them, or they get extra litter, or some extra grain (...) or some beets or carrots. (...) All to activate them"*. Producer D had previously experienced problems with rainwater running into the veranda and inside the stable. This ended in several dead hens a year or two until a solution was found: *"I got an area in front of the house covered with concrete with outlets for water removal, and the change was evident from one year to another"*.

3.1.3. Mortality cause 3: Infectious diseases

Infection with *E. coli* was seen as a main cause for high mortality, and systematic vaccination was mentioned as the main strategy to combat the *E. coli* problem, as Producer F states: *"We had a high mortality related to coli, it was gradually increasing and increasing, so we decided to vaccinate the hens against coli. The last two batches were not vaccinated, it had sort of disappeared"*. However, vaccination has not yet been the solution for Producer G: *"We have tried to minimise the coli problem. Five or six batches ago, we got an autovaccine, but it was evident that a new autovaccine should be prepared per batch. Last year, an autovaccine was prepared, and it revealed that there was an aggressive coli type present on the farm. However, an incorrect vaccine was delivered and we decided not to use it. The new batch had got the new coli-vaccine"*. Other initiatives such as avoiding wet litter and standing water in the free-range areas were focus areas for the producers in reducing the risk of *E. coli* infection. Keeping the supply and quality of the feed in focus was another prevention strategy. Producer G had experienced that other types of infections seemed to affect the risk of getting *E. coli*: *"E. coli is minimised incredibly when we deworm the hens (...) my idea is that if the hens are filled with parasites, they will become more weak"*.

3.1.4. Mortality cause 4: Smothering

Smothering was mentioned as a cause of death on all farms but one, either as something which had occurred previously or as cause of death happening under special circumstances. Producers experiencing smothering as a main cause of mortality now had focus on daily supervision of the hens and system: *"Daily supervision, what do you see? Do you find a problem? Can you find a solution to that problem? And can we solve it; we'll try to (Producer A)"*. The smothering mentioned was mainly social smothering with piling up in the middle of the floor area or nest smothering. The producers had the feeling of not being able to overcome the problem with social smothering: *"It mainly happened in the evening, and we can't be in the house until the hens go to bed. I do not believe it would help that we walk around in the house and get the hens away from one another when they start piling, as five minutes later they are piling up again. So I don't believe we can do much about it (Producer A)"*. With regard to nest smothering, the producer had experienced that the hens primarily died in the nest at the end of a row. Consequently, they included areas without nest in this row, and thereby increased the number of first and last nests, which led to a decrease in the number of hens per nest. He also looked at the how and when the nest was closed in the afternoon: *"When I experience a lot of nest smothering, I will often close the nest an hour later*

Theme 1: Pullets	Theme 2: Management and time	Theme 3: Feed
Get uniform pullets Good start after placement	Permanent daily routines Constant care Time in the hen house Sense, feel and being close to the hens	Stable supply of feed and water, including roughage

Fig. 1: The three identified themes in the interviews regarding management strategies for maintaining low mortality, including a description of the main focus areas within each theme.

(…) and increase the time it takes to close the nest, thereby being more gentle. I will also walk around the house and check the critical nests, where I, based on my experience, know that there is a risk of hens getting trapped, and I remove the hens from these nests (Producer C)".

3.1.5. Management strategies for maintaining low mortality

The producers had a number of practices to try to ensure low mortality, irrespective of which causes of mortality were identified on their farm. These practices are described as four main themes in Fig. 1 and explained in more detail below.

Theme 1: Pullet quality. The producers emphasise the importance of ensuring that the pullets are of a good and uniform standard: *"I have a really good pullet reared that delivered a uniform product (…) some of my colleagues get pullets that are a little doubtful, and that gives a poor start foundation (Producer F)".* At placement and the time immediately after, the producers emphasise the importance of getting hens the best possible start at the production site, including a clean house, and spend some extra time in the house in the beginning, as illustrated here: *"Especially in the beginning, I am walking around in the house six-eight-ten times per day, so the hens get familiar with me, and I can keep an eye on them. (…) During the first days, I will be in the house when the light is switched off and stay there until they are quiet. (…) They should also have a good start, start eating and eat what they are supposed to, and they don't get any poor quality feed (Producer C)".*

Theme 2: Management and Time. This theme is about the daily practices and everyday life on the farm. Overall, this theme is related to the management practices and time spent for daily routines and for taking precautionary action to avoid various conditions as described above. The producers emphasise the importance of having fixed routines, because *"animals are creatures of habits like us, so you should not change routines from day to day (Producer D)"* and *"All this with regularity is important regarding minimising the stress level (Producer F)"*. The producers mention several routines in relation to the constant care of the hens, and the system is performed inside the henhouse around the hens, like collecting eggs, roughage feeding or removing dead hens at regular basis to minimise contamination. The time spent in the henhouse will give an impression of potential problems, like described by Producer E: *"When we go into the house, we can hear if there is something wrong (...) if there is a strange sound and the hens are gathered where they shouldn't. It can be sensed, when we are the ones being with them the entire period"*. If a problem is identified, it seems to be important to have the time available to act immediately, as Producer C states: *"You must act quickly, react immediately. Tomorrow is too late, if you have a problem"*. Producer D mentions that on a busy day, it is possible to perform the essential daily routines only by leaving out the small nursing routines, allowing you also to have time for other tasks. However, some time spent in the house will benefit you later, such as time spent reducing the floor eggs: *"I remove floor eggs from the start, and if I see a hen trying to lay on the floor I move her to a nest. (...) I spend some time on this, but in return I save some time the rest of the year"*. The producers were aware of the importance of spending time with the animals, like illustrated in the statement from Producer C: *"In "the perfect world", I would be in the henhouse the whole time (...) to me is it about being close to the animals"*.

Theme 3: Feed. As a separate theme is feed, as the producers emphasise the importance of having a stable supply of feed, including roughage, and water throughout the production period in accordance with the norm of the hens. Producer F states that to ensure low mortality it is *"... first and foremost a stable supply of feed and water and litter material, and a regular working procedure in the stable"*. Producer B furthermore mentions *"Water, feed and the weight of the hens, those three things should fit with the norm figures"*. Feed is believed to be of great importance for getting the hens started, as mentioned in theme 1: Pullet Quality.

3.2. Endoparasite infections

At the time of the interview, the producers did not believe that they were confronted with current challenges related to endoparasite infections on their farm. They saw the awareness of and focus on endoparasites as a relative new issue within the organic poultry sector. Producer C expressed it as follows: *"I did not have so much focus on endoparasites earlier. The sector did not have so much focus on it"* and Producer F: *"It is not until during the last years that we have become aware of the problem (with endoparasite infections)"*. This also meant that producers, generally, had fairly new and little or no direct experience with what could be related to endoparasite infections.

However, they expressed awareness of potential negative side effects of endoparasite infections on the productivity of the hens: *"(they) lay fewer eggs; the hens need more feed to maintain life" (Producer D)"*. Another producer (Producer G) explained this in terms of 'general strength': *"if the hens are filled with worms they will be weakened"*. Two producers experienced to be informed that consumers had localised parasite worms in eggs from their farm: *"They called and said that they had found worms in the eggs. After that, we had to do something (Producer A)"*. The two producers seemed to have been surprised by the call and initiated an immediate treatment: *"You immediately start a powerful treatment for worms (Producer B)"*. In some cases, the producers were stimulated by the interviewer to think about general animal welfare in relation to the topic of endoparasite infections. As one producer explained, acknowledging the relevance of focusing on endoparasite infections: *"Of course I am thinking of the consumer that gets the worm in the egg, but I must say that my primary reason for acting is animal welfare (Producer C)"*.

Previous experiences with endoparasite infections were mentioned, like Producer G: *"We had a problem with worms, but it seems that now we are more aware of the problem, it has decreased"*. This producer mentioned that increased awareness and focus were the keys to solving the problem; thereby realising that endoparasites should not be regarded as an isolated problem on the farm: *"We need to link the worm problem with coli"*.

Management practices and strategies for controlling endoparasite infections were identified from the interviews, despite of the producers' lack of experience. These management practices and the identified different strategies are described in the model of understanding (Fig. 2) and explained in more detail below. The model of understanding explains three strategies: *regular testing*, *irregular testing* and *no testing*, where testing is the identification of the level of endoparasite infections.
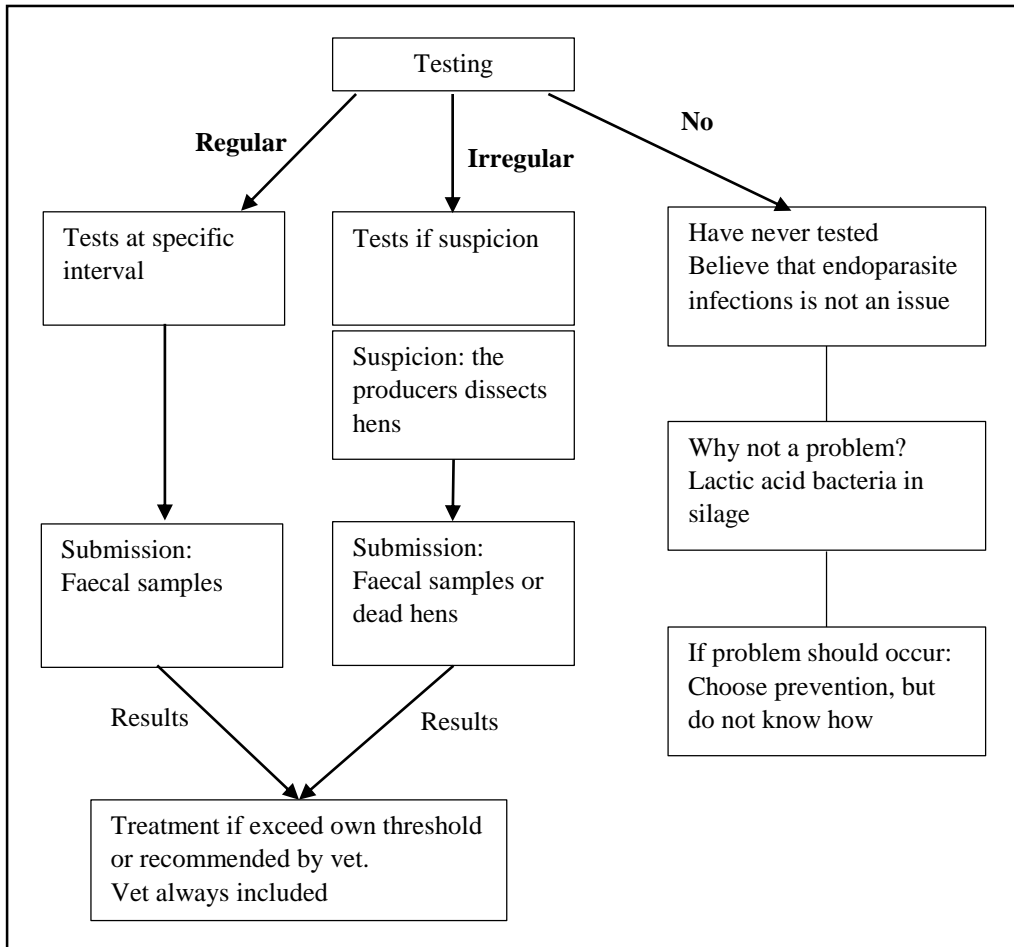
Fig. 2: A model of understanding developed on basis of the interviews with the organic egg producers, showing the three different situations related to managing endoparasite infection using tests to identify the level of infection. The three strategies were explained in the interviews: they had either no testing, or they tested regularly or irregularly, respectively. The no-testing strategy includes the potential option to test in case the problem should occur. The regular and irregular testing strategies are distinguished based on criteria for when and how to test and take action.

3.2.1 No testing

The producers stating that they do not use testing mention that they did not believe that endoparasite infections were a problem or issue on their farms. When talking about whether endoparasite infections are a problem on the farm, Producer D states: *"I don't know, I really don't know. I don't believe that it is"* and Producer E explains: *"It is not such a big problem that I know about it"*. The producers believed that lactic acid bacteria in silage and as a culture given to the hens is the main reason for why the occurrence of endoparasite infections apparently is not existing. Producer E

explains: *"The lactic acid in the silage is good for the hens, for their stomach"*. A similar statement was given by Producer D: *"The lactic acid bacteria is helping keeping the stomach alright and thereby the welfare of the hens (...) lactic acid is something I believe in, without knowing anything about it"*. These producers would prefer some preventive measures if they should get problems with endoparasite infections in the future, but with no idea of the possibilities for doing this: *"I would prefer not to treat the hens. So, it would probably be something with prevention, (but) I wouldn't know how I could prevent it, it is something I haven't acquainted myself with, but obviously I would do it, if it becomes a huge problem (Producer D)"*.

3.2.2 Regular testing.

Producers stating that they are using regular testing stated that tests of the endoparasite infection level based on faecal samples were conducted at certain time intervals, with the time interval being different from farm to farm. Producer G had the pullets tested before placement and after that every tenth week at the production site. Another producer was in the process of getting the testing perfectly scheduled: *"It is not completely scheduled yet, but it is something I try to do at a certain interval, around every six to eight weeks (Producer C)"*.

3.2.3 Irregular testing

Producers stated that they only conducted tests for endoparasite infections when there was a suspicion that the hens were infected, and only after the producers had dissected some of the hens themselves and checked the intestine for the presence of endoparasites. The suspicion could be increased mortality, as Producer F describes: *"If I believe that mortality is increasing, then I will dissect some of the hens"*. It could also be a general check of the status: *"This year, I have dissected some hens, and there was nothing (Producer A)"*. The producers' reason for dissecting the hens themselves is a way of having control and idea of what happens inside the hens, as stated by Producer F: *"You then have an idea of what is actually happening and whether there is something else at stake"*. The producers stated that they would have either faecal samples or dead hens tested for the presence of endoparasites.

3.2.4. Result of the test

The producers mention the same consideration regarding the result of the test independent of the test strategy (Fig. 2). They also mention that the decision concerning treatment is based on the result

of the test and in collaboration with the veterinarian. The producers seem to set their own thresholds in agreement with their veterinarian. The producers' threshold is different as expressed by Producer F: *"There are disagreements about how big the problems are: some think that some worms don't matter so much. However, how do you judge if there are few worms or many worms. I think it is a difficult balance"*. The producers are also aware of the responsibility in relation to resistance development, as indicated by Producer G: *"You should not treat just to treat (...) we will not conduct routine treatment every 10th week. I will not be part of that, as there is a risk of resistance. We will examine if there is a problem before we treat"*.

Not only the actual test results seem to be used when taking the decision whether to treat, as explained by Producer C in the following case, which also illustrates the lack of clear criteria for judging the test: *"I just talked with my veterinarian yesterday about the last test and got a result from him. We were in a dilemma not knowing what to do, as the prevalence was moderate, moderate to medium in his definition, but what that is, I do not know. He asked: do you have other animal welfare problems? And as there were no other problems, we decided not to deworm"*.

3.2.5. Other practices

Some of the producers expressed the use of other practices to reduce the endoparasite burden. One producer was treating the slats with a gas burner in between batches, as he explains: *"It should supposedly remove some (endoparasite) eggs. It seems to help (Producer A)"*. Whereas another producer disinfected the free-range area close to the house before placement of a new batch: *"I remove the top soil from the area close to the house and place new soil. After that, limestone is spread in the free-range to disinfect (Producer C)"*. The producers mention use of silicium powder mainly because of its documented effect in the control of red mites (*Dermanyssus gallinae*), but had also received information that it potentially could have an effect on endoparasites: *"We started using this silicium powder against red mites, it should also have an effect on endoparasites, but it is difficult to say if it works. They eat it if possible (Producer B)"*.

3.3. The connection between mortality and endoparasite infections

Based on the knowledge gained since the producers became aware of issues related to endoparasite infections, one producer described the potential associations between endoparasite burden and morality as follows: *"Parasites and mites and whatever else there could be, I believe that their presence has a high effect on the stress level, which eventually weakens the immune system followed*

by coli infections and other diseases (Producer F)". Another producer explained: *"Everything interacts, but you need to look at the hens all the time to see if they are well (Producer E)"*. These quotes give a good picture of how the producers generally perceived the mutual relationship between mortality and endoparasites, where management practices that potentially could reduce endoparasite burdens would influence mortality positively, and vice versa. Often, the latter was not revealed before encouraging the producers to reflect on management routines, which can influence both mortality and endoparasite infections simultaneously. When encouraged, they explained that there were no dilemmas between trying to avoid mortality and endoparasite infections at the same time, and reducing one also seemed to reduce the other, or referred back to practices mentioned during the mortality part of the interview: *"... yet again, give the hens a good start (Producer B)"*. Other actors such as colleagues, experience-exchange producer groups (called 'erfa-groups' in Danish), advisors and veterinarians seem to be involved in problem solving in relation to both mortality and endoparasites. The producers stated that veterinarians and advisory service were used in minor extent, while colleagues and erfa-groups were used in relation to discussing practical implementation of management practices and in the broader discussions and knowledge generation on the farms. Producer D describes this: *"I am using my erfa-group, I have been in that group for several years, over 10 years. So we just talk about everything (...) and I get some words of wisdom every time"*. The exchange within the producer groups gives a good feeling of support: *"I feel that, if there is a problem, it is possible to ask the other producers - at erfa-group meetings or at other meetings - what they have done in similar cases. (...) if we are not using one another, you would just be sitting in your own little world, not knowing what is happening outside (...) you will always take something with you home; when you feel that you have been there before, then another producer will tell about his new discovery (Producer B)"*.

4. Discussion

4.1. Mortality

The producers recognise that the relatively high mortality in the organic egg production is an important image problem, but they also emphasise that there are other more important challenges in the organic system.

According to the producers, who participated in this interview study, the main causes of mortality were *Predators*, *Effect of Weather*, *Infectious Diseases* and *Smothering*. These are also, except the *Effect of Weather*, reported in observational studies as the cause of death in commercial

organic egg production (Hegelund et al., 2006; Fossum et al., 2009; Stokholm et al., 2010). Hegelund, et al. (2006) reported that an average of 3.8% died due to smothering with a range between farms of 0 to 8% and an overall average mean farm mortality of 22.5%. Stokholm et al. (2010) found that mortality due to predation ranged from 0 to 3.7% based on farm registrations. However, mortality due to predation might be higher as it can be difficult to account for the loss of hens due to predation with farm registration. When mortality is calculated based on the difference between placed and slaughtered hens, the mortality rate is up to 5% higher than mortality rates based on farm registration (Stokholm et al., 2010). Predation is reported as a common cause of mortality. A Danish study reported a mean mortality of 6.4% by predators based on differences between placed and slaughtered (Hegelund et al., 2006), and a British questionnaire revealed that 80% of all respondents reported mortality due to predation (Moberly et al., 2004). The *Infectious Disease* mentioned by the producers in our study was mainly *E. coli* infection. Stokholm et al. (2010) found that the most common cause of death was *E.coli* infections and concluded that improved management and vaccination should be given priority to reduce the impact of coli. Vaccination was also the main solution mentioned by the producers together with improved management around standing water. The mortality related to *Effect of Weather* was described around times where the access to free-range area was deprived, which could lead to smothering due to overcrowding in areas around the popholes.

Three themes related to maintaining a low mortality were identified: *Pullet quality*, *Management and Time* and *Feed*. In relation to the theme *Pullet quality*, the producers experienced that the quality of the pullets in terms of health and 'fitness' was paramount for the production. The producers expressed that receiving healthy and uniform pullets, in terms of weight and appearance, together with maintaining the health and uniformity after placement at the production site were important. Studies have shown that problems that occur during rearing can be transferred to the production site (Bestman et al., 2009). In the theme *Management and Time,* producers expressed that the time spent with the hens, being able to incorporate a certain flexibility and allowing extra time when required for immediate problem solving are important for the organic egg producers when reducing hen mortality. A similar result was found in relation to calf mortality in dairy production in a study based on qualitative interviews (Vaarst and Sørensen, 2009). Removing dead hens is highly important, because dead hens impose a risk for the living hens in the group, as bacteria and potential pathogenic organism will develop in the carcass and will be released as the carcass breaks (Collett, 2013). A steady supply of appropriate feed and water was the focus in the

theme *Feed*. Appropriate diet should fulfil the hens' recruitment for nutrients and energy for maintenance and egg production (National Research Council, 1994).

4.2. Endoparasite infections

The producers argued for potential negative effects of endoparasite infections, although it had not been a focus area until recently. This is in accordance with results from observational studies. One of the most common helminth species is *Ascaridia galli* (Permin et al., 1999, Kaufmann et al., 2011) which can cause weight depression (Reid and Carmon, 1958; Kilpinen et al., 2005). Furthermore, endoparasite infections were associated with clinical symptoms such as loss of comb colour and strength (Ramadan and Abou Znada, 1991), and they were reported to affect the behaviour of the hens (Gauly et al., 2007).

Even though awareness of problems with endoparasite infections seems to be low, the producers seemed to have gained some experiences and have used it to generate different strategies for controlling the infections. The identified strategies are different ways of conducting test and treat (*regular* or *irregular testing*); a strategy relying on anthelmintic or a strategy without testing or use of anthelmintics (*no testing*). The producers who had chosen the *no testing* strategy did not believe that endoparasite infections caused problems in their herd, whereas producers who conducted tests had a strategy for treating relying on anthelmintics for controlling the endoparasite infections. Alternative control strategies that are not relying on the use of anthelmintics to control the infection risk in organic production are needed as concluded by Kaufmann et al. (2011), as most hens are subclinically infected with helminths. The producers only mentioned some few management practices, indicating that the producer either had a lack of knowledge about other possibilities for control of endoparasite infections or that effective alternative control strategies are lacking. In general, the producers' knowledge about possible alternative strategies and their potential effects should be improved if implementing alternative strategies. Further, alternative control strategies should be developed and investigated, which is already done in relation to stocking density, litter management and free-range management (Heckendorn et al., 2009; Maurer et al., 2009; Maurer et al., 2013).

The decision regarding treatment or not in the test and treat strategies (*regular* or *irregular testing*) where based on the producers' own perception of the current problem and recommendation for the veterinarian. In general, the producers stated that they allowed a low level of endoparasite

infections as long as no other problems were present. Treatment could thereby be avoided or postponed.

The producers applying the *no testing* strategy believed that the silage was beneficial for the hens as a preventative measure. However, Das et al. (2012) found that a diet rich on fibres could alter the gastrointestinal environment and thereby favour the establishment of *A. galli*. This is supported by the finding of Shreshtha et al. (2013), who found a tendency to higher worm count when providing silage supplement, indicating a higher susceptibility to *A. galli* infection.

4.3. The connection between mortality and endoparasite infections

With the knowledge and experience gained by the producers during the last couple of years, since endoparasite infections became a focus area, they perceived a mutual relationship between the management practices related to mortality and endoparasite infections. The producers stated that they perceived and believed that there was a connection between the two conditions, without being able to explain it further, which probably can be due to limited or lack of detailed and specific knowledge regarding endoparasite infections. With increased experience and knowledge about the potential effects of endoparasite infections, it is possible that current perception is revised or further developed.

The producers experienced that their erfa-groups were a source for gaining new relevant knowledge and for discussing current problems. Meetings with colleagues were seen as a way of getting support and help in certain situations. This could be a way of introducing alternative solutions related to endoparasite management also, when a suitable solution is ready and applicable in the production, either in combination with a 'test and treat strategy', or in the form of a completely alternative strategy. The success of implementing new practices or other initiatives depends on the producers' attitude, as Tuyttens et al. (2011) also stated regarding the transition from traditional cage systems to other systems in a questionnaire survey.

5. Conclusion

The producers identified four main cause for hen mortality: Predator attacks, effect of weather changes, infectious diseases and smothering, and different practices for maintaining a low mortality were stated and grouped into three theme: *Pullet quality*, *Management and time*, and *Feed*. Endoparasite infection, mainly related to helminth infection, were an area where the producers had limited knowledge and it at first recently emerged as a focus area. Despite this, three strategies for

controlling endoparasite infection were identified: *no*, *regular* or *irregular testing*. The last two were test-and-treat strategies. In conclusion, Danish egg producers appear to have three areas of focus in relation to mortality control and believe in a test-and-treat strategy in relation to controlling endoparasite infections.

Conflict of interest statement

There are no conflicts of interest.

References

Anonymous, 1991. Council Regulation (EEC) No 2092/91 of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs. Off. J. Eu. Commun., L198, 1-15.

Anonymous, 2013. Det danske fjerkræraad årsberetning 2012. (In Danish, "Danish poultry council annual report 2012"). Danish poultry council, 112 p.

Anonymous, 2014a. Statistics Denmark, http://www.statistikbanken.dk. Accessed September 2014.

Anonymous, 2014b. Tabelbilag til formændenes beretninger ved årsmødet den 4. februar 2014 (In Danish, " Appendix to the chairman report at the anual meeting 4th of February 2014"). Dansk Erhvervsfjerkræ. 43, 114-123.

Bestman, M., Koene, P., Wagenaar, J.P., 2009. Influence of farm factors on the occurrence of feather pecking in organic reared hens and their predictability for feather pecking in the laying period. Appl. Anim. Behav. Sci. 121, 120-125.

Collett, S.R., 2013. Principles of disease prevention, diagnosis, and control, in: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L., Nair, V.L. (Eds.), Diseases of Poultry. Wiley-blackwell, pp. 4-40.

Das, G., Abel, H., Humburg, J., Schwarz, A., Rautenschlein, S., Breves, G., Gauly, M., 2012. The effects of dietary non-starch polysaccharides on *Ascaridia galli* infection in grower layers. Parasitology. 139, 110-119.

Dawkins, M.S., 2012. Commercial scale research and assessment of poultry welfare. Br. Poult. Sci. 53, 1-6.

Fossum, O., Jansson, D.S., Etterlin, P.E., Vagsholm, I., 2009. Causes of mortality in laying hens in different housing systems in 2001 to 2004. Acta Vet Scand 51, 3.

Gauly, M., Duss, C., Erhardt, G., 2007. Influence of *Ascaridia galli* infections and anthelmintic treatments on the behaviour and social ranks of laying hens (*Gallus gallus domesticus*). Vet. Parasitol. 146, 271-280.

Heckendorn, F., Häring, D.A., Amsler, Z., Maurer, V., 2009. Do stocking rate and a simple run management practice influence the infection of laying hens with gastrointestinal helminths? Vet. Parasitol. 159, 60-68.

Hegelund, L., 2007. Management systems for organic egg production - Aiming to improve animal health and welfare (PhD thesis). DJF Animal Science 80, 1-123.

Hegelund, L., Sørensen, J.T., Hermansen, J.E., 2006. Welfare and productivity of laying hens in commercial organic egg production systems in Denmark. NJAS - Wagen J Life Sc 54, 147-155.

Hermansen, J.E., 2003. Organic livestock production systems and appropriate development in relation to public expectations. Livest. Prod. Sci. 80, 3-15.

Horseman, S.V., Roe, E.J., Huxley, J.N., Bell, N.J., Mason, C.S., Whay, H.R., 2014. The use of in-depth interviews to understand the process of treating lame dairy cows from the farmers' perspective. Anim. Welf. 23, 157-165.

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

Kaufmann, F., Das, G., Sohnrey, B., Gauly, M., 2011. Helminth infections in laying hens kept in organic free range systems in Germany. Livest. Sci. 141, 182-187.

Kilpinen, O., Roepstorff, A., Permin, A., Nørgaard-Nielsen, G., Lawson, L.G., Simonsen, H.B., 2005. Influence of *Dermanyssus gallinae* and *Ascaridia galli* infections on behaviour and health of laying hens (*Gallus gallus domesticus*). Bri. Poult. Sci. 46, 26-34.

Kvale, S., Brinkmann, S., 2008. InterViews: Learning the craft of qualitative research interviewing. Second ed. SAGE Publications., Califorina.

Maurer, V., Amsler, Z., Perler, E., Heckendorn, F., 2009. Poultry litter as a source of gastrointestinal helminth infections. Vet. Parasitol. 161, 255-260.

Maurer, V., Hertzberg, H., Heckendorn, F., Hördegen, P., Koller, M., 2013. Effects of paddock management on vegetation, nutrient accumulation, and internal parasites in laying hens. J. Appl. Poult. Res. 22, 334-343.

Moberly, R.L., White, P.C.L., Harris, S., 2004. Mortality due to fox predation in free-range poultry flocks in Britain. Vet. Rec. 155, 48-52.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Permin, A., Bisgaard, M., Frandsen, F., Pearman, M., Kold, J., Nansen, P., 1999. Prevalence of gastrointestinal helminths in different poultry production systems. Bri. Poult. Sci. 40, 439-443.

Ramadan, H.H., Abou Znada, N.Y., 1991. Some pathological and biochemical studies on experimental ascaridiasis in chickens. Nahrung. 35, 71-84.

Reid, W.M., Carmon, J.L., 1958. Effects of numbers of *Ascaridia galli* in depressing weight gains in chickens. Trop. Anim. Health. Prod. 44, 183-186.

Shreshtha, A., Norup, L.R., Juul-Madsen, H.R., Steenfeldt, S., Afrose, S., Engberg, R.M., 2013. Influence of maize silage supplementation on selected intestinal bacteria and the course of a *Ascaridia galli* infection on organic layers, in: Proceedings of the 19th European Symposium on Poultry Nutrition. http://www.wpsa.com/proceedings/ESPN_2013/assets/pdf/0299.pdf., 5 p.

Stokholm, N.M., Permin, A., Bisgaard, M., Christensen, J.P., 2010. Causes of mortality in commercial organic layers in Denmark. Avian Dis. 54, 1241-1250.

Transana 2.50 [Computer software], 2012. Madison, WI: The board of regents of the university of Wisconsin system. Available: http://www.transana.org.

Tuyttens, F.A.M., Sonck, B., Staes, M., Van Gansbeke, S., Van den Bogaert, T., Ampe, B., 2011. Survey of egg producers on the introduction of alternative housing systems for laying hens in Flanders, Belgium. Poult. Sci. 90, 941-950.

Vaarst, M., Sørensen, J.T., 2009. Danish dairy farmers' perceptions and attitudes related to calf-management in situations of high versus no calf mortality. Prev. Vet. Med. 89, 128-133.

Wier, M., Jensen, K.O., Andersen, L.M., Millock, K., 2008. The character of demand in mature organic food markets: Great Britain and Denmark compared. Food Policy 33, 406-421.

5 Discussion

The overall aim of this thesis was to investigate animal welfare in organic egg production with an emphasis on mortality and helminth (*A. galli* and *Heterakis* sp.) infections. This was investigated in three studies based on three hypotheses. Study I investigated how mortality and helminth infection are associated, Study II investigated how helminth infection and clinical welfare indicators are associated and Study III investigated how the producers manage mortality and helminth infections. In this chapter, the outcome of the research activities and methodologies will be discussed in relation to the hypotheses and overall aim of the PhD thesis.

5.1 Study population

The recruitment of the farms was based on voluntary participation, and the initial recruitment letter stated that the project aimed to investigate animal health and welfare. The participating farms may therefore have been farms without any recognised problems related to animal health and welfare. However, in the present study, the participating farms were recruited prior to the placement of the pullets at the egg production site, as the recruitment was conducted while the pullets were reared or with more than six months until placement. The early agreement to participate in the project means that the producers would have had no or only little knowledge about how the hens' appearance would be at the time of the farm visits. Further, a variation was observed among the participating farms in relation to the animal-based welfare indicators, especially for plumage condition and keel bone damage (Appendix 4). This indicates that the participating producers were not only producers that experience good animal welfare.

When comparing the participating farms with all Danish organic egg producers, there were some differences. Single-tiered systems were overrepresented among the participating farms compared to the general distribution between single-tiered and multi-tiered system in Denmark, as half of the organic egg producers have a single-tiered system and the other half have a multi-tiered system (Johansen, N.F., 2014 personal communication). However, the percentage of participating farms with a given size was relatively similar to the distribution size among all Danish organic egg farms during recruitment (14% had less than 3,000 hens, 44% had 3,001-9,000 hens, 32% had 9,001-15,000 hens, and 10% had more than 15,000 hens; Central Husbandry Register, February 2012). There was a slight overrepresentation of participating farms with more than 9,000 hens (53.3% and 42% among participating farms and all organic egg farms, respectively), but since the recruitment in

2012, the percentage of farms with more than 9,000 hens have increased to 55% of all organic egg farms (Central Husbandry Register, December 2014). This indicates that the participating farms showed a relatively representative sample of all the Danish organic egg farms regarding farm size, but not regarding housing system.

5.2   Association between mortality and helminth infection (Study I)

In Study I, the effect of helminth infection on the mortality rate was evaluated based on the producer's recordings of the weekly mortalities using a survival analysis model.

Previous studies including the effect of helminth infections on mortality found no significant effect (Häne et al., 2000; Sherwin et al., 2013). In the study by Häne et al. (2000), the average mortality rate per 28-days was used, and they found a similar mortality in flocks with and without helminth eggs in the droppings. This study included flocks with and without free-range access that were pooled for the analysis of the effect of helminth infections on the mortality rate, i.e. access or no access to free-range was not included in the analysis. The result also showed significantly higher mortality percentages in flocks with free-range access compared to flocks without free-range access (0.83% (SD: 0.65%) and 0.59% (SD: 0.49%), respectively). Furthermore, the helminth infection level was significantly higher in flocks with free-range access (75%) compared to flocks without free-range access (43%). This may indicate that taking access to free-range into account in the analysis of the effect of helminth infections on mortality rate might have given another result. The mortality rate may have been higher in helminth-infected flocks with free-range access compared to flocks with free-range access without a helminth infection, and the same for flocks without free-range access.

The study by Sherwin et al. (2013) investigated the association between helminth infections and mortality in free-range flocks. They reported no correlation between mortality and nematode infection, which is a taxonomic group of helminths. The level of helminth infections was determined within five weeks before depopulation, while mortality was the accumulated mortality for the entire production period. Other studies have reported that helminth infections fluctuate during the production period, with the maximum infection level (mean EPG) around peak of lay but the highest incidence of infections, i.e. highest prevalence of infected hens, at end of lay (Pennycott & Steel, 2001; Höglund & Jansson, 2011). This indicates that the determinaion of helminth infections at the end of lay might not be representative for the helminth infection level during the entire production period. Therefore, Sherwin et al. (2013) report of no correlation between helminth

infections at end of lay, and the accumlated mortality does not mean that there is no association between helminth infections and mortality.

Regardless of previous studies reporting that there is no association between mortality and helminth infections, mortality rates were expected to be different between low- and high-infected farms in the present study. This was expected due to the immune response related to *A. galli* infections and the increased severity of bacterial infections in dual infections with both helminth and bacterial infections (Dahl et al., 2002; Degen et al., 2005; Eigaard et al., 2006; Permin et al., 2006; Schwarz et al., 2011; Pleidrup et al., 2014).

5.2.1   Higher mortality rate in high-infected farms

The analyses in Paper I showed that the hazard functions for the low-infected farms (≤200 EPG) observed in summer and in winter were similar, i.e. there was no significant difference in the mortality rate between seasons in low-infected farms. However, the mortality rate in the high-infected farms (>200 EPG) was twice as high during summer compared to the mortality rate in the low-infected farms, whereas there was no significant difference between the high-infected farms observed in the winter and the low-infected farms. There were no apparent reasons for the different effects on the mortality rate in the high-infected farms in summer and winter. However, as stated in Paper I, the weather condition during the winter visits might have affected the results. Summer and winter visits were performed from August to September 2012 and from January to March 2013, respectively. The mean temperature in the summer months was similar to the norm temperature in these months, and the precipitation and sunshine hours seen in the two summer months were in the same range as the norm (DMI, 2014; Appendix 8), whereas the winter months were colder and had more days with frost compared to the norm (DMI, 2014; Appendix 8). This weather situation might have influenced the level of bacterial pathogens in the environment, as low temperatures may decrease bacterial growth (Perry et al., 2002). Consequently, the negative immunological effect of having a helminth infection might be decreased when the exposure to bacteria is reduced. A reduced effect of the weather conditions could have been achieved by including data from more than one year per season; however, this was not possible within the frame of the PhD study.

The risk of being infected with nematodes (helminths) is higher during summer (Kaufmann et al., 2011b; Sherwin et al., 2013), which means that it could be expected that more farms would be high-infected during summer. However, in the present study, the proportion of high-infected farms in winter and summer was similar or with a slightly higher proportion of high-infected farms

observed in summer (see Table 1 in Paper I). The number of farms included in the study is limited. It is, therefore, not possible to conclude if the risk of being high-infected was higher during summer compared to winter.

The findings in the present study indicate that mortality in organic egg production may potentially be reduced by measures to control the helminth infections, especially in high-infected farms. Control of helminth infection should not only be based on anthelmintics (deworming), but should also be based on alternative ways of controlling helminth infections. Höglund & Jansson (2011) reported that the EPG levels after deworming ended at a higher level than before treatment, which they suggest is due to impaired immunity in the time after deworming. This means that anthelmintics treatment is not the way to eradicate the problem, but only a procedure that deals with the problem for a limited period. The reliance should be on preventive strategies instead of anthelmintics, as also suggested by others (Jansson et al., 2010; Kaufmann et al., 2011b), and studies are testing possible alternative strategies like paddock rotation and litter management (Heckendorn et al., 2009; Maurer et al., 2009; Maurer et al., 2013).

5.2.2 Methodological considerations

In the present study, the mortality rates were evaluated in relation to the farms' helminth infection levels, based on the mean farm EPG that was the mean of the hens' individual FEC. A high variation between results from the individual FECs within a farm was observed; however, a high variation between animals and faecal samples collected in the same environment is normal. The number of worms detected in the intestines and the results of FECs in hens orally infected with the same dose is high (Gauly et al., 2001; Dahl et al., 2002; Gauly et al., 2007). Similar variation between samples is also seen under commercial settings (Pennycott & Steel, 2001; Höglund & Jansson, 2011; Kaufmann et al., 2011b).

The categorisation of farms, as either low- or high-infected, meant that it was the effect of the farms' helminth infection level that was evaluated. However, with mathematically arguments it can be shown that the results of an analysis using the hen's individual infection levels would give the same results as an analysis performed using the farms infection level. The arguments given in Appendix 9 state that if the mortality rate in high-infected farms is larger than the mortality rate in low-infected farms, then the mortality rate of infected hens will be larger than the mortality rate in non-infected hens. An investigation of the association between infection levels in individual hens

and weekly event status (death or alive) would therefore result in similar conclusions as an investigation conducted at farm level.

The observational study design caused that it was not possible to achieve a balanced distribution between low- and high-infected farms, as the infection status of the farms is not known prior to the initiation of the study. The producers themselves have a limited knowledge of the level of helminth infection (Paper III), and it could therefore not be expected that they could provide information about the potential infection status on their farm. A balanced design was not the main objective in the recruitment of farms; however, initiatives could have been included in an attempt to achieve a balanced design. This could have included pre-tests of the farms' helminth infection levels and based on the results, include farms that were expected to have a certain helminth infection level. However, this would have involved recruitment of several more farms than needed in the actual study and analysis of a larger number of faecal samples.

Even though the study represented more than 15% of the Danish organic hens in 2013 (see Paper I), and included 16% of the Danish farms with organic egg production at the time of recruitment (11 out of 66 farms; Anonymous, 2013), future studies are needed before the results can be generalised. This is both because the study did not take the variation between years into account and because of the limited number of farms included. However, generalisation within Danish organic egg farms, especially for the results of a higher mortality rate in high-infected farms during summer, might be reasonable, as this result agrees with the expected immunological response of an *A. galli* infection.

5.3 Helminth infection and clinical welfare indicators (Study II)

In Study II, the association between helminth infection and clinical welfare indicators were investigated using a graphical model to report whether helminth infection could be predicted from at least one clinical welfare indicator. This was investigated in an observational study with two sampling points where the individual hens were examined, i.e. a longitudinal study. Identification of the individual hens in the large flocks was required to keep track of the hens between the visits. Hens are not tagged individually as cattle, sheep and goats (Anonymous, 2014a), which means that the hens should be tagged specifically for this study. During the first visit, 100 hens in each flock were tagged with leg brands. The tagged hens should then be relocated at the second visit. Between 11 and 21 tagged hens per farm on 12 farms were relocated at the second visit, giving a total of 214 hens or almost 18% of the total number of tagged hens. As described in the methodology section (section 3.2), the aim was to have 20 hens examined twice, which only succeed on 5 of the 12 farms

(42%) (see Paper II). The producers reported finding lost leg brands in the litter in the period between the two visits, but as this was not recorded systematically, the proportions are not known. However, the lost leg brands have affected the chance of finding 20 tagged hens in the flocks of 3,000 hens, as the number of tagged hens will have decreased.

The identification of the individual hens was the main problem regarding this study, and previous statements concluded that identification of individual hens in large flocks is one reason for the limited number of studies of individual hens in large flocks (Freire et al., 2003). The leg brands showed to be a possible solution, which did not demand high-tech equipment, and it was possible to relocate the hens without too much effort. Further, the clinical assessment of the hens' feet indicated no increased level of damage to the feet or legs between placements of the leg brands and until the hens were examined at end of lay, which were 28 to 41 weeks after the leg brands were placed. This indicates that the leg brands did not become a pecking target.

5.3.1 Prediction of helminth infection by a clinical welfare indicator

The analysis in Paper II showed an association between back feathering at the end of lay and helminth infections diagnosed at end of lay, but not between helminth infections and any of the other indicators included in the study. Thus, back feathering at end of lay were the only indicator that could provide any information about helminth infections. The direction of the association was that hens with a good plumage condition on the back had a higher incidence of helminth infections compared to hens with a poor plumage condition on the back (see Paper II). This indicates that hens with a good plumage condition on the back have a higher risk of being infected with helminths. This suggests that hens with a good plumage condition on the back might spend more time in areas that potentially are contaminated with parasites, i.e. the littered area and the free-range area (Bray & Lancaster, 1992; Heckendorn et al., 2009; Maurer et al., 2009).

It has been reported that the more time hens spend outside, the better the plumage condition (Bestman & Wagenaar, 2003; Nicol et al., 2003; Mahboub et al., 2004; Lambton et al., 2010; Bestman & Wagenaar, 2014). However, the causation is unclear, but there could be two possible explanations: *i)* the use of the free-range area decreases plumage damage, or *ii)* mainly hens with good plumage condition use the free-range area. Either explanation would be in relative good agreement with the present findings. Following the first explanation *i)*, it could be suggested that the hens with a good plumage condition at the end of lay are those that use the free-range, thereby the level of plumage damage is decreased in the users compared to non-users of the free-range, and

at the same time, these hens will have an increased risk of infection, as the free-range is potentially contaminated with helminth eggs. Following the second explanation *ii)*, it could be suggested that hens with a poor plumage condition stay inside and thereby experience a reduced risk of infection, whereas hens with good plumage condition experience an increased risk of infection by using the free-range. This indicates that regardless the causation, it is important to limit the infection pressure from the free-range areas. Paddock rotation has shown to have a positive effect on the parasite infection load together with the application of wood chips or gravel in front of the house (Heckendorn et al., 2009; Maurer et al., 2013), and the area just in front of the house is especially important as it has the highest parasite contamination (Bray & Lancaster, 1992; Heckendorn et al., 2009).

The results stating that a good plumage condition on the back can predict or indicate a helminth infection might not be useful in relation to on-farm management. The producers are aiming at having hens with a good plumage condition and a good plumage condition is included in the evaluation of the animal welfare in the official control (Anonymous, 2014d). This means that good plumage condition is supposed to be the standard and the goal and, therefore, not practicable as an indicator of the invisible helminth infection. Instead, if the helminth infection would have been indicated by a negative measure it could potentially have been more useful in realtion to on-farm management.

The analysis in Paper II included the FEC results of *A. galli/Heterakis*; however, the post mortem worm count conducted in the same hens as in Paper II (results presented in Appendix 7), indicates that the hens in the study were mainly infected with *A. galli*. Of the 214 hens, three hens had a *Heterakis* sp. infection, but on the same time, they also had *A. galli* worms. This indicates that the detected effect, in Study II, mainly relates to *A. galli* infections, and that *A. galli* is the dominating helminth in Danish organic hens. This is contrary to the findings by Permin et al., (1999), who reported that in Danish free-range and organic systems, *Heterakis gallinarum* was dominating with a prevalence of 72.5% while *A. galli* had a prevalence of 63.8%. This potentially means that the incidence of *Heterakis* sp. infection has decreased since 1994-1995, where Permin et al. (1999) investigated the infection level on Danish farms.

The investigation in Paper II showed that between peak of lay and end of lay, the prevalence of keel bone deformities increased and that the plumage condition deteriorated in the 214 tagged hens, which is in agreement with other studies (Fleming et al., 2004; Nicol et al., 2006; Drake et al., 2010; Richards et al., 2012). This association was also displayed in the graphical model with direct

connection between the indicators at peak and end of lay. The prevalence of bumble foot (dorsal swellings) and pale combs decreased between the visits; however, the incidence level was low at both visits. A report states that acute bumble foot occurs around 30-40 weeks of age (EFSA, 2005), which corresponds with the age during the peak of lay visits where a higher prevalence of dorsal swellings was reported. Low prevalence of hens with feet problems at end of lay (0.5-1.9%), indicates that the dorsal swellings healed between peak and end of lay and that hens with dorsal swellings at peak of lay did not have other problems with their feet at end of lay.

5.3.2   Methodological considerations

The on-farm study design presented some limitation and benefits. Firstly, the number of included hens per flock was limited; however, the number of hens included in total was in the same range as experimental setting potentially would have included. Secondly, when studying hens on commercial farms, the hens are exposed to life in large flocks and the management practices related to that, whereas an experimental setting would only include small flocks that potentially could affect the conclusion. Small flock size is known to affect the hens' use of the free-range area (Bubier & Bradshaw, 1998; Hirt et al., 2000; Hegelund et al., 2005), and hens in an experimental setting would potentially not be exposed to the same parasite contamination as hens in commercial settings, which could necessitate initiatives to ensure that helminth infections are established. Finally, conclusions from experimental studies might not always be directly applicable to commercial on-farm settings (Ersbøll & Toft, 2004; Dawkins, 2012), whereas on-farm observational studies are directly applicable as they study the actual condition on the included farms. The hens included in the present study were all hens that had experienced the life of a high productive hen living with around 3,000 other hens, and the randomised selection of the hens from the beginning should ensure that the included hens represented the conditions of the other hens as well.

Instead of conducting the study on individual hen level, the study could have been performed at flock level using a representative sample from each flock, thereby analysing the response between flocks instead of between hens. Each flock could contribute with being either in poor or good welfare status for each indicator. However, this would require a defined cut-off point for when the status is good or poor at flock level for each indicator, or each flock could contribute with the percentage of individual examined hens being in good or poor welfare status as a numerical value instead of a binary variable. However, that would require a higher number of participating farms in order to achieve enough statistical power.

5.4 Management related to mortality and helminth infection (Study III)

In Study III, the producer's daily practices and management strategies for maintaining a low mortality and controlling endoparasite infections were identified based on qualitative interviews. The qualitative interview method explores the range within a certain area and is context specific. The results of the qualitative interviews cannot be quantified, i.e. the percentages of producers conducting a certain practice or having a certain belief cannot be stated. If quantification is important, a questionnaire study should be performed instead. Sparks et al. (2008) used a questionnaire in order to gain understanding of the production environment, key constrains and factors affecting the health among organic pullet rearers, while Tuyttens et al. (2011) used a questionnaire to quantify, among other, reasons for selecting a certain production system. In these studies, the percentage of producers stating a certain action can be mentioned. This is not possible in the present study as it is based on qualitative interviews among a group of producers. Further, it should not be generalised to be the opinion of all Danish organic egg producers as the result only reflects the opinion of the interviewed persons. However, with cautiousness, it can be seen as a reflection of the opinion from all participating producers, or more precisely, it can be seen as what would be anticipated if all participating producers were interviewed.

If the aim is to generalise the results of the qualitative interviews, a mixed method approach could be applied. This means that the results of the qualitative interviews were transformed into a questionnaire, which could be sent to a higher quantity of producers, while another mixed method approach could be to combine the results obtained in the observational studies with the producers statements in the interviews. However, this was not the aim of this study, so the study did not try to quantify the results.

5.4.1 Identified management practices

The main identified causes of mortality were *Predators*, *Effect of weather*, *Infectious diseases* and *Smothering*. Mortality related to the *Effect of weather* was often due to events of smothering, which occurred under special weather conditions. Predation, infectious diseases and smothering have previously been reported as causes of death in commercial organic egg productions (Hegelund et al., 2006b; Fossum et al., 2009; Stokholm et al., 2010). The producers stated that getting a good start at the egg producing site and getting pullets of a good quality (*Pullet quality*) are important for maintaining a low mortality. It is known that the condition of the pullets can affect the condition of the hens later in the production. Bestman et al. (2009) showed that pullets with plumage damage

were likely to have plumage damage at the egg production site, meaning that problems starting at the rearing facility might continue at the egg production site, thereby potentially affecting the production results. The other two themes for maintaining a low mortality were *Management and time* and *Feed*, both related to the daily management and routines on the farm. Having time for unexpected work tasks and problem solving was important, which also has been reported in a study of calf mortality by Vaarst & Sørensen (2009).

Regarding the control of endoparasite infections, three strategies were identified: no testing, regular testing and irregular testing; the last two being test and treat strategies. In general, the producers had limited knowledge about alternative strategies, but were open-minded towards trying them out. Therefore, knowledge about alternative strategies should be provided to the producers. Among the Danish producers, knowledge often spread through experience-exchange producer groups (called 'erfa-groups') and meetings, where other producers participate. Therefore, providing knowledge to some producers may be a potential way of spreading the knowledge to many producers, especially if a producer can inform about his/her own experience with the practice/knowledge, which should be spread.

The hypothesis of this study was that it is possible to identity management strategies for maintaining low mortality and controlling endoparasite infections. The producers consider that management strategies and practices related to maintaining low mortality and controlling endoparasite infections had a mutual effect. The mutual effect between management strategies related to mortality and endoparasite infections indicates, that it would be possible to make a management strategy that includes both mortality and endoparasite infections, without having to compromise on the one or the other. The results of the interviews show that a management strategy should include focus on the start-up phase, the health and "fitness" of the pullets, time spent with the hens, daily routines, flexible time management, feed quality and prevention of endoparasite infections.

5.4.2 Methodological considerations

For the qualitative study, an interview guide was constructed. The guide included a theme about endoparasite infections, but as mentioned in Paper III, the producers' reply mainly concerned *A. galli* infections. The producers' awareness of endoparasite infections was new (within the last years), which might explain why their focus and awareness primarily were related to one species (*A. galli*). This can also be related to the fact that within the last year, information about *A. galli* has

been provided to the producers in magazines and at conferences (Kabell, 2012, Mikél & Kabell, 2013, Kabell, 2014). The post mortem worm count (see Appendix 7) indicated that *A. galli* was mainly responsible for the endoparasite infections on the participating farms, which indicates that the producers' primary focus on *A. galli* might help decrease the problems related to helminth infections in the participating farms.

Of the 15 participating producers, seven were interviewed. The process of conducting interviews was finalised when it was concluded that the point of saturation was reached. The point of saturation is the point where a new interview does not contribute with any new information, i.e. a new interview only repeats or supports statements already stated in other interviews and the interviews seem quite repetitious (Kvale & Brinkmann, 2008). It was decided that the interview process should be finalised when the point of saturation was achieved. Another decision could have been to interview all participating producers; thereby conducting interview after the point of saturation was reached. However, this might not have provided any new information, and it would have prolonged the transcribing and analytical phase without contributing with extra knowledge.

The seven producers that were interviewed were chosen among the participating producers from a randomly generated list, where the producers were interviewed according to the order in which they were listed. The random list ensured that the producers were randomly chosen; thereby eliminating the possible effects of allowing a known prehistory related to the farms and the producers to influence which producers that were interviewed, which may have affected the outcome. Four farms were excluded from the random list due to travel distances or shift in farm manager; no other exclusion criteria were set. However, the interview study ended up only including farms with single-tiered systems. It is, however, not expected that the producer's perception and belief would differ depending on the housing system within the organic production. Therefore, the results from the interviews would not be expected to have been different if producers with multi-tiered system had been included, but it might have provided other perspectives or explanations.

The design of the present interview study did not include any prehistory of the farms; however, the interviewees could have been selected according to one or several parameters, e.g. mortality level, endoparasite level or a combination of the two. Previous qualitative studies have reported differences in attitude between farmers experiencing a high or low calf mortality (Vaarst & Sørensen, 2009), suggesting that grouping of farm according to prehistory of the farms may show difference between groups. However, such a grouping would be difficult within the egg production

sector, as there is no central registration of mortality or endoparasite infection levels. In addition, there may be variation between batches on the same farm. This potential variation might also mean that the producers would have experienced different levels of mortality and endoparasite infections.

5.5   General discussion across papers

The overall aim of this PhD thesis was to investigate animal welfare in organic egg production with emphasis on mortality and helminth infections. Three studies were carried out considering different aspect of animal welfare in organic egg production, focussing on animal-based welfare indicators that measure the hens' responses to the environment they are living in. All three studies indicated that there is a need for effective preventive methods for controlling helminth infections. Firstly, the mortality rate might be decreased in low-infected farms compared to high-infected farms, especially during summer. Secondly, the incidence of helminth infections was higher in hens with a good plumage condition on the back compared to hens with a poor plumage condition on the back, indicating that the producers cannot use this association to predict or as an indicator of the hens' helminth infection status. Thirdly, the producers lack knowledge about how to prevent helminth infections and depend on test and treat strategies if they have any focus on endoparasite infections at all. Improvement of and finding possible preventive strategies to controlling helminth infections could potentially increase the welfare of the organic hens by a decrease in mortality and reduced negative consequences of the helminth infections.

The results from each study may influence or be related to the results of another study, as the three studies investigate approximately the same flock (Appendix 1). The reported associations between mortality and helminth infections in Paper I indicate that prevention and control of helminth infections could reduce mortality. These results are in agreement with the producers' beliefs, that management practices related to maintaining a low mortality would help controlling endoparasite infections (mainly *A. galli*) and vice versa (Paper III). Further, the producers state that helminth infections weaken the hens and connect the helminth infections with other bacterial infections, which could result in the increased mortality rate as reported in Paper I.

The producers using test and treat strategies for controlling helminth infections stated that decision of treatment was multi-factorial based on test results, veterinarian advice, own beliefs and the status of the hens. One producer (Producer C in Paper III) explained that when the hens had a mild to moderate infection without any other welfare problems, then the decision was not to deworm. However, the results from Paper II showed an association between helminth infections and

back fathering with hens having a good plumage condition having the highest incidence of helminth infection. Therefore, judging whether to treat or not, based on the hens' general welfare appearance, might not be the best possible solution as the hens will not display visible useful symptoms of the infection.

Paper II reported that the incidence of helminth infections was higher in hens with a good plumage condition on the back compared to hens with a poor plumage condition. It could be postulated that hens with a good plumage condition on the back have an increased mortality risk, as the helminth infected hens would have a higher mortality rate than non-infected hens (see section 5.2; Appendix 9). Thereby, apparently healthy hens might die due to problems associated with helminth infections. It is suggested that hens are being infected in the free-range area (see section 5.3), showing the importance of decreasing the infection pressure in the free-range area.

6 Conclusion

An association between helminth (*A. galli*/*Heterakis*) infection and mortality rate at peak of lay was found in Study I. However, hypothesis 1 was not completely confirmed, as an increased mortality rate in high-infected farms compared to low-infected farms only was demonstrated for high-infected farms observed in summer and not for high-infected farms observed during winter.

In Study II, helminth (*A. galli*/*Heterakis*) infection diagnosed at end of lay was associated with back feathering at end of lay, with an increased incidence of helminth infection in hens with a good back feathering. Between peak and end of lay, the prevalence of keel bone deformities increased, and the plumage condition deteriorated, whereas prevalence of bumble foot and pale combs decreased. No significant differences for the other feet or skin damage indicators between the two visits were found. Thus, Study II confirmed hypothesis 2, as it was possible to predict *Ascaridia galli*/*Heterakis* sp. infections based on at least one clinical welfare indicator. However, as the association was that hens with good plumage on the back had a higher incidence of helminth infections, the association may not be very useful in relation to daily on-farm management.

Study III showed that it was possible to identify management strategies for maintaining a low mortality and controlling endoparasite infections, thereby confirming hypothesis 3. Three themes for maintaining low mortality were identified together with two different practices related to endoparasites, i.e. producers not noticing the issue (no testing) and producers using a test and treat strategy with regular or irregular test intervals.

The findings of this PhD study showed that organic egg producers lack knowledge of how to control helminth infections, beside test and treat strategies, and that control of helminth infections potentially could reduce mortality and improve animal welfare. Therefore, focus on helminth infections is important, and there is a need to develop preventive management strategies to control helminth infections applicable for commercial organic systems.

7 Perspectives

The results of this PhD study showed the importance of controlling helminth infections in relation to reducing mortality and improving the animal welfare of hens. In relation to the organic egg production, the current dependence of anthelminthics for controlling the helminth infections is not a sustainable method. Instead, alternative control strategies should be developed and implemented, which is also concluded by others (Jansson et al., 2010; Kaufmann et al., 2011b). This could be strategies related to management of the free-range (Heckendorn et al., 2009; Maurer et al., 2013) or use of genetic resistance (Permin & Ranvig, 2001) or plant supplements that could help the hens repel parasites from the intestine. In an experiment with broilers in a free-range system, Almeida et al. (2012) reported that *Artemisia annua* could reduce the oocyst excretion. The control of helminth infections would not only improve the welfare of Danish layers, but also of European layers, as they experience similar problems with helminth infections (see Appendix 5). However, it is not possible to state that the association found between mortality and helminth infections in Danish layers can be generalised to all organic layers. But reducing helminth infections, even if there is no effect on mortality, will improve the hens' welfare as helminth infection is an indicator for animal welfare. Further, studies that are more comprehensive are needed in order to fully understand the association between helminth infections and mortality. Such studies may potentially include the immunological response, as this might be the actual cause of the increased mortality rate in high-infected farms during summer, that was found in Paper I. New studies could benefit from extending the study to include data from more calendar months than the present, and including data from more than one year in order to limit the effect of weather and year.

An increased incidence of helminth infections was reported in hens with a good plumage condition on the back. Despite the unknown causality, the reportings of better plumage condition in flock where more hens are outside (Bestman & Wagenaar, 2003; Nicol et al., 2003; Mahboub et al., 2004; Lambton et al., 2010; Bestman & Wagenaar, 2014) suggest that the hens might become infected with helminth in the free-range area. This indicates that focus on reducing the parasite contamination in the free-range areas is needed for the continuous development of the organic production. The free-range area does not only provide a risk of infections (parasite and bacterial) and predation, but also provides behavioural and physiological stimuli (Knierim, 2006; Lay et al., 2011). However, the proportion of the flock that is outside at a given time is under 30% (Bubier & Bradshaw, 1998; Zeltner & Hirt, 2003; Hegelund et al., 2005; Hermansen et al., 2005; Hegelund et al., 2006a). Most hens stay near the house (Bassler et al., 2000; Hirt et al., 2000; Zeltner & Hirt,

2003), which is also the area with the highest parasite contamination (Bray & Lancaster, 1992) and also has an increased nutrient load (Hermansen et al., 2005; Aarnink et al., 2006). A more even distribution of the hens in free-range potential could decrease the parasite contamination and the nutrient load, especially when the general use of the free-range area is increased. Some producers state that they replace the topsoil in the area in front of the house, use seashells and disinfect the area, so practices for limiting the contamination are applied on the farms. However, a more even distribution of the hens in the free-range area and a continuous focus on solutions for how to limit the parasite contamination are needed.

The involvement of producers when new strategies are to be implemented, or when knowledge should be distributed, could be a possibility under Danish circumstances as the producers share information and new practices with one another (Paper III). New preventive strategies for controlling helminth infections could also be implemented on a few farms as a start, and the producers' experiences with the strategies will then spread to other producers, especially if the experiences are positive.

Most of the clinical animal-based welfare indicators had a low prevalence; however, the prevalence of keel bone deformities and poor plumage condition was relatively high (20-44% at end of lay, Paper II). This suggests that the Danish organic layers have a relative good animal welfare in some areas, but the results also show that there are possibilities for continuous improvement. A guide for solving feather pecking problems was publish at the end of 2013 (Johansen et al., 2013), and it included parameters that potentially influence feather pecking and a list of corrective actions that could minimise the problem in flocks with feather pecking and be used for preventive adjustments. The target audience of the guide was the producers, and the guide can be used as a helper; both as prevention or when action plans are ordered by the Danish AgriFish Agency (see section 2.1), and could potentially improve animal welfare by reducing feather pecking.

In general, improving the welfare of organic layers in Denmark and Europe should include focus on minimising the prevalence of helminth infections. It should also be investigated whether the association between mortality and helminth infections is present in other countries and between years, and the cause of the association should be localised. Further investigation on how to prevent keel bone deformities and decrease damage to the plumage should also be performed. In addition, distributing knowledge to and among producers of how to prevent and reduce the prevalence of helminth infections, keel bone deformities and plumage damage may be a powerful tool in improving animal welfare.

8 References

Aarnink, A. J. A., Hol, J. M. G., & Beurskens, A. G. C. 2006. Ammonia emission and nutrient load in outdoor runs of laying hens. NJAS - Wageningen Journal of Life Sciences 54:223-233.

Abreu, G. C. G., & Labouriau, R. 2010. Characterization of differentially expressed genes using high-dimensional co-expression networks. arXiv:1011.3805v1.

Ackert, J. E., & Herrick, C. A. 1928. Effects of the nematode *Ascaridia Lineata* (schneider) on growing chickens. The Journal of Parasitology 15:1-13.

Aerni, V., Brinkhof, M. W. G., Wechsler, B., Oester, H., & Frohlich, E. 2005. Productivity and mortality of laying hens in aviaries: A systematic review. Worlds Poultry Science Journal 61:130-142.

Almeida, G. F. d., Horsted, K., Thamsborg, S. M., Kyvsgaard, N. C., Ferreira, J. F. S., & Hermansen, J. E. 2012. Use of *Artemisia annua* as a natural coccidiostat in free-range broilers and its effects on infection dynamics and performance. Veterinary Parasitology 186:178-187.

Andersen, J. P. 2013. *A. Galli* infection in chickens - immunological and imuno-modulatory aspects. PhD Thesis. Science and Technology, Aarhus University. 123 pages.

Anonymous. 1991. Council regulation (EEC) no 2092/91 of 24 June 1991, on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs. Official Journal of the European Communities L198:1-15.

Anonymous. 1998. Council directive 98/58/EC of 20 July 1998, concerning the protection of animals kept for farming purposes. Official Journal of the European Communities L 221/23:1-5.

Anonymous. 1999. Council directive 1999/74/EC of 19 July 1999, laying down minimum standards for the protection of laying hens. Official Journal of the European Communities L 203/53:1-5.

Anonymous. 2002. Commission directive 2002/4/EC of 30 January 2002, on the registration of establishments keeping laying hens, covered by council directive 1999/74/EC. Official Journal of the European Communities L30/44:3.

Anonymous. 2008a. Commission regulation (EC) no 589/2008 of 23 June 2008, laying down detailed rules for implementing council regulation (EC) no 1234/2007 as regards marketing standards for eggs. Official Journal of the European Union L 163/6:1-18.

Anonymous. 2008b. Commission regulation (EC) no 889/2008 of 5 September 2008, laying down detailed rules for the implementation of council regulation (EC) no 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. Official Journal of the European Union L 250:1-84.

Anonymous. 2012a. Bekendtgørelse om økologisk jordbrugsproduktion m.v. BEK nr 716 af 27/06/2012 (In Danish: "Act on organic agricultural production")

Anonymous. 2012b. Commission implementing regulation (EU) no 505/2012 of 14 June 2012, amending and correcting regulation (EC) no 889/2008, laying down detailed rules for the implementation of council regulation (EC) no 834/2007, on organic production and labelling of organic products with regard to organic production, labelling and control. Official Journal of the European Union L 154/12 1-8.

Anonymous. 2012c. Det danske fjerkræraad - årsberetning 2009-2011. 120 pages. (In Danish: "Danish poultry councils annual report 2009-2011")

Anonymous. 2013. Det danske fjerkræraad - årsberetning 2012. 112 pages. (In Danish: "Danish poultry councils annual report 2012").

Anonymous. 2014a. Bekendtgørelse om mærkning, registrering og flytning af kvæg, svin, får eller geder. BEK nr 1090 af 08/10/2014 (In Danish: "Act om tagging, registration and relocation of cattle, pig, sheep, and goats).

Anonymous. 2014b. Statistics Denmark. http://www.Statistikbanken.Dk/statbank5a/default.Asp?W=1920 (Accessed September 2014).

Anonymous. 2014c. Laying hens by way of keeping. https://circabc.europa.eu (Accessed July 2014).

Anonymous. 2014d. The ministry of food, agriculture and fishery. Vejledning on økologisk jordbrugsproduktion 1-178. (In Danish: "Guide for organic agriculture")

Anonymous. 2014e. Tabelbilag til formændenes beretninger ved årsmødet den 4. Februar 2014. Dansk Erhvervsfjerkræ 43:114-123. (In Danish: "Appendix for the presidents report at the annual meeting 4th of February 2014").

Arnould, C., Butterworth, A., & Knierim, U. 2009. Standardisation of clinical scoring in poultry. Pages 7-30 in Assessment of animal welfare measures for layers and broilers. Welfare quality report no. 9. Forkman B., & Keeling. L. (eds.)

Barnett, J. L. & Hemsworth, P. H. 2009. Welfare monitoring schemes: using research to safeguard welfare of animals on the farm. Journal of applied animal welfare science, 12: 114-131

Bartussek, H. 2001. Animal needs index for laying hens ANI 35-l/2001 – laying hens. Bundesantalt für Alpenländishe Landwirtschaft, Gumpenstein, Austria:30.

Bassler, A., Ciszuk, P., & Sjelin, K. 2000. Management of laying hens in mobile houses - a review of experiences. Pages 45-50 in Darcof report no. 2. Ecological animal husbandry in the Nordic countries. Proceedings from NJF-seminar no. 303 Horsens, Denmark 16-17 September 1999. Hermansen, J. E., Lund V., & Thuen, E. eds. Danish Research Centre for Organic Agriculture, DARCOF.

Berg, C. 2001. Health and welfare in organic poultry production. Acta veterinaria Scandinavica. Supplementum 95:37-45.

Bestman, M., Koene, P., & Wagenaar, J. P. 2009. Influence of farm factors on the occurrence of feather pecking in organic reared hens and their predictability for feather pecking in the laying period. Applied Animal Behaviour Science 121:120-125.

Bestman, M., & Wagenaar, J. P. 2012. Health and welfare in organic laying hens in the Netherlands. 159-159, Proceedings in 2nd Organic Animal Husbandry Conference. Hamburg/Trenthorst, Germany.

Bestman, M., & Wagenaar, J. P. 2014. Health and welfare in Dutch organic laying hens. Animals 4:374-390.

Bestman, M. W. P., & Wagenaar, J. P. 2003. Farm level factors associated with feather pecking in organic laying hens. Livestock Production Science 80:133-140.

Bilcik, B., & Keeling, L. J. 1999. Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. British Poultry Science 40:444-451.

Blokhuis, H. J. 1986. Feather-pecking in poultry: Its relation with ground-pecking. Applied Animal Behaviour Science 16:63-67.

Blæsild, P., & Granfeldt, J. 2002. Statistics with applications in biology and geology. Chapman & Hall/CRS.

Bray, T. S., & Lancaster, M. B. 1992. The parasitic status of land used by free range hens. British Poultry Science 33:1119-1120 (Abstract).

Bright, A., Brass, D., Clachan, J., Drake, K. A., & Joret, A. D. 2011. Canopy cover is correlated with reduced injurious feather pecking in commercial flocks of free-range laying hens. Animal Welfare 20:329-338.

Broom, D. M. 1996. Animal welfare defined in terms of attempts to cope with the environment. Acta Agriculturae Scandinavica Section A Animal Science 0:22-28.

Bubier, N. E., & Bradshaw, R. H. 1998. Movement of flocks of laying hens in and out of the hen house in four free range systems. British Poultry Science 39 Suppl:S5-S6.

Central Husbandry Register. Ministry of food, agriculture and fisheries, Danish veterinary and food administration, central husbandry register https://chr.Fvst.Dk/chri/faces/frontpage?Adf-window-id=w0&_afrloop=3244114658091000&_afrwindowmode=0&_adf.Ctrl-state=5ke08dxx4_3&_afrredirect=3244114774682000.

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

Clark, W. D., Cox, W. R., & Silversides, F. G. 2008. Bone fracture incidence in end-of-lay high-producing, noncommercial laying hens identified using radiographs. Poultry Science 87:1964-1970.

Cloutier, S., Newberry, R. C., Forster, C. T., & Girsberger, K. M. 2000. Does pecking at inanimate stimuli predict cannibalistic behaviour in domestic fowl? Applied Animal Behaviour Science 66:119-133.

CORE organic HealthyHens project 2014. HealthyHens, good health and welfare in organic laying hens depends on good management http://www.Coreorganic2.Org/healthyhens (Accessed September 2014).

Cox, F. E. G. 2001. Concomitant infections, parasites and immune responses. Parasitology 122:S23-S38.

Croxall, R. A., & Elson, H. A. 2007. Comparative welfare of laying hens in a wide range of egg production systems as assessed by criteria in Swedish animal welfare standards. British Poultry Abstracts 3:15-16.

Dahl, C., Permin, A., Christensen, J. P., Bisgaard, M., Muhairwa, A. P., Petersen, K. M. D., Poulsen, J. S. D., & Jensen, A. L. 2002. The effect of concurrent infections with *Pasteurella multocida* and *Ascaridia galli* on free range chickens. Veterinary Microbiology 86:313-324.

Dawkins, M. S. 2012. Commercial scale research and assessment of poultry welfare. British Poultry Science 53:1-6.

Degen, W. G. J., van Daal, N., Rothwell, L., Kaiser, P., & Schijns, V. E. J. C. 2005. Th1/th2 polarization by viral and helminth infection in birds. Veterinary Microbiology 105:163-167.

DMI. 2014. Månedens, sæsonen og årets vejr (In danish: "Weather of the month, season and year"). http://www.dmi.dk/vejr/arkiver/maanedsaesonaar/ (Accessed November 2014).

Drake, K. A., Donnelly, C. A., & Dawkins, M. S. 2010. Influence of rearing and lay risk factors on propensity for feather damage in laying hens. British Poultry Science 51:725-733.

Duncan, I. J. H. 1996. Animal welfare defined in terms of feelings. Acta Agriculturae Scandinavica Section A-Animal Science. Supplement 27: 29-35.

EFSA. 2005. Scientific report: The welfare aspects of various system of keeping laying hens (EFSA-Q-2003-92). The EFSA Journal 197.

Eigaard, N. M., Schou, T. W., Permin, A., Christensen, J. P., Ekstrom, C. T., Ambrosini, F., Cianci, D., & Bisgaard, M. 2006. Infection and excretion of *Salmonella enteritidis* in two different chicken lines with concurrent *Ascaridia galli* infection. Avian Pathology 35:487-493.

El-Lethey, H., Aerni, V., Jungi, T. W., & Wechsler, B. 2000. Stress and feather pecking in laying hens in relation to housing conditions. British Poultry Science 41:22-28.

Ersbøll, A. K., & Toft, N. 2004. Experimental studies. Pages 61-75 in Introduction to veterinary epidemiology. Houe, H., Ersbøll, A. K., & Toft, N. (eds.) Biolofia.

Ersbøll, A. K., Tolf, N., & Bruun, J. 2004. Observational studies. Pages 47-61 in Introduction to veterinary epidemiology. Houe, H., Ersbøll, A. K., & Toft, N. (eds.) Biolofia.

FAWC. 1979. Press statement from the farm animal welfare council on Dec. 5, 1979. http://www.fawc.org.uk/pdf/fivefreedoms1979.pdf.

Fleming, R. H., McCormack, H. A., McTeir, L., & Whitehead, C. C. 2004. Incidence, pathology and prevention of keel bone deformities in the laying hen. British Poultry Science 45:320-330.

Fossum, O., Jansson, D. S., Etterlin, P. E., & Vagsholm, I. 2009. Causes of mortality in laying hens in different housing systems in 2001 to 2004. Acta Veterinaria Scandinavica 51.

Fraser, D. 2008. Understanding animal welfare. Acta Veterinaria Scandinavica 50 (SUPPL. 1):S1.

Fraser, D., Weary, D. M., Pajor, E. A., & Milligan, B. N. 1997. A scientific conception of animal welfare that reflects ethical concerns. Animal Welfare 6:187-205.

Freire, R., Wilkins, L. J., Short, F., & Nicol, C. J. 2003. Behaviour and welfare of individual laying hens in a non-cage system. British Poultry Science 44:22-29.

Fuller, F. 2004. The encyclopaedia of farm animal nutrition. CABI Pub.

Gauly, M., Bauer, C., Mertens, C., & Erhardt, G. 2001. Effect and repeatability of *Ascaridia galli* egg output in cockerels following a single low dose infection. Veterinary Parasitology 96:301-307.

Gauly, M., Duss, C., & Erhardt, G. 2007. Influence of *Ascaridia galli* infections and anthelmintic treatments on the behaviour and social ranks of laying hens (*Gallus gallus domesticus*). Veterinary Parasitology 146:271-280.

Gentle, M. J., & Hunter, L. N. 1990. Physiological and behavioural responses associated with feather removal in *Gallus gallus* var *domesticus*. Research in Veterinary Science 50:95-101.

Gilani, A. M., Knowles, T. G., & Nicol, C. J. 2013. The effect of rearing environment on feather pecking in young and adult laying hens. Applied Animal Behaviour Science 148:54-63.

Green, L. E., Lewis, K., Kimpton, A., & Nicol, C. J. 2000. Cross-sectional study of the prevalence of feather pecking in laying hens in alternative systems and its associations with management and disease. Veterinary Record 147:233-238.

Gunnarsson, S., Algers, B., & Svedberg, J. 2000. Description and evaluation of a scoring system of clinical health in laying hens in laying hens in loose housing systems. In Gunnarsson, S. Doctoral thesis, Swedish university of agricultural sciences, Uppsala 2000. Acta universitatis agriculturae sueciae veterinaria 73.

Gunnarsson, S., Odèn, K., Algers, B., Svedberg, J., & Keeling, L. 1995. Poultry health and behaviour in a tiered system for loose housed layers. Report 35. Swedish University of Agricultural Sciences, Faculty of Veterinary Medicine, Department of Animal Hygiene.

Heckendorn, F., Häring, D. A., Amsler, Z., & Maurer, V. 2009. Do stocking rate and a simple run management practice influence the infection of laying hens with gastrointestinal helminths? Veterinary Parasitology 159:60-68.

Hegelund, L. 2007. Management systems for organic egg production - aiming to improve animal health and welfare. DJF Animal Science no.80. PhD thesis:1-123.

Hegelund, L., Hermansen, J. E., Sørensen, J. T, & Horsted, K. 2006a. Økologisk ægproduktion: Produktion, sundhed, velfærd og næringsstofhusholdning. 49 pages. (In Danish: "Organic egg production: productivity, health, welfare and nutrient management")

Hegelund, L., Sørensen, J. T., & Hermansen, J. E. 2006b. Welfare and productivity of laying hens in commercial organic egg production systems in Denmark. NJAS - Wageningen Journal of Life Sciences 54:147-155.

Hegelund, L., Sørensen, J. T., & Johansen, N. F. 2003. Developing a welfare assessment system for use in commercial organic egg production. Animal Welfare 12:649-653.

Hegelund, L., Sørensen, J. T., Kjær, J. B., & Kristensen, I. S. 2005. Use of the range area in organic egg production systems: Effect of climatic factors, flock size, age and artificial cover. British Poultry Science 46:1-8.

Hermansen, J. E. 2003. Organic livestock production systems and appropriate development in relation to public expectations. Livestock Production Science 80:3-15.

Hermansen, J. E., Horsted, K., Hegelund, L., Frantzen, C., & Johansen, N. F. 2005. Forbedrede udearealer i økologisk ægproduktion. DJF rapport Husdyrbrug nr. 67, 71 pages. (In Danish: "improved free-range areas in organic egg production")

Hess, M., & McDougald, L. R. 2013. Histomoniasis (blackhead) and other protpzoan diseases of the intestinal tract. Pages 1172-1182 in Diseases of poultry. Swayne, D. E., Glisson, J. R., McDougald, L. R., Nolan, L. K., Suarez, D. L., & Nair, V. L. (eds.) Wiley-Blackwell.

Hirt, H., Hördegen, P., & Zeltner, E. 2000. Laying hen husbandry: Group size and use of hen-runs. International IFOAM Scientific Conference, Basel, 28 to 31 August 2000.

Horseman, S. V., Roe, E. J., Huxley, J. N., Bell, N. J., Mason, C. S., & Whay, H. R. 2014. The use of in-depth interviews to understand the process of treating lame dairy cows from the farmers's perspective. Animal Welfare 23:157-165.

Huber-Eicher, B., & Wechsler, B. 1998. The effect of quality and availability of foraging materials on feather pecking in laying hen chicks. Animal Behaviour 55:861-873.

Hughes, B. O., & Duncan, I. J. H. 1972. Influence of strain and environmental factors upon feather pecking and cannibalism in fowls. British Poultry Science 13:525-547.

Häne, M., Huber-Eicher, B., & Frohlich, E. 2000. Survey of laying hen husbandry in Switzerland. World's Poultry Science Journal 56:21-31.

Höglund, J., & Jansson, D. S. 2011. Infection dynamics of *Ascaridia galli* in non-caged laying hens. Veterinary Parasitology 180:267-273.

Hørning, G., Rasmussen, S., Permin, A., & Bisgaard, M. 2003. Investigations on the influence of helminth parasites on vaccination of chickens against Newcastle disease virus under village conditions. Tropical Animal Health and Production 35:415-424.

Ikeme, M. M. 1971. Observations on the pathogenicity and pathology of *Ascaridia galli*. Parasitology 63:169-179.

Jansson, D. S., Nyman, A., Vågsholm, I., Christensson, D., Göransson, M., Fossum, O., & Höglund, J. 2010. Ascarid infections in laying hens kept in different housing systems. Avian Pathology 39:525-532.

Jensen, K. O., Lund, T. B., Andersen, L. M., Chistensen, V. T., Krarup, S., Christensen, T., Denver, S., Bossen, H., Hindborg, H., Roland, T., & Øllgaard, G. 2008. Hvorfor køber forbrugerne økologi? Pages 91-129 in Udvikling, vækst og integritet i den danske økologisektor. Alrøe, H. F., and Halberg, N. (eds.) Internationalt Center for Forskning i Økologisk Jordbrug og Fødevaresystemer (ICROFS). (In Danish: "Why are consumers buying organic products?")

Johansen, N. F., Kjær, J., Kyed, S., & Larsen, J. N. 2013. Fjerpilningsnøgle (In Danish: "Feather pecking guide").

Jones, R. B., Blokhuis, H. J., de Jong, I. C., Keeling, L. J., McAdie, T. M., & Preisinger, R. 2004. Feather pecking in poultry: The application of science in a search for practical solutions. Animal Welfare 13:S215-S219.

Kabell, S. 2012. Noget udsætning af fasaner og spolorm hos høns. Dansk Erhvervsfjerkræ 41:220-221. (In Danish: "Note on release of pheasant and *A. galli* in hens").

Kabell, S. 2014. Projekt sundhed og velfærd 2012. Dansk Erhvervsfjerkræ 43:452-453 (In Danish: "Project health and welfare 2012").

Kappeli, S., Gebhardt-Henrich, S. G., Frohlich, E., Pfulg, A., & Stoffel, M. H. 2011. Prevalence of keel bone deformities in Swiss laying hens. British Poultry Science 52:531-536.

Kaufmann-Bart, M., & Hoop, R. K. 2009. Diseases in chicks and laying hens during the first 12 years after battery cages were banned in Switzerland. Veterinary Record 164:203-207.

Kaufmann, F., Das, G., Preisinger, R., Schmutz, M., Konig, S., & Gauly, M. 2011a. Genetic resistance to natural helminth infections in two chicken layer lines. Veterinary Parasitology 176:250-257.

Kaufmann, F., Das, G., Sohnrey, B., & Gauly, M. 2011b. Helminth infections in laying hens kept in organic free range systems in Germany. Livestock Science 141:182-187.

Kaufmann, F., & Gauly, M. 2009. Prevalence and burden of helminths in laying hens kept in free range systems. Tribun EU, Brno, Czech Republic.

Keeling, L. J. 2005. Healthy and happy: Animal welfare as an integral part of sustainable agriculture. Ambio 34:316-319.

Kilpinen, O., Roepstorff, A., Permin, A., Nørgaard-Nielsen, G., Lawson, L. G., & Simonsen, H. B. 2005. Influence of *Dermanyssus gallinae* and *Ascaridia galli* infections on behaviour and health of laying hens (*Gallus gallus domesticus*). British Poultry Science 46:26-34.

Kjær, J., & Hocking, P. M. 2004. The genetics of feather pecking and cannibalism. Pages 109-121 in Welfare of the laying hen, proceedings of the world's poultry science association meeting held in Bristol, England, 2003. Perry, G. C. (ed.) CABI Publishing, Wallingford, Oxfordshire, UK.

Kjær, J. B., & Sørensen, P. 1997. Feather pecking behaviour in White Leghorns, a genetic study. British Poultry Science 38:333-341.

Kjær, J. B., & Sørensen, P. 2002. Feather pecking and cannibalism in free-range laying hens as affected by genotype, dietary level of methionine plus cystine, light intensity during rearing and age at first access to the range area. Applied Animal Behaviour Science 76:21-39.

Kleinbaum, D. G., & Klein, M. 2012. Survival analysis: A self-learning text. Third ed. Springer.

Knierim, U. 2006. Animal welfare aspects of outdoor runs for laying hens: A review. NJAS-Wageningen Journal of Life Sciences 54:133-145.

Knierim, U., Staack, M., Gruber, B., Keppler, C., Zaludik, K., & Niebuhr, K. 2008. Risk factors for feather pecking in organic laying hens – starting points for prevention in the housing environment. Poster at: Cultivating the Future Based on Science: 2nd Conference of the International Society of Organic Agriculture Research ISOFAR, Modena, Italy, June 18-20, 2008:32-35.

Kristensen, E. R., Jørgensen, E., & Tolf, N. 2010. Herd management science II. Advanced topics. 2010 Edition ed. Academic books.

Kvale, S., & Brinkmann, S. 2008. Interviews: Learning the craft of qualitative research interviewing. 2nd. Ed. SAGE Publications Inc, California.

Lambton, S. L., Knowles, T. G., Yorke, C., & Nicol, C. J. 2010. The risk factors affecting the development of gentle and severe feather pecking in loose housed laying hens. Applied Animal Behaviour Science 123:32-42.

Lauritzen, S.L., 1996. Graphical Models. Oxford University Press, New York.

Lay, D. C., Fulton, R. M., Hester, P. Y., Karcher, D. M., Kjær, J. B., Mench, J. A., Mullens, B. A., Newberry, R. C., Nicol, C. J., O'Sullivan, N. P., & Porter, R. E. 2011. Hen welfare in different housing systems. Poultry Science 90:278-294.

Leenstra, F., Maurer, V., Bestman, M., van Sambeek, F., Zeltner, E., Reuvekamp, B., Galea, F., & van Niekerk, T. 2012. Performance of commercial laying hen genotypes on free range and organic farms in Switzerland, France and the Netherlands. British Poultry Science 53:282-290.

Leeson, S., & Morrison, W. D. 1978. Effect of feather cover on feed efficiency in laying birds. Poultry Science 57:1094-1096.

Lund, V. 2006. Natural living-a precondition for animal welfare in organic farming. Livestock Science 100:71-83.

Lund, V., & Algers, B. 2003. Research on animal health and welfare in organic farming - a literature review. Livestock Production Science 80:55-68.

Mahboub, H. D. H., Müller, J., & von Borell, E. 2004. Outdoor use, tonic immobility, heterophil/lymphocyte ratio and feather condition in free-range laying hens of different genotype. British Poultry Science 45:738-744.

Main, D. C. J., Kent, J. P., Wemelsfelder, F., Ofner, E. & Tuyttens, F. A. M. 2003. Application for methods of on-farm welfare assessment. Animal welfare 12:523-528.

Main, D. C. J., Mullan, S., Atkinson, C., Bond, A., Cooper, M., Fraser, A., & Browne, W. J. 2012. Welfare outcomes assessment in laying hen farm assurance schemes. Animal Welfare 21:389-396.

Marcos-Atxutegi, C., Gandolfi, B., Aranguena, T., Sepulveda, R., Arevalo, M., & Simon, F. 2009. Antibody and inflammatory responses in laying hens with experimental primary infections of *Ascaridia galli*. Veterinary Parasitology 161:69-75.

Margolis, L., Esch, G. W., Holmes, J. C., & Kuris, A. M. S. G. A. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American society of parasitologists). Journal of Parasitology 68:131-133.

Maurer, V., Amsler, Z., Perler, E., & Heckendorn, F. 2009. Poultry litter as a source of gastrointestinal helminth infections. Veterinary Parasitology 161:255-260.

Maurer, V., Hertzberg, H., Heckendorn, F., Hördegen, P., & Koller, M. 2013. Effects of paddock management on vegetation, nutrient accumulation, and internal parasites in laying hens. Journal of Applied Poultry Research 22:334-343.

McAdie, T. M., & Keeling, L. J. 2000. Effect of manipulating feathers of laying hens on the incidence of feather pecking and cannibalism. Applied Animal Behaviour Science 68:215-229.

McAdie, T. M., & Keeling, L. J. 2002. The social transmission of feather pecking in laying hens: Effects of environment and age. Applied Animal Behaviour Science 75:147-159.

McDougald, L. R. 2005. Blackhead disease (histomoniasis) in poultry: A critical review. Avian Diseases 49:462-476.

McDougald, L. R. 2013. Internal parasites. Pages 1117-1146 in Diseases of poultry. Swayne, D. E., Glisson, J. R., McDougald, L. R., Nolan, L. K., Suarez, D. L., & Nair, V. L. (eds.) Wiley-Blackwell.

Menke, P., Waiblinger, S., Studnitz, M., & Bestman, M. 2004. Mutilations in organic animal husbandry: Dilemmas involving animal welfare, humans and environmental protection. Pages 163-188 in Animal health and welfare in organic agriculture. Vaarst, M., Roderick, S., & Lockeretz, W. (eds). CABI Publishing, Wallingford, UK.

Mielenz, N., Spilke, J., & von Borell, E. 2010. Analysis of ordered categorical data with threshold models exemplified by plumage damage scores from laying hens differing in their genotype and rearing environment. Poultry Science 89:2521-2534.

Mikél, A., & Kabell, S. 2013. Kontrol af spolorm og blodmider i konsumægproduktion. Oral presentation at the Danish poultry conference, Brædstrup, Denmark 2013. (In Danish: "Control of A. galli and red mites in commerical egg production").

Moberly, R. L., White, P. C. L., & Harris, S. 2004. Mortality due to fox predation in free-range poultry flocks in Britain. Veterinary Record 155:48-52.

Mollenhorst, H., Rodenburg, T. B., Bokkers, E. A. M., Koene, P., & De Boer, I. J. M. 2005. On-farm assessment of laying hen welfare: A comparison of one environment-based and two animal-based methods. Applied Animal Behaviour Science 90:277-291.

Nicol, C. J., Brown, S. N., Glen, E., Pope, S. J., Short, F. J., Warriss, P. D., Zimmerman, P. H., & Wilkins, L. J. 2006. Effects of stocking density, flock size and management on the welfare of laying hens in single-tier aviaries. British Poultry Science 47:135-146.

Nicol, C. J., Pötzsch, C., Lewis, K., & Green, L. E. 2003. Matched concurrent case-control study of risk factors for feather pecking in hens on free-range commercial farms in the UK. British Poultry Science 44:515-523.

Niebuhr, K., Zaludik, K., Baumung, R., Lugmair, A., & Troxler, J. 2005. Injurious pecking in free-range and organic laying hen flocks in Austria. Animal Science Papers and Reports 23:195-201.

Nordquist, R. E., Heerkens, J. L. T., Rodenburg, T. B., Boks, S., Ellen, E. D., & Staay, F. J. v. d. 2011. Laying hens selected for low mortality: Behaviour in tests of fearfulness, anxiety and cognition. Applied Animal Behaviour Science 131:110-122.

Norup, L. R., Dalgaard, T. S., Pleidrup, J., Permin, A., Schou, T. W., Jungersen, G., Fink, D. R., & Juul-Madsen, H. R. 2013. Comparison of parasite-specific immunoglobulin levels in two chicken lines during sustained infection with *Ascaridia galli*. Veterinary Parasitology 191:187-190.

Oliver, E., Caspari, C., & Biggs, C. 2009. The availability of organic reared livestock in the European Union. JRC54488, EUR 24108 EN, Luxembourg, Publications Office of the European Union.

Pennycott, T. W., & Steel, F. 2001. Parasitic worms in commercial free-range poultry flocks in England and Wales. Veterinary Record 149:428.

Permin, A., Bisgaard, M., Frandsen, F., Pearman, M., Kold, J., & Nansen, P. 1999. Prevalence of gastrointestinal helminths in different poultry production systems. British Poultry Science 40:439-443.

Permin, A., Bojesen, M., Nansen, P., Bisgaard, M., Frandsen, F., & Pearman, M. 1997. *Ascaridia galli* populations in chickens following single infections with different dose levels. Parasitology Research 83:614-617.

Permin, A., Christensen, J., & Bisgaard, M. 2006. Consequences of concurrent *Ascaridia galli* and *Escherichia coli* infections in chickens. Acta Veterinaria Scandinavica 47:43-54.

Permin, A., & Hansen, J. W. 1998. Epidemiology. Diagnosis and control of poultry parasites. FAO Animal Health Manual.

Permin, A., & Ranvig, H. 2001. Genetic resistance to *Ascaridia galli* infections in chickens. Veterinary Parasitology 102:101-111.

Perry, J. J., Staley, J.T., & Lory, S. 2002. Microbial life. Sinauer Associates, Sunderland, USA

Pleidrup, J., Dalgaard, T. S., Norup, L. R., Permin, A., Schou, T. W., Skovgaard, K., Vadekær, D. F., Jungersen, G., Sørensen, P., & Juul-Madsen, H. R. 2014. *Ascaridia galli* infection influences the development of both humoral and cell-mediated immunity after Newcastle disease vaccination in chickens. Vaccine 32:383-392.

Potzsch, C. J., Lewis, K., Nicol, C. J., & Green, L. E. 2001. A cross-sectional study of the prevalence of vent pecking in laying hens in alternative systems and its associations with feather pecking, management and disease. Applied Animal Behaviour Science 74:259-272.

Ramadan, H. H., & Abou Znada, N. Y. 1991. Some pathological and biochemical studies on experimental ascaridiasis in chickens. Die Nahrung 35:71-84.

Reid, W. M., & Carmon, J. L. 1958. Effects of numbers of *Ascaridia galli* in depressing weight gains in chickens. Tropical Animal Health and Production 44:183-186.

Reid, W. M., Mabon, J. L., & Harshbarger, W. C. 1973. Detection of worm parasites in chicken eggs by candling. Poultry Science 52:2316-2324.

Richards, G. J., Nasr, M. A., Brown, S. N., Szamocki, E. M. G., Murrell, J., Barr, F., & Wilkins, L. J. 2011. Use of radiography to identify keel bone fractures in laying hens and assess healing in live birds. Veterinary Record 169:279

Richards, G. J., Wilkins, L. J., Knowles, T. G., Booth, F., Toscano, M. J., Nicol, C. J., & Brown, S. N. 2012. Pop hole use by hens with different keel fracture status monitored throughout the laying period. Veterinary Record 170:494

Rodenburg, T. B., Tuyttens, F. A. M., de Reu, K., Herman, L., Zoons, J., & Sonck, B. 2008. Welfare assessment of laying hens in furnished cages and non-cage systems: An on-farm comparison. Animal Welfare 17:363-373.

Rousing, T., Sørensen, J. T., Knage-Rasmussen, K., Thomsen, P. T., & Houe, H. 2013. Vurdering af dyrevelfærden baseret på besøg i den enkelte besætning. Pages 15-21 in Vurdering af dyrevelfærd i en husdyrbesætning. Sørensen, J. T., Houe, H., Rousing T., & Sandøe, P. (eds.) Center for bioetik og risikovurdering, projektrapport 18. (In Danish: "Assessment of animal welfare based on visits in each herds")

Sandilands, V., Moinard, C., & Sparks, N. H. C. 2009. Providing laying hens with perches: Fulfilling behavioural needs but causing injury? British Poultry Science 50:395-406.

Savory, C. J. 1995. Feather pecking and cannibalism. Worlds Poultry Science Journal 51:215-219.

Savory, C. J., & Hughes, B. O. 2010. Behaviour and welfare. Pages 13-22 in British Poultry Science. Taylor & Francis.

Schwarz, A., Gauly, M., Abel, H., Daş, G., Humburg, J., Rohn, K., Breves, G., & Rautenschlein, S. 2011. Immunopathogenesis of *Ascaridia galli* infection in layer chicken. Developmental & Comparative Immunology 35:774-784.

Sherwin, C. M., Nasr, M. A. F., Gale, E., Petek, M., Stafford, K., Turp, M., & Coles, G. C. 2013. Prevalence of nematode infection and faecal egg counts in free-range laying hens: Relations to housing and husbandry. British Poultry Science 54:12-23.

Sherwin, C. M., Richards, G. J., & Nicol, C. J. 2010. Comparison of the welfare of layer hens in 4 housing systems in the UK. British Poultry Science 51:488-499.

Sommer, F. 2001. A decade of experience with free-range poultry farming in Austria. Is this the future? Proceedings of the western poultry disease conference 50:95-96.

Sparks, N. H. C., Conroy, M. A., & Sandilands, V. 2008. Socio-economic drivers for UK organic pullet rearers and the implications for poultry health. British Poultry Science 49:525-532.

Stokholm, N. M., Permin, A., Bisgaard, M., & Christensen, J. P. 2010. Causes of mortality in commercial organic layers in Denmark. Avian Diseases 54:1241-1250.

Sørensen, J. T., Houe, H., Rousing, T., & Sandøe, P. 2013. Vurdering af dyrevelfærd i en husdyrbesætning. Hvad er det, og hvem har glæde af det? Pages 7-13 in Vurdering af dyrevelfærd i en husdyrbesætning. Sørensen, J. T., Houe, H., Rousing, T., & Sandøe, P. (eds.) Center for bioetik og risikovurdering, projektrapport 18. (In Danish: "Assessment of animal welfare in livestock herds. What is it and who benefit from it?").

Tablante, N. L., Vaillancourt, J. P., Martin, S. W., Shoukri, M., & Estevez, I. 2000. Spatial distribution of cannibalism mortalities in commercial laying hens. Poultry Science 79:705-708.

Tauson, R. 2005. Management and housing systems for layers - effects on welfare and production. World's Poultry Science Journal 61:477-490.

Tauson, R., & Abrahamsson, P. 1994. Foot and skeletal disorders in laying hens. Effects of perch design, hybrid, housing system and stocking density. Acta Agricultura Scandinavica. Section A, Animal Science 44:110-119.

Tauson, R., Ambrosen, T., & Elwinger, K. 1984. Evaluation of procedures for scoring the integument of laying hens - independent scoring of plumage condition. Acta Agriculturae Scandinavica, 34:400-408.

Tauson, R., Elwinger, G. F., Holm, K., & Wall, H. 2006. Analyses of a database for health parameters in different housing systems. LayWel WP3-Health Deliverables D.3.2-D.3.3. http://www.laywel.eu/web/pdf/deliverables%2031-33%20health-3.pdf

Tauson, R., Kjær, J., Maria, G., Cepero, R., & Holm, K. 2005. Applied scoring of integument and health in laying hens. Animal Science Papers and Reports 23:153-159.

Thomsen, P. T., Kjeldsen, A. M., Sørensen, J. T., Houe, H., & Ersbøll, A. K. 2006. Herd-level risk factors for the mortality of cows in Danish dairy herds. Veterinary Record 158:622-626.

Torgerson, P. R., Paul, M., & Lewis, F. I. 2012. The contribution of simple random sampling to observed variations in faecal egg counts. Veterinary Parasitology 188:397-401.

Tuyttens, F. A. M., Sonck, B., Staes, M., Van Gansbeke, S., Van den Bogaert, T., & Ampe, B. 2011. Survey of egg producers on the introduction of alternative housing systems for laying hens in Flanders, Belgium. Poultry Science 90:941-950.

van de Weerd, H. A., & Elson, A. 2006. Rearing factors that influence the propensity for injurious feather pecking in laying hens. World's Poultry Science Journal 62:654-664.

van de Weerd, H. A., Keatinge, R., & Roderick, S. 2009. A review of key health-related welfare issues in organic poultry production. World's Poultry Science Journal 65:649-684.

van der Meulen, J., van der Werf, J. T. N., & Kijlstra, A. 2007. Questionnaire survey of disease prevalence and veterinary treatments in organic layer husbandry in the Netherlands. Tijdschrift Voor Diergeneeskunde 132:292-295.

Vannier, P., Michel, V., & Keeling, L. J. 2014. Science-based management of livestock welfare in intensive systems: Looking to the future. Revue Scientifique et Technique - Office International des Epizooties 33:153-160.

Vaarst, M., Nissen, T. B., Østergaard, S., Klaas, I. C., Bennedsgaard, T. W., & Christensen, J. 2007. Danish stable schools for experiential common learning in groups of organic dairy farmers. Journal of Dairy Science 90:2543-2554.

Vaarst, M., & Sørensen, J. T. 2009. Danish dairy farmers' perceptions and attitudes related to calf-management in situations of high versus no calf mortality. Preventive Veterinary Medicine 89:128-133.

von Borell, E., & Sørensen, J. T. 2004. Organic livestock production in Europe: Aims, rules and trends with special emphasis on animal health and welfare. Livestock Production Science 90:3-9.

Weeks, C. A., & Nicol, C. J. 2006. Laywell. Deliverable 7.1. Overall strengths and weaknesses of each defined housing system for laying hens, and detailing the overall welfare impact of each housing system. Http://www.Laywel.Eu/web/pdf/deliverable%2071%20welfare%20assessment-2.Pdf

Weeks, C. A., Nicol, C. J., Haslam, S. M., & Whay, R. C. 2006. Laywel. Deliverable 7.2. Manual that can be used to audit the welfare of laying hens at a farm level in whatever housing system they are held. (Complied by Weeks, C & Nicol, C.). Http://www.Laywel.Eu/web/pdf/deliverable%2072%20manual-3.Pdf

Welfare Quality. 2009. Welfare quality assessment protocol for poultry. Welfare Quality Consortium, Lelystad, Netherlands:119.

Whay, H. R., Main, D. C. J., Green, L. E., Heaven, G., Howell, H., Morgan, M., Pearson, A., & Webster, A. J. F. 2007. Assessment of the behaviour and welfare of laying hens on free-range units. Veterinary Record 161:119-128.

Whitehead, C. C. 2004. Overview of bone biology in the egg-laying hen. Poultry Science 83:193-199.

Whittaker, J., 1990. Graphical models in applied multivariate statistics. John Wiley & Sons, Chichester, UK

Wier, M., Jensen, K. O., Andersen, L. M., & Millock, K. 2008. The character of demand in mature organic food markets: Great Britain and Denmark compared. Food Policy 33:406-421.

Wilkins, L. J., Brown, S. N., Zimmerman, P. H., Leeb, C., & Nicol, C. J. 2004. Investigation of palpation as a method for determining the prevalence of keel and furculum damage in laying hens. Veterinary Record 155:547-549.

Wilkins, L. J., McKinstry, J. L., Avery, N. C., Knowles, T. G., Brown, S. N., Tarlton, J., & Nicol, C. J. 2011. Influence of housing system and design on bone strength and keel bone fractures in laying hens. Vet Rec 169:414.

Zeltner, E., & Hirt, H. 2003. Effect of artificial structuring on the use of laying hen runs in a free-range system. British Poultry Science 44:533-537.

Zeltner, E., & Maurer, V. 2009. Welfare of organic poultry. Proceedings of 8th Poultry Welfare Symposium, Cervia, Italy, 18-22 May 2009:104-112.

Zimmerman, P. H., Lindberg, A. C., Pope, S. J., Glen, E., Bolhuis, J. E., & Nicol, C. J. 2006. The effect of stocking density, flock size and modified management on laying hen behaviour and welfare in a non-cage system. Applied Animal Behaviour Science 101:111-124.

9 List of additional publications from the PhD programme

Published publications:

Almeida, G. F. d., **Hinrichsen, L. K.**, Horsted, K., Thamsborg, S. M., & Hermansen, J. E. 2012. Feed intake and activity level of two broiler genotypes foraging different types of vegetation in the finishing period. Poultry Science 91:2105-2113.

Hinrichsen, L.K. 2012. Hvorfor dør den økologiske høne? Økologi og erhverv, 495, p. 17

Hinrichsen, L.K. & Sørensen, J.T. 2012. Færre døde høner i økologisk ægproduktion. Dansk Erhvervsfjerkræ, vol. 1, p. 20-21

Publication (expected or in preparation; authors with main responsibility for the given publication are written in italics and co-authors are listed alphabetical)

Bestman, M., Verwer, C., Brenninkmeyer, C., Ferrante, V., Gunnarsson, S., Heerkens, J., **Hinrichsen, L.K.**, Smajlhodzic, F, Willet, A. Working title: HealthyHens WP3 - Feather pecking

Ferrante, V., Ferrari, P., Lolli, S., Brenninkmeyer, C., Gunnarsson, S., Heerkens, J., **Hinrichsen, L.K**, Smajlhodzic, F., Verwer, C., Willet, A. Working title: HealthyHens WP1 – red mites

Niebuhr, K., Smajlhodzic, F., Bestman, M., Brenninkmeyer, C., Ferrari, P., Gunnarsson, S., Heerkens, J., **Hinrichsen, L.K.**, Willet, A. Working title: HealthyHens WP4 - Keel bone and food pads damage

Riber, A.B., Steenfeldt,S., **Hinrichsen, L.K.** Working title: Feather pecking and the nutritional link

Thapa, S., Hinrichsen, L.K., Brenninkmeyer, C., Grilli, G., Gunnarsson, S., Heerkens, J., Niebuhr, K., Verwer, C., Willet, A., *Mejer, H., Thamsborg, S.M*. Working title: HealthyHens WP1 – endoparasite; Management factors affecting prevalence and intensity of *Ascaridia galli* infections in organic laying hens in Europe

Willet, A., Edge, S., Phelps, E., Bestman, M., Brenninkmeyer, C., Gunnarsson, S., Heerkens, J., **Hinrichsen, L.K.**, Lolli, S., Niebuhr, K. Working title: HealthyHens WP2 - Range use

10 **Appendix**

Appendix 1: Description of the 15 Danish farms included in the PhD study

Appendix 2: HealthyHens protocol for clinical welfare assessment

Appendix 3: Inter-assessor reliability testing based on the HealthyHens protocol

Appendix 4: Prevalence of the clinical welfare indicators in Denmark in 15 farms

Appendix 5: Endoparasite infections in European organic egg production

Appendix 6: Interview guide used in the qualitative study

Appendix 7: Preliminary result of the post mortem examination

Appendix 8: Weather condition in Denmark during peak of lay visits

Appendix 9: Memo – A trivial argument on mortality rates

10.1 Appendix 1: Description of the 15 Danish farms included in the PhD study

Table 1.1: Description of the 15 Danish farms according to size, housing system, hybrids, free-range, roughage, and feed together with information about which paper each farm contribute to.

Farm size	Housing system[a]	Veranda	Hybrids	Free-range, m²/hens	Paddock rotation	Roughage[b], g/hen/day	Feed[c]	Additional calcium	Included in paper
3,000	single-tiered	no	Lohmann LB-lite	8	no	33	supplement	yes	I, II, III
4,300	single-tiered	yes	Hisex White	8	no	30	complete	yes	I, II, III
6,000	single-tiered	yes	Lohmann LSL	8	no	25	complete	yes	II
6,000	single-tiered	yes	Lohmann LSL	8	no	30	supplement[d]	yes	I, II, III
6,000	single-tiered	yes	Lohmann LB-lite	8	no	20	supplement	yes	
6,000	single-tiered	no	Lohmann LSL	4	yes	35	complete	yes	I, II, III
9,000	single-tiered	no	Lohmann LB-lite	4	yes	11	supplement[d]	yes	I, II, III
10,500	single-tiered	no	Hisex White	4	yes	100	complete	no	I, II
11,200	multi-tiered	yes	Lohmann LSL	4	yes	25	complete	yes	I, II
12,000	single-tiered	yes	Lohmann LSL	4	yes	160	complete	yes	I, II, III
12,000	single-tiered	yes	Lohmann LSL	8	no	24	complete	no	III
12,000	single-tiered	no	Lohmann LB-lite	8	no	25	supplement	yes	I, II
12,000	multi-tiered	yes	Hisex White	8	no	28	complete	yes	I, II
18,000	multi-tiered	yes	Hisex White	8	no	-	complete	yes	II
51,000	multi-tiered	yes	Hisex White	8	no	50	complete	yes	I

a: Multi-tiered (aviaries) and single-tiered (littered floor area with different level of raised area/slats)
b: Silage and/or vegetables
c: Supplement: add feedstuff (grains) to a supplement ration bought from the feed mill; complete: buy complete ration from the feed mill
d: 100% organic feed

10.2 Appendix 2: HealthyHens protocol for clinical welfare assessment

The assessment protocol was developed for the HealthyHens project are presented in Table 2.1 (CORE organic HealthyHens project, 2014). Smajlhodzic, F. and Niebuhr, K. prepared the description for the scoring of comb, keel bone, and feet, and Bestman, M. prepared the description of plumage condition and skin damage scoring (plumage condition scoring modified from the LayWel protocol (Tauson et al., 2005)).

Table 2.1: Principles of scores in the protocol: the higher score, the better status

Body part	Score
Weight	Body weight in gram
Comb	Score 1: pale
	Score 2: red (normal)
Keel bone deviation[a]	Score 1: > 1 cm
	Score 2: > 0.5 cm and ≤ 1 cm
	Score 3: ≤ 0.5 cm
Keel bone fracture[a]	Score 1: fracture
	Score 2: no fracture
Keel bone tip[a]	Score 1: deviated
	Score 2: not deviated
Keel bone skin	Score 1: haematomas
	Score 2: no haematomas
Footpad – hyperkeratosis[b]	Score 1: hyperkeratosis
	Score 2: no hyperkeratosis
Footpad – lesions	Score 1: dorsal swelling
	Score 2: larger lesion > 0.2 cm
	Score 3: small lesion < 0.2 cm
	Score 4: no lesion
Missing toes/claws	Score 1: missing toes or claws
	Score 2: no missing toes or claws
Toe wounds	Score 1: wounds (fresh and old)
	Score 2: no wounds
Discharge from cloaca	Score 1: white-yellowish discharge
	Score 2: no discharge

Plumage: neck, back and belly (see figure 2.1 for definition)	Score 1: featherless areas > 5 cm^2 AND 75% to complete featherless Score 2: featherless areas ≥ 5 cm^2 and up to 75% featherless Score 3: few feathers damaged. Featherless areas < 5 cm^2 Score 4: no or very few feathers damaged
Plumage: tail (see figure 2.1 for definition)	Score 1: > 13 tail feather highly damaged[c] and/or almost bare Score 2: 9-12 tail feathers damaged[c] Score 3: 6-10 tail feather damaged[c] Score 4: ≤ 5 tail feather damaged[c]
Skin wounds – belly and back	Score 1: wounds > 2.2 cm Score 2: wounds < 2.2 Score 3: wounds < 0.5 cm or haematoma Score 4: no wounds

a: examination with palpation

b: excessive growth, thickening of the outermost layer of the epidermis

c: damaged tail feathers are feathers that are either broken off or showing at least more than 1 cm bare area alongside the main shaft
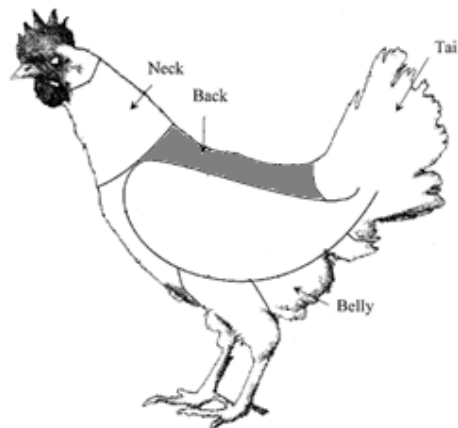

Figure 2.1: Illustration of body parts for the plumage condition scoring

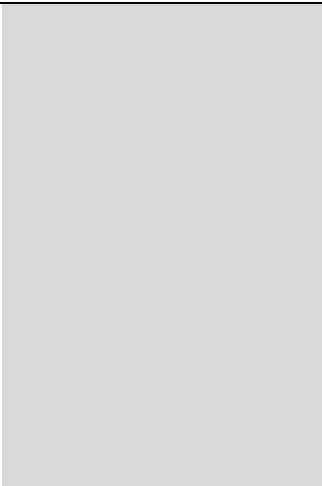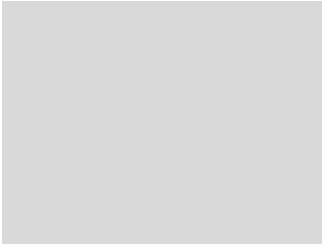(Modified from Bilcik & Keeling (1999))

References

CORE organic HealthyHens project 2014. Healthyhens, good health and welfare in organic laying hens depends on good management http://www.Coreorganic2.Org/healthyhens (accessed september 2014).

Tauson, R., Kjær, J., Maria, G., Cepero, R., & Holm, K. 2005. Applied scoring of integument and health in laying hens. Animal Science Papers and Reports 23:153-159.

10.3 Appendix 3: Inter-observer reliability testing based on the HealthyHens protocol

HealthyHens arranged an observer training workshop to train and test the agreement among twelve observers from eight countries (two from Denmark) that were involved in data collection. Inter-observer reliability were calculated as a prevalence adjusted bias adjusted Kappa (PABAK) with a PABAK ≥ 0.4 being the least acceptable agreement and presented in Table 3.1 (Brenninkmeyer, 2013).

Table 3.1: Result of the inter-observer reliability testing

Welfare Indicator (see Appendix 2)	Result workshop (PABAK)	Result photo assessment (PABAK)
Plumage - neck	< 0.4	
Plumage – back	< 0.4	
Plumage – belly	≥ 0.4 → acceptable	
Plumage – tail	≥ 0.4 → acceptable	
Wounds – back	≥ 0.4 → acceptable	
Wounds – belly	≥ 0.4 → acceptable	
Footpad – hyperkeratosis	≥ 0.4 → acceptable	
Footpad – lesions	≥ 0.4 → acceptable	
Missing toes/claws	≥ 0.4 → acceptable	
Toe wound	≥ 0.4 → acceptable	
Keel bone deviation	< 0.4 → *Photo*	< 0.4
Keel bone fracture	≥ 0.4 → acceptable	
Keel bone tip	< 0.4	
Keel bone skin	≥ 0.4 → acceptable	
Discharge from cloaca	≥ 0.4 → acceptable	
Beak treatment	≥ 0.4→ acceptable	
Comb colour	< 0.4 → *Photo*	≥ 0.4 → acceptable

Comments on indicators without least acceptable agreement:

Neck and back plumage scoring:

At the workshop four observers in disagreement were retained, therefore an acceptable agreement were gained.

Keel bone deviation scoring:

After the photo assessment were the least acceptable agreement still not fulfilled. Half of the observers had an acceptable agreement, this included one of the Danish observers. Each country was advised to use the observers with acceptable agreement, while in countries with no observer with acceptable agreement were they advised to read the instructions and retake the test (results not represented).

Inter-observer reliability testing of the Danish observer for scoring of keel bone deviations

Based on photo scoring the two Danish observer conducted an inter-observer reliability test for the keel bone deviation scoring. The agreement were judged based on the Kappa coefficient ($k = \frac{p_0 - p_r}{1 - p_r}$). The level of agreement are (Landis & Koch, 1977):

Poor if kappa coefficient are < 0.00

Slight if kappa coefficient are 0.00-0.20

Fair if kappa coefficient are 0.21-0.40

Moderate if kappa coefficient are 0.41-0.60

Substantial if kappa coefficient are 0.61-0.80

Almost perfect if kappa coefficient are 0.81-1.00

The level of agreement between the two Danish observers were $k = 0.818$, thereby being almost in perfect agreement.

Overall conclusion of the training and inter-observer reliability testing

Based on the training and inter-observer reliability it were concluded that both Danish observers were capable of performing the clinical assessment based on the HealthyHens protocol with good agreement.

References

Brenninkmeyer, C. 2013. Personal communication; included in the HealthyHens midterm report

Landis, J. R., & Koch, G. G. 1977. The measurement of observer agreement for categorical data. Biometrics 33:159-174.

10.4 Appendix 4: Prevalence of the clinical welfare indicators in Denmark in 15 farms

Prevalence of the animal-based welfare indicators (definition in Appendix 2) at peak of lay, at 60-65 weeks of age (used for the HealthyHens project) and end of lay in 15 commercial organic egg productions in Denmark are presented in Table 4.1 and the range (min.-max) between farms in Table 4.2.

Table 4.1: Prevalence of animal-based welfare indicators

Parameter	Category/score	Visit 1 peak of lay	Visit 2 HealthyHens	Visit 3 end of lay
Age in weeks of the hens at the visit		30-38	58-66	66-77
Number of farm		15	15	11[a]
Number of hens		1502	1244[b]	1109
Weight, gram	Hissex white	1671	1718	1730
	Lohmann LSL	1687	1727	1751
	Lohmann LB-lite	1920	1900	1911
Prevalence (percentages of all hens having)				
Comb colour	Score 1: pale	5.5	2.2	1.0
	Score 2: red	94.5	97.8	99.0
Keel bone deviation	Score 1: deviated	2.0	5.5	8.6
	Score 2: slight deviated	4.9	9.6	15.8
	Score 3: no deviation	93.1	84.8	75.7
Keel bone fracture	Score 1: fracture	4.8	7.8	17.8
	Score 2: no fracture	95.2	92.1	82.2
Keel bone tip	Score 1: deviated	0.2	1.5	0.6
	Score 2: not deviated	99.8	98.5	99.4
Keel bone skin	Score 1: haematomas	0.5	1.7	0.2
	Score 2: no haematomas	99.5	98.3	99.8
Hyperkeratosis	Score 1: hyperkeratosis	0.7	0.5	0.2
	Score 2: no hyperkeratosis	99.3	99.5	99.8
Footpad lesion	Score 1: dorsal swellings	2.7	3.1	1.2
	Score 2: larger lesion	0.3	0.5	0.5
	Score 3: small lesion	0.1	0.6	0.3
	Score 4: no lesion	96.8	95.9	98.0
Missing toe	Score 1: missing toe/claw	2.2	2.3	3.2
	Score 2: no missing	97.8	97.7	96.8

Toe wounds	Score 1: wounds	1.3	0.7	0.2
	Score 2: no wounds	98.7	99.3	99.8
Discharge[c]	Score 1: discharge	1.4	12.1	12.0
	Score 2: no discharge	98.6	87.9	88.0
Plumage condition[d]	Severe (total score 4-8)	3.3	16.2	20.8
	Moderate (total score 9-11)	5.3	17.1	21.3
	Good (total score 12-16)	91.4	66.7	57.9
Plumage – back (incl. rump)	Score 1: large featherless areas	3.0	7.2	8.7
	Score 2: featherless areas	6.5	15.4	14.8
	Score 3: damaged feathers	6.0	17.3	16.1
	Score 4: good condition	84.6	60.1	60.3
Plumage – tail	Score 1: large featherless areas	0.3	19.8	30.5
	Score 2: featherless areas	4.8	22.7	29.0
	Score 3: damaged feathers	10.7	42.6	31.4
	Score 4: good condition	84.2	15.0	9.1
Plumage – neck	Score 1: large featherless areas	0.9	2.3	3.6
	Score 2: featherless areas	4.1	9.2	9.9
	Score 3: damaged feathers	10.1	20.6	16.8
	Score 4: good condition	85.0	67.8	69.7
Plumage – belly	Score 1: large featherless areas	3.3	26.4	34.0
	Score 2: featherless areas	7.1	10.7	9.7
	Score 3: damaged feathers	6.7	11.3	11.0
	Score 4: good condition	82.9	51.7	45.3
Skin wounds[e]	Score 1: wounds	6.4	10.0	3.4
	Score 2: no wounds	93.6	90.0	96.6
Skin wounds – back (incl. rump)	Score 1: large wounds	0.0	0.2	0.0
	Score 2: wounds	0.1	0.2	0.1
	Score 3: small wounds	0.7	1.0	0.6
	Score 4: no wounds	99.1	98.6	99.3
Skin wounds – belly (incl. vent)	Score 1: large wounds	0.7	1.0	0.1
	Score 2: wounds	1.2	1.9	0.5
	Score 3: small wounds	4.0	6.3	2.2
	Score 4: no wounds	94.1	90.8	97.3
Faecal egg count (% with infection)[f]	*Ascaridia galli*/*Heterakis* spp.	64.2	65.7	78.2
	Capillaria spp.	46.7	25.5	12.2
	Coccidia	57.8	20.8	28.9

a: On two farms the end of lay visit were conducted together with the second visit (HealthyHens), and in this table they are included as the second visit, however, in Paper II they are included as end of lay visits.

b: On six farms minimum were 50 hens assessed and on nine farms minimum were 100 hens assessed, at some farm were the actual number of assessed hens higher than the minimum.

c: Discharge from the cloaca has been assessed incorrect at visit 2 (HealthyHens) and visit 3 (end of lay).

d: Total plumage score for the four body parts (back, neck, belly and tail) categorised as good, moderate and severe based on definition in Tauson et al. (2005) .

e: Combined prevalence for skin wounds at back and belly as a binary variable (wounds or no wounds).

f: 297 samples, 309 samples and 247 samples for visit 1, visit 2 and visit 3, respectively.

Table 4.2: Minimum and maximum farm prevalence for the animal-based welfare indicators at the best score and range of the parasite infection (percentage of positive samples).

Parameter	Category/score	Visit 1 peak of lay	Visit 2 HealthyHens	Visit 3 end of lay
Comb colour	Score 2: red	70-100%	84-100%	93-100%
Keel bone deviation	Score 3: no deviation	72-100%	52-97%	52-97%
Keel bone fracture	Score 2: no fracture	85-100%	80-100%	60-98%
Keel bone tip	Score 2: not deviated	99-100%	94-100%	97-100%
Keel bone skin	Score 2: no haematomas	97-100%	83-100%	99-100%
Hyperkeratosis	Score 2: no hyperkeratosis	95-100%	96-100%	99-100%
Footpad lesion	Score 4: no lesion	86-100%	68-100%	94-100%
Missing toe	Score 2: no missing	92-100%	93-100%	89-100%
Toe wounds	Score 2: no wounds	93-100%	96-100%	99-100%
Discharge	Score 2: no discharge	93-100%	46-100%	67-100%
Plumage condition	Good (total score 12-16)	30-100%	3.7-100%	2-100%
Skin wounds	Score 2: no wounds	39-100%	50-100%	84-100%
Faecal egg count	*Ascaridia galli*/*Heterakis* spp.	0-100%	0-95%	47-95%
	Capillaria spp.	0-100%	0-60%	0-35%
	Coccidia	0-95%	0-80%	0-68%

References

Tauson, R., Kjær, J., Maria, G., Cepero, R., & Holm, K. 2005. Applied scoring of integument and health in laying hens. Animal Science Papers and Reports 23:153-159.

10.5 Appendix 5: Endoparasite infections in European organic egg production

HealthyHens – Endoparasite infection in European organic egg production   August 2014

Hinrichsen, L.K. & Sørensen, J.T.

HealthyHens workshop 4, Vienna, August 2014

Endoparasite infections in European organic egg production

- A note on the level of endoparasite infections based on faecal egg counts in the project

HealthyHens

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Introduction

The risk of getting an endoparasite infection seems to be higher in organic egg production as higher prevalence has been found in organic layer flocks compared to other production system (Permin et al., 1999). Most organic hens seems to be subclinical infected with helminths (Kaufmann et al., 2011b). The most prevalent helminths found in organic production are *Ascaridia galli* and *Heterakis gallinarum* (Permin et al., 1999). Helminth infections may cause weight depression, diarrhoea, obstruction of intestine, depressed in feed intake, reduced locomotion and weakens the layer (Ackert & Herrick, 1928; Reid & Carmon, 1958; Ramadan & Abou Znada, 1991; Permin & Ranvig, 2001; Kilpinen et al., 2005; Gauly et al., 2007).

The aim of this note is to provide preliminary descriptive result from an investigation of level of endoparasite infections based on faecal egg count (FEC) in organic egg production in eight European countries.

Material and method

A total of 113 flocks from eight European countries were involved in the project. Each flock were visited twice, aiming at first between 30 to 40 weeks of age and second at 60 to 65 weeks of age. During both visit a minimum 15 individual faecal samples were collected and subsequently analysed using a simple McMaster method (Permin & Hansen, 1998). For each individual sample,

Table 5.1: Number of flocks visited in the eight countries.

	First visit	Second visit
	30-40 week of age	60-65 weeks of age
Austria	25	25
Belgium	8	7
Germany	22	19
Denmark	15	15
Italy	15	15
Netherlands	7	3
Sweden	9	9
United Kingdom (UK)	12	10
All countries	113	103

For Austria, Denmark, Italy and Sweden were faecal samples collected from all flocks at both visit. While three, two and one flock from Germany, United Kingdom and Belgium, respectively, only had faecal samples from first visit. In the Netherlands six flocks were only sampled at first visit and one flock that only were sampled at second visit, given two flocks with samples from both visit.

the eggs per gram of faeces (EPG) were registered and used for calculation of a flock level EPG. Due to similar size and shape of eggs from *Ascaridia galli* and *Heterakis spp.* (McDougald, 2013) these species were counted together. Faecal samples were obtained from first visit in 113 flocks and second visit in 103 flocks (Table 5.1). One flock from Netherland had no faecal sample collected at first visit and 11 flocks had no faecal sampling at second visit (Belgium: 1, Germany: 3, Netherlands: 5, United Kingdom: 2). The Netherlands had two flocks only with faecal samples from both visit.

Result and discussion

Ascaridia galli/Heterakis sp. and coccidia were the most common endoparasites found in all countries during both visit (Table 5.2 and 5.3). *Capillaria* were present in almost all countries, whereas *Tetrameres*, *Strongyloides*, *Cestodes* and *Trishostronylus* had low EPG values and were practically not present.

The flock prevalence of endoparasite infections were around 84% at both ages (Table 5.4 and 5.5). The flock prevalence of being infected with helminth, excluded coccidia, was 62% and 68% at first and second visit, respectively. The highest prevalence was found for coccidia followed by *A.*

galli/Heterakis and *Capillaria*. Whereas the prevalence was very low for *Tetrameres*, *Strongyloides*, *Cestodes* and *Trishostronylus*.

The flock EPG is illustrated in Figure 5.1 for *A. galli/Heterakis* Figure 5.2, for *Capillaria* and in Figure 3 for Coccidia. It appears that in each country and in general between flocks is there are high level of variance in the EPG level. The highest EPG for *A. galli/Heterakis* sp. were 4950 EPG in a Danish flock, which already had ordinated deworming from the veterinarian before the visit. Figure 5.4-5.7 shows the number of flock having either a faecal egg count of under 100 EPG or over 100 EPG for or *A. galli/Heterakis* (Figure 5.4), for *Capillaria* (Figure 5.5), for Coccidia (Figure 5.6) and for all helminth species (Figure 5.7). Most flocks were having an EPG of over 100 when considering *A. galli/Heterakis* sp. and coccidia, whereas only few flocks had an EPG over 100 for *Capillaria*. It appears that the pattern of categorisation helminth species into over and under an EPG of 100 follows the pattern of the most common helminth *A. galli/Heterakis*.

Potential explanatory variables for mean EPG and the prevalence of *A. galli/Heterakis* sp. are shown in Table 5.6. There seems to be an effect of season and system at visit 1 in the mean *A. galli/Heterakis* EPG. White birds tends to have a higher mean *A. galli/Heterakis* EPG, but the prevalence are similar. Litter quality and veranda does not seem to affect the mean EPG or prevalence of *A. galli/Heterakis*. Roughage seem to increase the both the mean EPG and prevalence, which are in line with other findings (Das et al., 2012; Shreshtha et al., 2013). Straw increases the prevalence at both visit, but only mean EPG at visit 2.

Table 5.2: Country mean EPG for each endoparasite at first visit (30 to 40 weeks of age) and the mean EPG across counties (mean ±standard deviation)

	A. galli / Heterakis	*Capillaria*	*Tetra- meres*	*Strongy- loides*	*Cestodes*	*Tricho- strongylus*	Coccidia
Austria	524 ± 571	67 ± 90	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2436 ± 7304
Belgium	324 ± 683	27 ± 33	0 ± 0	0.8 ± 1.4	1.2 ± 3.5	5.4 ± 14	1288 ± 1104
Germany	391 ± 495	40 ± 60	4.7 ± 20	1.5 ± 2.9	0.1 ± 0.4	0.1 ± 0.4	1078 ± 1266
Denmark	1039 ± 1327	200 ± 279	0 ± 0	0 ± 0	0 ± 0	0 ± 0	743 ± 885
Italy	148 ± 239	10 ± 30	0 ± 0	0 ± 0	0 ± 0	0 ± 0	736 ± 1305
Netherlands	189 ± 375	33 ± 72	0.5 ± 1.3	0 ± 0	0 ± 0	0 ± 0	123 ± 325
Sweden	476 ± 620	0.6 ± 1.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	556 ± 276
UK	464 ± 713	115 ± 130	7.9 ± 16	0.8 ± 2.9	0 ± 0	1.1 ± 2.2	1101 ± 1820
All	472 ± 773	67 ± 134	1.8 ± 10	0.4 ± 1.8	0.1 ± 1.0	0.5 ± 3.8	1205 ± 3600

Table 5.3: Country mean EPG for each endoparasite at second visit (60 to 65 weeks of age) and the mean EPG across counties (mean ±standard deviation)

	A. galli / Heterakis	*Capillaria*	*Tetra- meres*	*Strongy- loides*	*Cestodes*	*Tricho- strongylus*	Coccidia
Austria	360 ± 467	42 ± 66	0 ± 0	0 ± 0	3.1 ± 12	0 ± 0	744 ± 1855
Belgium	333 ± 459	34 ± 36	0 ± 0	0.5 ± 1.3	0 ± 0	1.9 ± 3.8	824 ± 482
Germany	476 ± 475	19 ± 31	0 ± 0	13 ± 26	2.2 ± 5	0 ± 0	257 ± 217
Denmark	706 ± 456	24 ± 19	0 ± 0	0 ± 0	0.3 ± 0.9	0.2 ± 0.5	49 ± 93
Italy	202 ± 242	9.2 ± 17	0 ± 0	0 ± 0	0 ± 0	0 ± 0	692 ± 988
Netherlands	960 ± 1299	7.8 ± 13.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sweden	585 ± 564	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	191 ± 159
UK	416 ± 486	80 ± 86	0 ± 0	0 ± 0	3.3 ± 11	0.3 ± 1.1	224 ± 155
All	449 ± 500	29 ± 50	0 ± 0	2.5 ± 12	1.5 ± 7.2	0.2 ± 1.1	430 ± 1028

Table 5.4: Country prevalence for each endoparasite at the first visit (30 to 40 weeks of age) and an overall prevalence for having an endoparasite infection in each country, together with prevalence across countries (prevalence in percentage ±standard deviation)

	A. galli / Heterakis	*Capillaria*	*Tetra- meres*	*Strongy- loides*	*Cestodes*	*Tricho- strongylus*	Coccidia	Overall
Austria	54.1 ± 25	24.0 ± 30	0 ± 0	0 ± 0	0 ± 0	0 ± 0	52.5 ± 27	82.9 ± 16
Belgium	35.4 ± 39	25.8 ± 27	0 ± 0	0.8 ± 2.4	1.7 ± 4.7	5.0 ± 12	84.2 ± 17	90.0 ± 16
Germany	75.9 ± 32	35.3 ± 33	4.1 ± 14	4.3 ± 9	0.3 ± 1.4	0.3 ± 1.5	76.9 ± 21	94.3 ± 12
Denmark	64.2 ± 29	46.7 ± 37	0 ± 0	0 ± 0	0 ± 0	0 ± 0	57.8 ± 28	90.1 ± 15
Italy	32.4 ± 33	10.2 ± 28	0 ± 0	0 ± 0	0 ± 0	0 ± 0	58.7 ± 30	67.1 ± 29
Netherlands	26.7 ± 25	17.1 ± 32	1.0 ± 2.5	0 ± 0	0 ± 0	0 ± 0	13.3 ± 35	44.8 ± 38
Sweden	61.5 ± 35	2.2 ± 7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	88.1 ± 14	94.1 ± 9
UK	59.3 ± 33	43.3 ± 26	7.0 ± 13	0.6 ± 1.9	0 ± 0	1.7 ± 3	64.5 ± 25	87.3 ± 20
All	54.9 ± 34	27.4 ± 32	1.6 ± 8	0.9 ± 4.2	0.2 ± 1.4	0.6 ± 3.4	62.7 ± 30	83.5 ± 23

Table 5.5: Country prevalence for each endoparasite at the second visit (60 to 65 weeks of age) and an overall prevalence for having an endoparasite infection in each country, together with prevalence across countries (prevalence in percentage ±standard deviation)

	A. galli / Heterakis	Capillaria	Tetra- meres	Strongy- loides	Cestodes	Tricho- strongylus	Coccidia	Overall
Austria	50.4 ± 25	21.3 ± 25	0 ± 0	0 ± 0	1.3 ± 4.7	0 ± 0	54.9 ± 22	80.0 ± 13
Belgium	45.7 ± 33	33.3 ± 22	0 ± 0	1.0 ± 2.5	0 ± 0	1.9 ± 3.3	77.1 ± 22	91.4 ± 10
Germany	79.3 ± 34	26.0 ± 33	0 ± 0	13.3 ± 17	4.9 ± 10	0 ± 0	58.0 ± 21	91.4 ± 20
Denmark	65.7 ± 27	25.5 ± 18	0 ± 0	0 ± 0	0.7 ± 1.8	0.3 ± 1.3	20.8 ± 22	80.0 ± 22
Italy	48.4 ± 35	12.4 ± 24	0 ± 0	0 ± 0	0 ± 0	0 ± 0	64.4 ± 26	79.1 ± 20
Netherlands	73.3 ± 31	11.1 ± 19	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	80.0 ± 20
Sweden	73.3 ± 38	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	57.8 ± 26	87.4 ± 17
UK	57.3 ± 26	51.3 ± 22	0 ± 0	0 ± 0	3.3 ± 11	0.7 ± 2.1	50.0 ± 22	86.0 ± 12
All	60.7 ± 32	23.1 ± 27	0 ± 0	2.5 ± 9	1.7 ± 6	0.2 ± 1.2	51.6 ± 27	84.0 ± 18
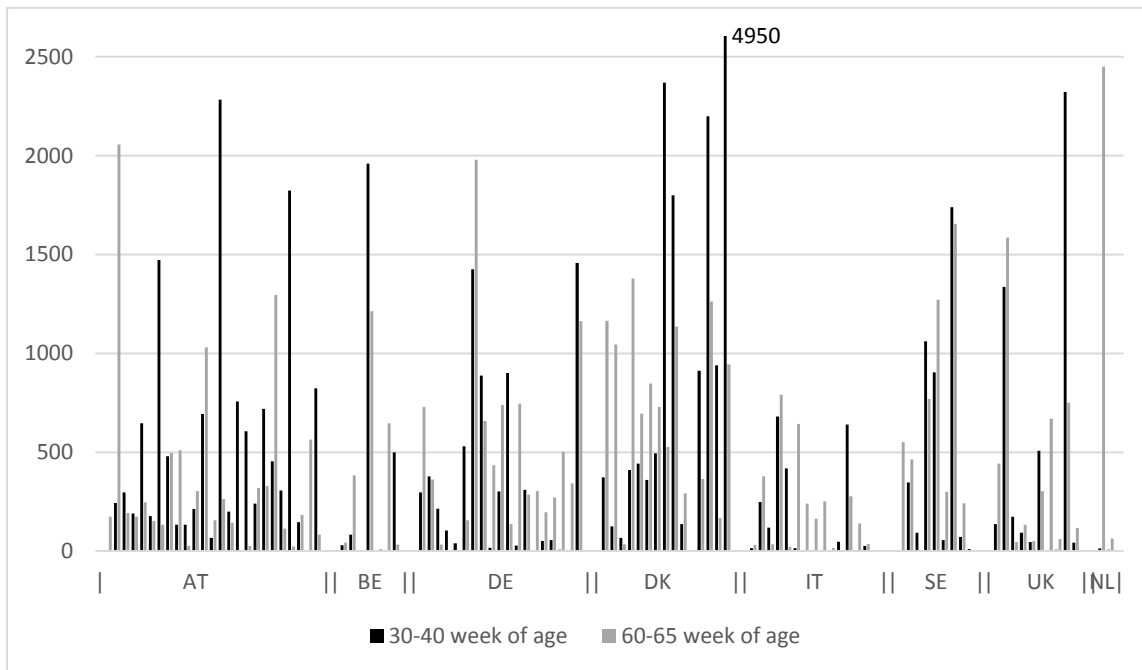
Figure 5.1: Mean EPG per flock of *Ascaridia galli/Heterakis sp*. grouped into country for each visit. The dark line is from visit at 30 to 40 weeks of age and the lighter column is from second visit at 60 to 65 weeks of age. The number beside the column indicates the actual value of the faecal egg count for that given farm, as it exceed the values on the y-asks. With AT=Austria, BE=Belgium, DE= Germany, DK=Denmark, IT=Italy, SE=Sweden and NL=Netherlands
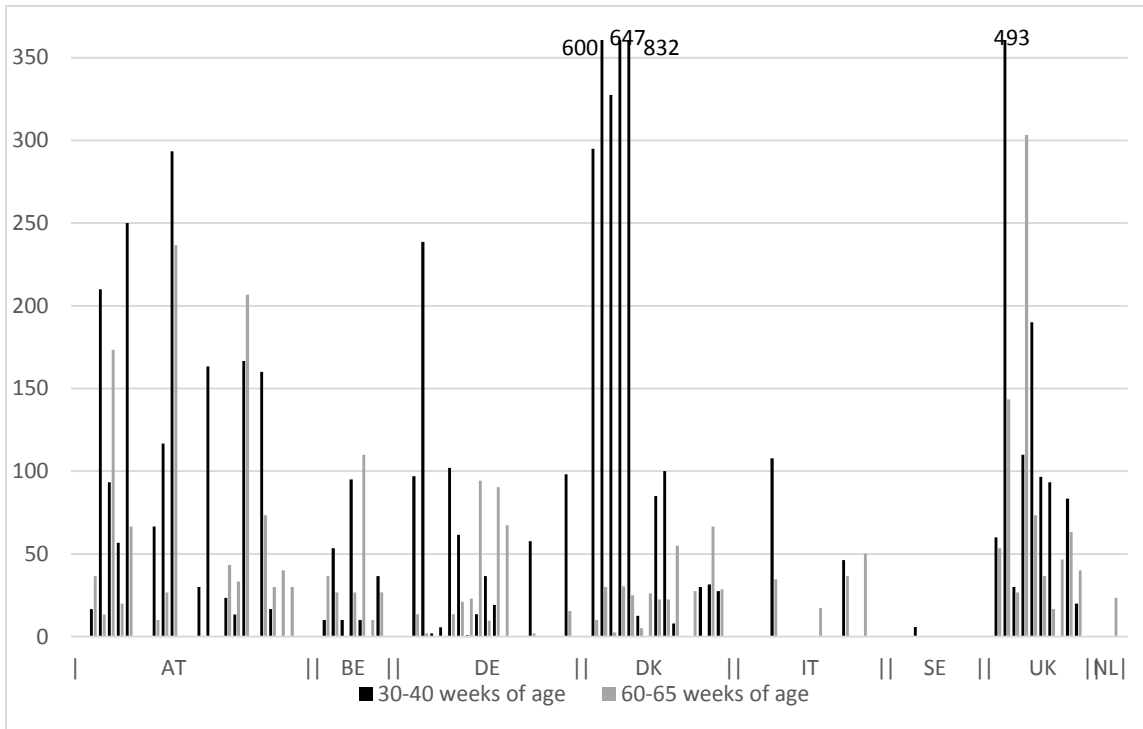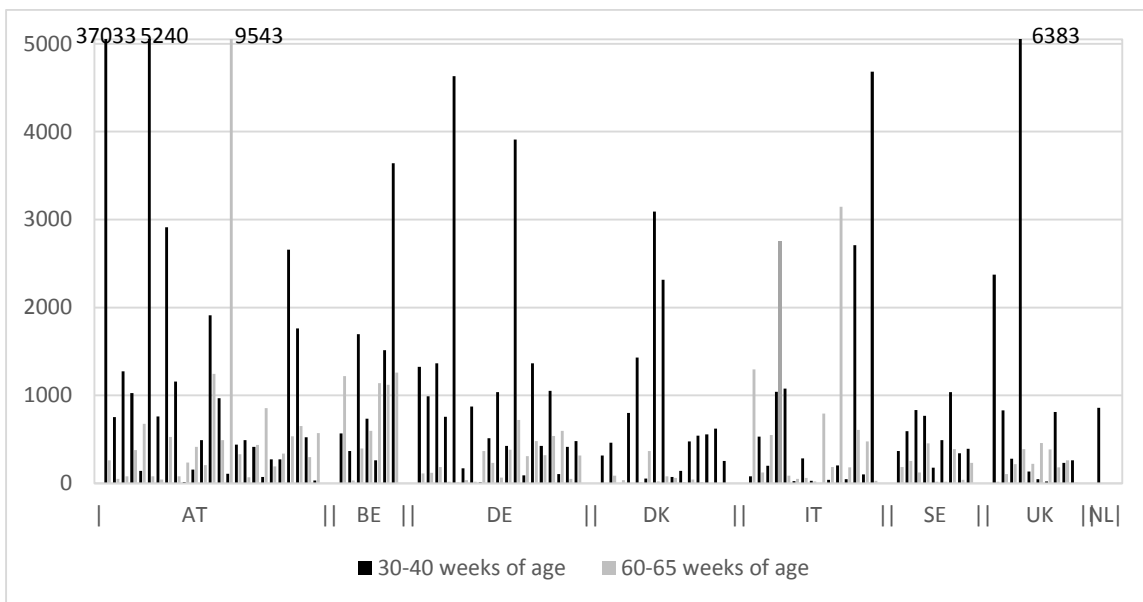
Figure 5.2: Mean EPG per farm of *Capillaria sp.* grouped into country for each visit. The dark line is from visit at 30 to 40 weeks of age and the lighter column is from second visit at 60 to 65 weeks of age. The number beside the column indicates the actual value of the faecal egg count for that given farm, as it exceed the values on the y-asks. With AT=Austria, BE=Belgium, DE= Germany, DK=Denmark, IT=Italy, SE=Sweden and NL=Netherlands

Figure 5.3: Mean EPG per farm of coccidia grouped into country for each visit. The dark line is from visit at 30 to 40 weeks of age and the lighter column is from second visit at 60 to 65 weeks of age. The number beside the column indicates the actual value of the faecal egg count for that given farm, as it exceed the values on the y-asks. With AT=Austria, BE=Belgium, DE= Germany, DK=Denmark, IT=Italy, SE=Sweden and NL=Netherlands
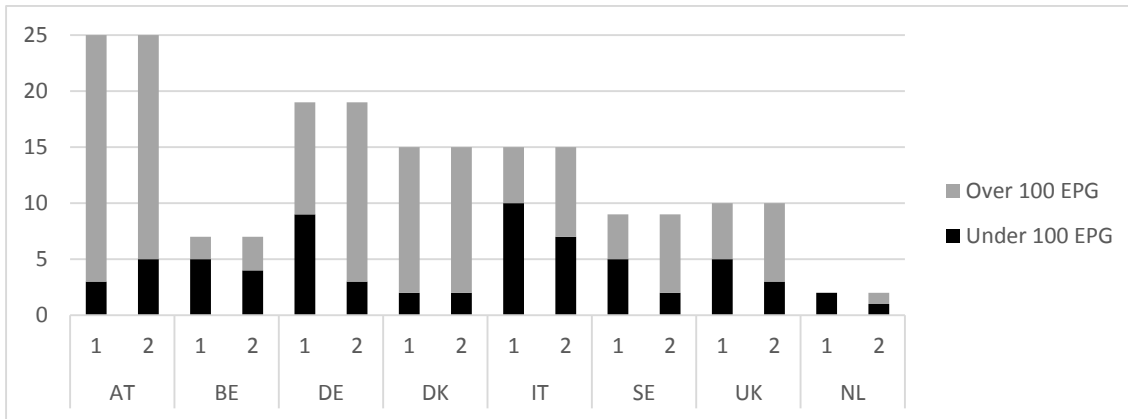
Figure 5.4: Total number of flocks per country per visit (1: 30-40 weeks of age; 2: 60-65 weeks of age), with the black represents flocks with a mean EPG for *A. galli* and *Heterakis* sp. under 100 and the grey represents flocks with a mean EPG for *A. galli* and *Heterakis* sp. over 100. With AT=Austria, BE=Belgium, DE= Germany, DK=Denmark, IT=Italy, SE=Sweden and NL=Netherlands
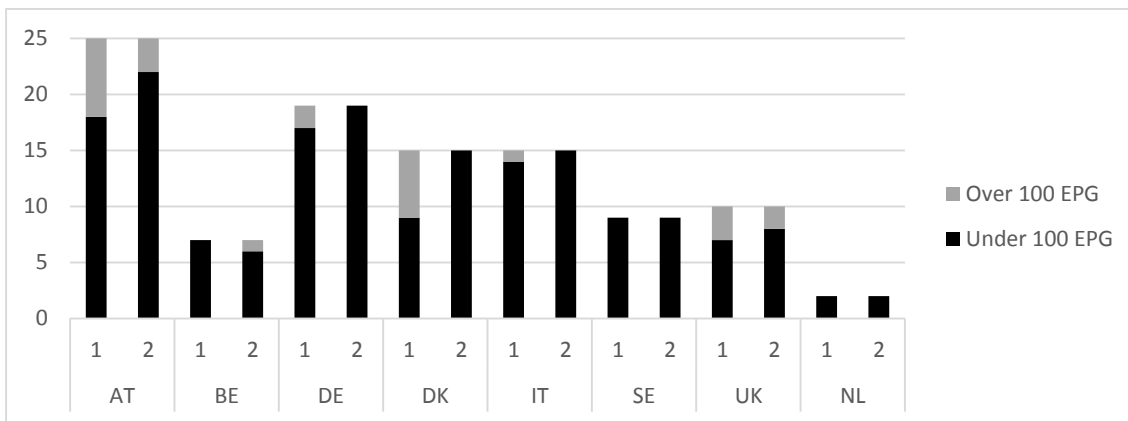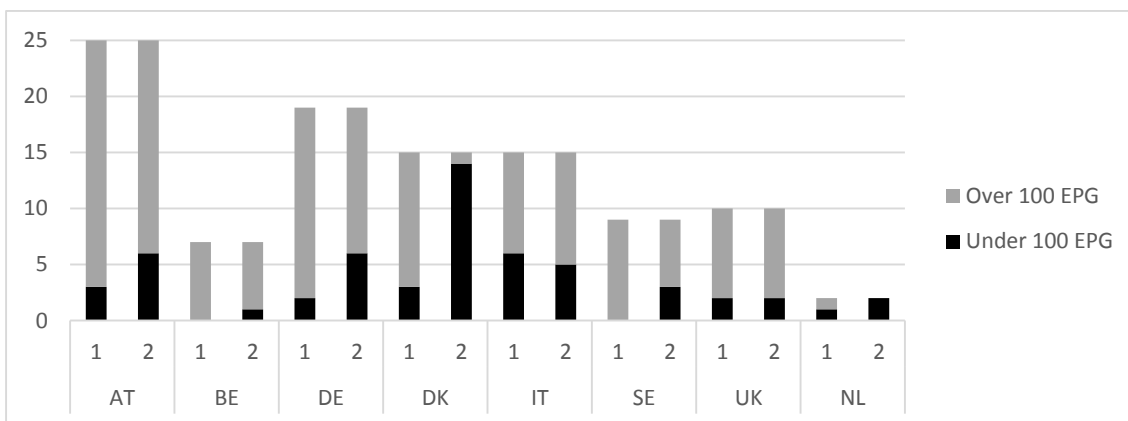

Figure 5.5: Total number of flocks per country per visit (1: 30-40 weeks of age; 2: 60-65 weeks of age), with the black represents flocks with a mean EPG for *Capillaria* under 100 and the grey represents flocks with a mean EPG for *Capillaria* over 100. With AT=Austria, BE=Belgium, DE= Germany, DK=Denmark, IT=Italy, SE=Sweden and NL=Netherlands

Figure 5.6: Total number of flocks per country per visit (1: 30-40 weeks of age; 2: 60-65 weeks of age), with the black represents flocks with a mean EPG for coccidia under 100 and the grey represents flocks with a mean EPG for coccidia over 100. With AT=Austria, BE=Belgium, DE= Germany, DK=Denmark, IT=Italy, SE=Sweden and NL=Netherlands
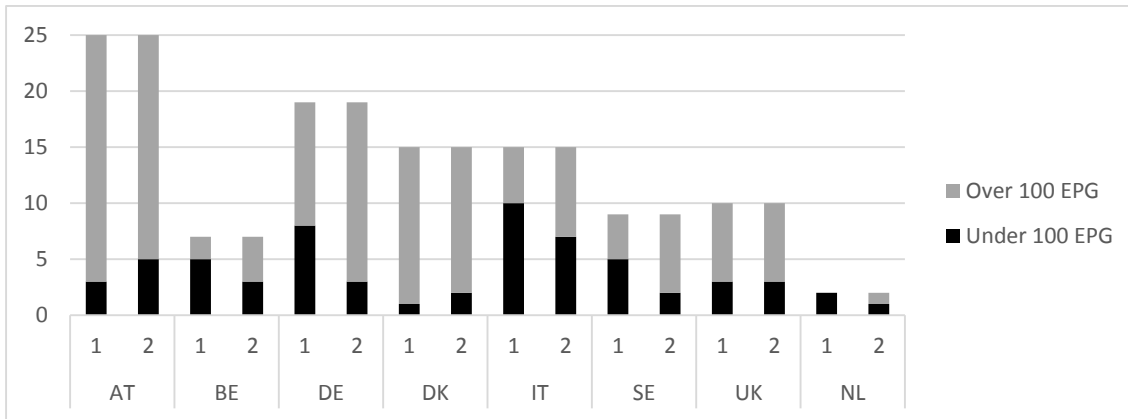
Figure 5.7: Total number of flocks per country per visit (1: 30-40 weeks of age; 2: 60-65 weeks of age), with the black represents flocks with a mean EPG for helminth under 100 and the grey represents flocks with a mean EPG for helminth over 100. With AT=Austria, BE=Belgium, DE= Germany, DK=Denmark, IT=Italy, SE=Sweden and NL=Netherlands

Table 5.6: Mean *A. galli/Heterakis* EPG (mean (standard deviation)) and prevalence of *A. galli/Heterakis* for potential explanatory variables in relation to visit (including 107 and 102 first and second visit farm, respectively)

Variable		Mean EPG		Prevalence, %	
		Visit 1	Visit 2	Visit 1	Visit 2
Season	Summer	331 ± 471	427 ± 515	54.5 ± 32.2	55.0 ± 32.0
	Winter	624 ± 902	476 ± 486	54.6 ± 35.7	68.4 ± 30.3
System	Single-tiered	520 ± 813	437 ± 493	52.7 ± 34.9	56.1 ± 31.5
	Multi-tiered	391 ± 548	482 ± 524	58.1 ± 31.6	70.5 ± 30.8
Veranda	Veranda	467 ± 738	463 ± 519	55.9 ± 34.0	62.7 ± 32.7
	No veranda	501 ± 727	421 ± 449	50.2 ± 33.3	55.8 ± 28.8
Breed	Brown	431 ± 564	388 ± 461	54.6 ± 33.7	57.3 ± 31.6
	White	601 ± 1078	633 ± 571	54.4 ± 34.5	71.4 ± 30.8
Litter quality*	Good	493 ± 751	480 ± 515	57.3 ± 31.5	62.8 ± 29.4
	Bad	450 ± 699	452 ± 538	50.6 ± 35.3	58.1 ± 36.1
Roughage	Silage, veg., hay	564 ± 905	525 ± 568	59.4 ± 33.1	65.0 ± 31.3
	None	410 ± 575	403 ± 476	50.9 ± 34.0	56.4± 30.0
Straw as occupation	Yes	436 ± 509	842 ± 746	70.0 ±36.8	89.1 ± 19.8
	No	481 ± 762	386 ± 439	52.2 ± 32.9	54.9 ± 29.4
Other occupation**	Yes	476 ± 988	268 ± 240	58.4 ± 34.8	51.4 ± 35.1
	No	475 ± 625	486 ± 546	53.2 ± 33.5	61.4 ± 29.6

*Good = dry and free-flowing, Bad= conglomerates and plaques

** plastic bottles or cans, blocks, stone, string

References

Ackert, J. E., & Herrick, C. A. 1928. Effects of the nematode *Ascaridia lineata* (schneider) on growing chickens. JOUR PARASITOL 15:1-13.

Das, G., Abel, H., Humburg, J., Schwarz, A., Rautenschlein, S., Breves, G., & Gauly, M. 2012. The effects of dietary non-starch polysaccharides on ascaridia galli infection in grower layers. Parasitology 139:110-119. doi 10.1017/s0031182011001636

Gauly, M., Duss, C., & Erhardt, G. 2007. Influence of *Ascaridia galli* infections and anthelmintic treatments on the behaviour and social ranks of laying hens (gallus gallus domesticus). Veterinary Parasitology 146:271-280. doi 10.1016/j.vetpar.2007.03.005

Kaufmann, F., Das, G., Sohnrey, B., & Gauly, M. 2011. Helminth infections in laying hens kept in organic free range systems in germany. Livestock Science 141:182-187. doi DOI 10.1016/j.livsci.2011.05.015

Kilpinen, O., Roepstorff, A., Permin, A., Nørgaard-Nielsen, G., Lawson, L. G., & Simonsen, H. B. 2005. Influence of *Dermanyssus gallinae* and *Ascaridia galli* infections on behaviour and health of laying hens (*Gallus gallus domesticus*). British Poultry Science 46:26-34. doi 10.1080/00071660400023839

McDougald, L. R. 2013. Internal parasites. Pages 1117-1146 in Diseases of poultry. D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez, and V. L. Nair eds. Wiley-Blakwell.

Permin, A., Bisgaard, M., Frandsen, F., Pearman, M., Kold, J., & Nansen, P. 1999. Prevalence of gastrointestinal helminths in different poultry production systems. British Poultry Science 40:439-443. doi 10.1080/00071669987179

Permin, A., & Hansen, J. W. 1998. Epidemiology, diagnosis and control of poultry parasites. FAO Animal Health Manual.

Permin, A., & Ranvig, H. 2001. Genetic resistance to *Ascaridia galli* infections in chickens. Veterinary Parasitology 102:101-111. doi http://dx.doi.org/10.1016/S0304-4017(01)00525-8

Ramadan, H. H., & Abou Znada, N. Y. 1991. Some pathological and biochemical studies on experimental ascaridiasis in chickens. Die Nahrung 35:71-84.

Reid, W. M., & Carmon, J. L. 1958. Effects of numbers of *Ascaridia galli* in depressing weight gains in chickens. Tropical Animal Health and Production 44:183-186.

Shreshtha, A., Norup, L. R., Juul-Madsen, H. R., Steenfeldt, S., Afrose, S., & Engberg, R. M. 2013. Influence of maize silage supplementation on selected intestinal bacteria and the course of a *Ascaridia galli* infection on organic layers. Proceedings of the 19th European Symposium on Poultry Nutrition. http://www.wpsa.com/proceedings/ESPN_2013/assets/pdf/0299.pdf.

Acknowledgement

10.6 Appendix 6: Interview guide used in the qualitative study

Introduction (formalities: tape-recording, structure of interview, anonymity)

Introduction to the objectives of the study and the process during the interview

Start question: How would you describe the batch, participating in the observational studies?

Theme 1: Mortality

Think of a flock with exceptional low mortality

- were there any particular situation behind (cause, management practices)?

Think of a flock with exceptional high mortality

- did something special occur?

- are there a known cause?

- was it a unexpected incident or were it long-term problem?

Have you tried difference management practices, what was the effect?

What do you think are important to maintaining a low mortality and what do you do?

What role do other actors (erfa-group (experience-exchange producer group), adviser, and veterinarians) play in problem solving?

If there are no limits (economical or production level) – which solution could you suggest?

What does mortality mean for you (in relation to health and animal welfare)?

What does mortality mean for the organic egg production in general?

Theme 2: Endoparasite infection (producers mainly talk about helminth infection)

What are your experience of the level of infections, and is it a current problem?

How does it affect the hens (health and animal welfare)?

Which management practices do you practise in relation to endoparasite infections?

What is important to ensure a low endoparasite infection level and what do you do?

What role do other actors play in the problem solving (erfa-group, adviser, and veterinarians)?

Theme 3: connection between mortality and endoparasite infection

Is there a connection or opposite between prevention of high mortality and endoparasite infection?

Is there other health problems that affects the production at the moment or previously?

Ending and summing up

The person behind the production (narrative interview approach – life history):

- your history in relation to organic egg production

- previous experience

- why were chosen organic production?

Give the interviewee an opportunity to think and reflect over the interview, and let them speak if they have forgotten to say something during the interview.

The list of question is a guideline to ensure that all point were covered in each interview, however the order of the questions might differ between interviews.

10.7 Appendix 7: Preliminary result of the post mortem examination

A veterinarian (Engberg, R.M.) performed the post mortem examination on the 214 tagged hens from the longitudinal study (Paper II) and the 50 tagged hens that died during the study. The findings were categorised into four main categories and subdivided within category (Table 7.1). Table 7.2 presents the result and preliminary conclusions are that death hens had higher incidences of inflammation of the abdominal cavity and pathological changes to the ovary (or not developed the ovary), and a lower incidence of cyst compared to slaughtered hens at end of lay. There are a tendency to a lower prevalence of irritation and prolapse at the cloaca in the death hens.

Table 7.1: Categorise for the findings in the post mortem examination

Abdominal cavity	Ovary	Remnant of the Müllers duct	Cloaca
no remarks	fully developed	none	no remarks
peritonitis	pathological changes	little cyst	Irritation
salpingitis	not developed	big cyst	little prolapse
aerosacculitis			severe prolapse

Table 7.2: Prevalence of finding from the post mortem examination in slaughtered hens at end of lay and dead hens (fallen stock), and the level of significance (Fisher's exact test) for the difference between groups.

	Hens slaughtered at end of lay	Death hens (fallen stock)	Fisher's exact test
Abdominal cavity	3.7%	36.7%	$p < 0.001$
- peritonitis	3.3%		
- salpingitis	0.0%		
- aerosacculitis	0.5%		
Ovary	6.5%	20.4%	$p = 0.005$
- pathological changes	5.6%		
- not developed	0.9%		
Remnant of the Müllers duct	18.2%	0.0%	$p < 0.001$
- little cyst	15.4%		
- big cyst	2.8%		
Cloaca	14.0%	4.1%	$p = 0.056$
- irritation	3.3%		
- little prolapse	10.7%		
- severe prolapse	0.0%		

The post mortem examination included a parasite worm count conducted in the 214 tagged hens, were the result for *Ascaridia galli* and *Heterakis* sp. are presented in Table 7.3 and the number of hens with a certain number of *A. galli* worms (Figure 7.1).

Table 7.3: Prevalence of hens being infected and non-infected with *A. galli* and *Heterakis* sp. based on post mortem worm counts

	Non-infected	Infected[a]
A. galli (immature)	37.9%	62.1%
A. galli (adult)	29.4%	70.6%
A. galli (immature and mature)	19.6%	80.4%
Heterakis sp.	98.6%	1.4%[b]

a: one or more worms detected

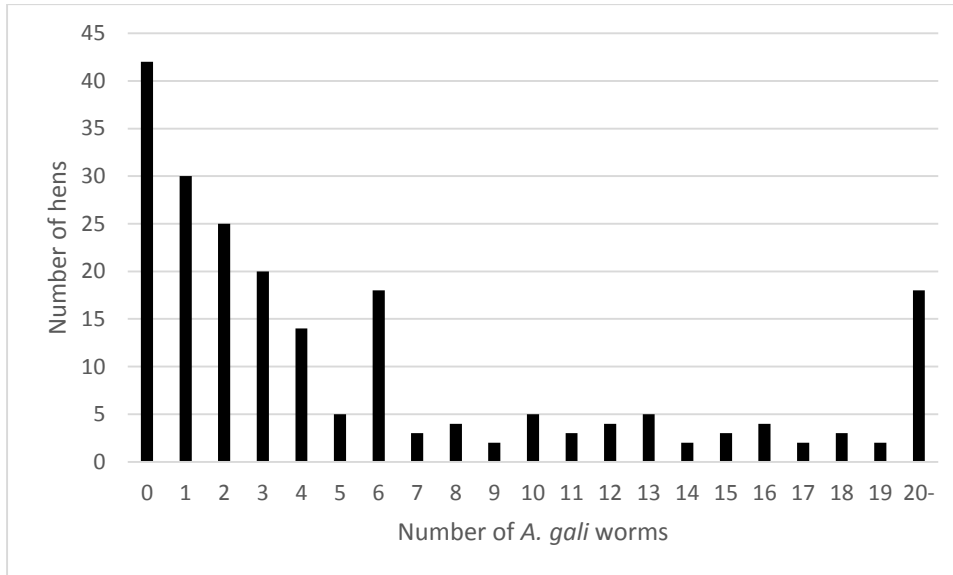b: if a hen had *Heterakis* sp. infection it also were infection with *A. galli*

Figure 7.1: Number of hens with different level of *A. galli* worms in the intestine, maximum number of worms found in one hen were 103.

10.8 Appendix 8: Weather condition in Denmark during peak of lay visits

The weather condition in Denmark during the peak of lay visits are presented in Table 8.1. This are the weather condition for all observation included in Study I, i.e. observation in summer (August and September) and observation in winter (January, February, and March); this were at the same time the peak of lay visit in Study II.

Table 8.1: The weather conditions in Denmark in the time of the peak of lay visits and the norm values for the period 2001-2010 given (DMI, 2014)

	August		September		January		February		March	
	2012	norm	2012	norm	2013	norm	2013	norm	2013	norm
Mean temperature, °C	16.7	17.2	13.0	13.8	0.1	1.5	-0.4	1.2	-0.8	3.0
Precipitation, mm	69	91	95	62	57	66	22	50	9	43
Sunshine hours, hour	215	196	115	162	49	47	51	71	190	146
Days with frost, days[a]					18	19[b]	23.8	19[b]	29.1	15[b]

a: days with frost are days were the mean temperature

b: norm value for the period 1961-1990

References

DMI. 2014. Månedens, sæsonen og årets vejr (In danish: "Weather of the month, season and year"). http://www.dmi.dk/vejr/arkiver/maanedsaesonaar/ (Accessed November 2014).

10.9 Appendix 9: Memo – A trivial argument on mortality rates

Memo - A Trivial Argument on Mortality Rates

Rodrigo Labouriau, December 8th 2014

Here I prove using mathematically trivial arguments (definition of discrete time hazard function, basic probability theory and high-school elementary algebra) that under mild general regularity conditions *if the mortality rate in highly infected farms is larger than the mortality rate in low infected farms, then the mortality rate of infected animals is larger than the mortality rate of non-infected animals.*

Notation:
h/H = h(. I H) = discrete time hazard function for death in highly infected farms
h/L = h(. I L) = discrete time hazard function for death in low infected farms
h/I   = h(. I I) = discrete time hazard function for death of infected animals
h/N = h(. I N) = discrete time hazard function for death of non-infected animals
h(. I I, H) = discrete time hazard function for death of infected animals in highly infected farms
h(. I N, H), h(. I I, L) and h(. I N, L) defined with the (obvious) analogous convention.

Assumptions:
· P/H > P/L
· h(. I I, H) = h(. I I, L) = h/I, i.e. the discrete-time hazard function for an infected animals die in a highly infected farm is equal to the hazard function for an infected animal die in a low infected farm
· Analogously, h(. I N, H) = h(. I N, L) = h/N.

Proposition - Under the assumptions above
    h/H - h/N < h/I - h/N.
In particular,
h/H > h/N => h/I > h/N

Proof:
Since the discrete time hazard functions are probabilities, using the total probability theorem yields:
    h/H = h/I P/H + h/N (1-P/H)
and
     h/L = h/I P/L + h/N (1-P/L).
Therefore,
   h/H - h/L = h/I P/H + h/N (1-P/H) - h/I P/L - h/N (1-P/L)
          = h/I (P/H - P/L) + h/N (P/L - P/H)
          = h/I (P/H - P/L) - h/N (P/H - P/L)
          = (P/H - P/L) (h/I - h/N)
          < (h/I - h/N)

QED

The aim of this PhD project was to investigate animal welfare in organic egg production, with emphasis on mortality and helminth infections. The study were conducted on Danish commercial organic egg farm and included three studies. A study of the interactions between hen mortality and helminth infection at peak of lay, a study investigating whether helminth infections at the end of lay can be predicted by clinical welfare indicators examined at peak of lay and end of lay, and a study identifying management strategies for maintaining low mortality and controlling helminth infections by using qualitative interviews.