The epidemiology of enteric disorders in broiler flocks in the United Kingdom

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

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Abstract

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Enteric disorders of poultry appear to have gained in importance in recent years, possibly as a result of the decreased use of antimicrobial growth promoters in animal feed. Necrotic enteritis (NE) is a prime example of this. The study described in this thesis had as an original objective to determine the prevalence and associated risk factors of NE but was expanded to also include descriptions of the epidemiology of wet litter and coccidiosis in UK broiler flocks. The study of NE consisted of two components. First, the use of the systematic review process to construct an evidence-based case definition for NE and to review putative risk factors for the disease from experimental studies. Secondly, the use of an observational study to estimate the prevalence of NE in UK broiler flocks and to identify associated risk factors.

The case definition of NE was constructed by the systematic review of case reports and case series describing the clinical, gross and histo-pathological signs of naturally occurring NE. Twenty-one papers were included and 24 papers were excluded from the review on the basis of pre-defined inclusion criteria. The following case definition was obtained: necrotic enteritis of chickens is a disease characterized clinically by depression, loss of appetite and a sudden increase in mortality, pathologically by distended intestines and a pseudomembrane covering the intestinal mucosa of the duodenum, jejunum and ileum and histo-pathologically by a fibrino-necrotic enteritis and the presence of basophilic rod-like bacteria. Experimental studies aiming to reproduce NE were reviewed, using the derived case definition of the disease as an objective diagnostic standard. Nineteen papers were included and 68 papers excluded from the review. Based on the available evidence, an experimental model featuring the inoculation of *Clostridium perfringens* via the feed preceded by an oral challenge of *Eimeria acervulina* oocysts was most successful and consistent in terms of NE production.

In order to study the epidemiology of NE, wet litter and coccidiosis, a cross-sectional study was conducted among 857 farms, rearing broilers for nine UK poultry companies. Data were collected with a postal questionnaire directed at farm managers. Additional information was collected from veterinary post-mortem reports. The response rate to the questionnaire was 75%. For all three disorders, the disease was most often reported during the months October to February. Farm manager reported point prevalence (disease occurrence in the most recently reared crop) was 12.3% (95% C.I. 9.8 – 15.2) for NE, 56.1% (95% C.I. 52.0-60.0) for wet litter and 5.8% (95% C.I. 4.1-8.1) for clinical coccidiosis. Multilevel logistic regression analyses with poultry company included as a random effect, using the occurrence of NE, wet litter and clinical coccidiosis in the farm's most recently reared crop as dependent variables, demonstrated strong associations between all three outcome variables (NE and wet litter O.R.=2.39, NE and coccidiosis O.R.=4.68 and wet litter and coccidiosis OR=9.14). Other identified risk factors for the occurrence of NE included the use of ammonia as a disinfectant (O.R.=3.44) and the use of plasterboard walls in poultry houses (O.R.=3.72).

An increased risk for the occurrence of wet litter was found with the use of side ventilation systems (O.R.=1.74), feed equipment failures (O.R.=2.02), thinning of the flock (O.R.=3.86), an increased number of rodent baits (O.R.=1.02), an increased number of people working on the farm (non-linear), having plastic over boots for each house (O.R. 2.11) and having pigs on the farm (O.R.=4.15). A decreased risk of wet litter was found with the availability of separate farm clothing for each house (O.R.=0.33), an increased age at slaughter (O.R.=0.91), and using a certain broiler breed (O.R.=0.43). A separate analysis was carried out using only those cases of wet litter with an implicated disease aetiology as dependent variable. Additional associations were found between this variable and always using hand sanitizers before entering the house compared to never using hand sanitizers (O.R.=8.15) and house walls made of concrete (O.R.=3.66).

The random effects model with clinical coccidiosis as dependent variable demonstrated increased risks for coccidiosis with visits by representatives of the feed mill (O.R.=11.47) and an increased age at slaughter (non-linear). A separate analysis using a population average model confirmed these two risk factors and additionally found an increased risk of coccidiosis with an increased number of chicks at placement (O.R.=1.05 for an increase of 10000 chicks) and a decreased risk of coccidiosis for those farms that had bacterial counts taken prior to the last crop (O.R.=0.34).

This study has demonstrated the novel application of the systematic review process to construct a case definition and to review experimental studies. Although the limitations of the cross-sectional study design are acknowledged and discussed, its use as a hypotheses generating tool and informant for future epidemiological study design are emphasised.

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List of abbreviations

ACNV	Automatically Controlled Natural Ventilation
CAV	Chicken Anaemia Virus
CE	Competitive Exclusion Products
CI	Confidence Interval
EPEF	European Production Efficiency Factor
FCR	Feed Conversion Ratio
GEE	Generalized Estimating Equations
IB	Infectious Bronchitis
IBD	Infectious Bursal Disease
ICC	Intra-cluster Correlation Coefficient
IQR	Inter-Quartile Range
LRT	Likelihood Ratio Test
NCD	Newcastle Disease
NE	Necrotic Enteritis
OR	Odds Ratio
ROC	Receiver Operating Characteristic
SD	Standard Deviation
SE	Standard Error
SPF	Specific Pathogen Free
TRT	Turkey Rhinotracheitis

Chapter 1: Introduction

Background to and overview of the thesis

Of all food animals, the modern broiler chicken is perhaps the most intensively reared and fastest growing. The average broiler chicken will take just six weeks from the time of hatching to a slaughter weight of two kilogram or more, enabling farmers to complete six or seven cycles (crops) per year. This rapid turn-over of chickens allows for large genetic advances to be made in terms of growth rate, carcass composition and disease resistance in a relatively short time. Arguably, the accelerated growth rate with its accompanying large feed intake, makes the bird extremely susceptible to digestive disorders. One of these, necrotic enteritis, has received increasing attention in recent years.

Necrotic enteritis

Necrotic enteritis (NE) is an enteric disease of poultry capable of causing major animal welfare problems and substantial economic losses (Ficken & Wages, 1997). The disease occurs primarily in broiler-type chickens (Long, 1973; Bernier *et al.*, 1974) but has also been reported in both floor-reared and cage-reared layer-type birds (Helmboldt & Bryant, 1971; Broussard *et al.*, 1986; Payne, 1987). NE was reported as early as 1930 in a Black Orpington pullet in Australia (Bennetts, 1930) but was first described in detail in 1961 in England (Parish, 1961a,b,c). Thereafter, NE was soon reported worldwide and developed into a economically significant poultry disease during the 1960's. In later years, disease incidence and severity decreased, which was thought to be due in large part to the intensive use of antimicrobial growth promoters. However, the use of these substances in animal feed has become increasingly controversial and official bans and voluntary removal of growth promoters appears to have led to an increased incidence of NE, although frequency data from before and after the removal of these compounds are not widely available.

The occurrence of NE in commercial poultry is reported in several case reports. Although many of these reports describe the disease in adequate detail, they are not necessarily consistent and it was felt there existed a need for a summation of the clinical, pathological, and histopathological signs to arrive at a case definition for the disease. In order to make such a case definition as objective as possible, it was decided to use the systematic review process. Systematic reviews were developed to combine the results of randomised clinical trials but its basic principles can be applied to the review of any type of evidence. Importantly, these types of reviews aim to minimise bias by using explicit protocols, collecting all the available evidence, and collating this evidence on the basis of predefined inclusion criteria. The Cochrane Collaboration, formed in 1993 and now the world's largest organization devoted to the production and maintenance of systematic reviews, issues a set of guidelines intended to help reviewers to be systematic and explicit in the way they review research evidence (Alderson *et al.*, 2004). Chapter 2 describes the construction of a case definition for NE by reviewing case reports and case series, using Cochrane Collaboration guidelines.

Case definitions are an important component of epidemiological studies. In addition, they are used in studies that aim to reproduce disease under experimental conditions. The study of the pathogenesis and risk factors for NE has mostly been carried out in experimental settings. Parish's earliest experiments made it apparent that there are multiple interacting causes for NE (Parish, 1961c). Although he isolated a strain of *Clostridium welchii* from diseased birds, which was later re-named *Clostridum perfringens*, he was unable to reproduce NE in healthy birds unless he created an intestinal inbalance by treating birds with opium and mineral salts prior to the inoculation of Clostridia. Over the years, numerous experiments have been carried out implicating several risk factors. Many of these risk factors are consistently reiterated when the disease is discussed but it is not always clear to what extent they are based on valid evidence. In Chapter 3, we objectively assess the role of various risk factors in the pathogenesis of NE, by using the Cochrane Collaboration guidelines to review intervention studies aiming to reproduce NE. The case definition which was constructed in Chapter 2, is used to evaluate the outcome of these intervention studies. By combining studies which use similar experimental methods we attempt to come to a more precise assessment of the relevance of the risk factor.

Data on the frequency of NE are usually based on records of diagnostic laboratories (Long, 1973; Köhler *et al.*, 1977; Kaldhusdal & Skjerve, 1996). As such these estimates are not based on a representative sample of the actual population at risk and can be potentially misleading. Perceived increased, decreased or unaltered frequency measures of NE are often used as arguments for or against the presence of antimicrobial growth enhancers in animal feed, but at best these frequency measures are based on data from diagnostic laboratories and more often on anecdotal evidence from the field. To validate the assumption that NE is a re-emerging disease, possibly associated with a reduced use of growth promoters, population-based frequency measures of NE are essential but lacking. In addition, representative data on disease occurrence are important informants of future epidemiological study design. In Chapter 4, we report the results of a cross-sectional study which had as an objective to estimate the prevalence of NE in UK broiler flocks and to determine some of the factors which were associated with its occurrence under natural conditions.

Wet litter

The majority of broiler rearing takes place in sheds on concrete or earth-packed floors. These floors are usually covered with bedding material such as wood shavings, chopped straw, or saw dust (Butcher & Miles, 1996). The mixture of bedding material and excreted faeces is termed litter which under normal conditions is friable. When litter reaches its saturation threshold and is unable to absorb more moisture it loses its friability and a wet cap may form on the surface. Wet litter results from excess moisture being added to the litter and not enough moisture being removed through heating and ventilation. Climate control within the poultry house is therefore of utmost importance and becomes more difficult during the winter months. Moist cold air directed unto the litter surface can lead to condensation as can uinsulated pipes or water tanks (Pattison, 1987). Other sources of excess moisture include leaking drinkers and roofs, and excessive amounts of water in the bird's faeces. Watery droppings or diarrhoea can have infectious or nutritional causes. Infectious agents that have been associated with wet litter in poultry include

Campylobacter jejuni (Neill *et al.*, 1984), *Eimeria spp.* (Hoerr, 1998), spirochaetes (Stephens & Hampson, 2001) and several viruses (Collins *et al.*, 1989; Ziegler *et al.*, 2002). Other organisms, like *Escherichia coli*, can cause diarrhoea not so much through a direct effect on the gut mucosa but rather by increasing the water intake of the septicaemic birds (Pattison, 1987). Feed components can lead to watery droppings, either by causing excessive thirst resulting in increased water intake or through poor digestibility. High sodium, chloride and potassium levels in the feed all encourage increased water intake (Pattison, 1987; Butcher & Miles, 1996). Grains such as wheat, barley, and rye have indigestible components (non-starch polysaccharides) which can have adverse effects on digestion and nutrient absorption leading to wet or sticky droppings (Choct & Annison, 1990; Hoerr, 1998). Wet litter can have severe consequences for the health, welfare, and productivity of broiler flocks in that it has been demonstrated to be associated with the occurrence of foot-pad, breast, and hock lesions (Greene *et al.*, 1985; Martland, 1985; McIlroy *et al.*, 1987; Menzies *et al.*, 1998).

During the preparation stages of a cross-sectional survey of NE, after discussions with poultry veterinarians and agricultural managers, it became apparent that broiler rearing in the UK, and indeed elsewhere, was increasingly accompanied by very wet conditions of the litter. However, exact prevalence figures were unavailable and the risk factors for this wet litter had not been properly investigated. Consequently, the cross-sectional study described in Chapter 4 was not only designed to investigate the occurrence and risk factors of NE but also that of wet litter, the results of which are presented in Chapter 5.

Coccidiosis

Coccidiosis is an intestinal disease of poultry caused by parasites of the genus *Eimeria* (Apicompexa: Eimeriorina). It is now generally accepted that seven species (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella*) parasitize the domesticated fowl (*Gallus gallus*) with varying pathogenicity (Shirley, 1986). The identification of the different species is typically based on the location of the lesions in the intestine, the appearance of the gross lesion, and the size, shape and colour of the oocysts (McDougald & Reid, 1997). Three forms of disease in the chicken have been described: coccidiasis, being a mild infection with no adverse effects, subclinical coccidiosis, characterised by slight but economically important reductions in growth and feed utilization, and clinical coccidiosis or frank disease (Williams, 1999). The outcome of clinical coccidiosis can vary from diarrhoea, morbidity, reduction of weight gain and poor feed conversion in milder infections to intestinal haemorrhage and death in more severe infections (Williams, 2005).

In 1995, economic losses to the UK poultry industry due to coccidiosis were estimated to be £38.6 million of which 46% was attributed to reduced weight gain in broilers and 34% was as a result of poor feed conversion (Williams, 1999). From an economic viewpoint therefore, avian coccidiosis is one the most important diseases affecting commercial poultry. To complement a study of the major enteric disorders of modern broilers, Chapter 6 records some of the observations made on this disease in a cross-sectional survey of UK broiler flocks.

Overview of the UK broiler industry

The UK broiler industry is largely controlled by integrated companies. These companies own or control the breeding flocks, hatcheries, feed mills, fattening farms and the abattoirs. It is estimated that at the time of the study described in this thesis, four companies processed 70% of the UK broiler production and produced almost 50% of those birds themselves on company owned farms. Most of the other broilers were raised on farmer owned holdings but with chicks, feed, or some other input either supplied or closely controlled by a company. The UK produces approximately 800 million broiler birds annually (Sheppard, 2004).

The majority of these broilers are reared in closed houses on solid floors with controlled ventilation, heating and lighting. Organic and free-range production systems are relatively rare, constituting about 5% of total UK production. Most broilers are slaughtered between 35 and 42 days of age, weighing approximately 1600 to 2200 grams. It is not uncommon that chickens within a flock are removed for slaughter in two batches whereby the second batch is reared longer and allowed to reach a higher slaughter weight. This process is called thinning. About half of the broilers are reared separated by sex. During thinning, one sex, usually the pullets, are removed. Allowing for a cleaning and disinfection period of about seven to ten days between two rearing cycles, most farms produce six to seven batches of broilers each year. Over 80% of the broilers produced in the UK are grown under a quality assurance scheme, most commonly Assured Chicken Production (ACP). This scheme lays down specific hygiene, biosecurity and welfare codes (Sheppard, 2004).

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Chapter 2: A case definition of necrotic enteritis in chickens; a systematic review.

Summary

Background

There is a need for a case definition of necrotic enteritis for use in observational and experimental studies. Such a case definition should be based on all the available evidence and obtained with a minimum of bias.

Objectives

The objective of this review is to construct a case definition of necrotic enteritis using Cochrane Collaboration guidelines and to report it in the standardised Cochrane Collaboration format.

Search strategy

Three electronic databases were searched (Medline:1958 – 2001, Web of Science:1981 – 2001 and CAB abstracts:1993 – 2001) using the following search strategy: (Poultry OR broiler* OR chicken* OR fowl* OR avian OR bird* OR flock*) AND (Necroti* enteritis OR *Clostridium perfringens*). Index Veterinarius was hand-searched from 1961-1993 looking under the following headings: avian*, birds, broiler, chick*, *Clostridium perfringens*, digestive system diseases, enteritis, flock, fowl, necrosis, poultry, poultry diseases. The proceedings of the Western Poultry Disease Conference were hand-searched from 1951 to 2001 and research institutes were contacted for unpublished reports.

Selection criteria

Case reports and case series describing the clinical, gross and histo-pathological signs of naturally occurring necrotic enteritis were included in this review.

Data collection

Two reviewers independently assessed whether the papers fulfilled the inclusion criteria and extracted data based on the clinical, gross and histo-pathological signs.

Descriptive signs were placed in synonymous groups by three independent researchers.

Main results

The following case definition was obtained: Necrotic enteritis of chickens is a disease characterized clinically by depression, loss of appetite and a sudden increase in mortality, pathologically by distended intestines and a pseudomembrane covering the intestinal mucosa of the duodenum, jejunum and ileum and histo-pathologically by a fibrino-necrotic enteritis and the presence of basophilic rod-like bacteria.

Conclusions

This review demonstrates the novel use of the systematic review process in the development of a case definition. There is a need for improved standardisation of clinical and pathological recording. The case definition establishes a criterion for the systematic review of experimental studies of NE and a validation standard for future experimental models of the disease.

Background

Necrotic enteritis (NE) in poultry was first described in detail in 1961 (Parish, 1961a,b,c) and has since been reported world-wide. Although the aetiology and pathogenesis of NE remain to be fully elucidated, a variety of case reports have provided detailed descriptions of the disease, and as such can be used to formulate a case definition. Case definitions are an essential component of all observational and experimental epidemiological studies.

Systematic reviews were developed during the 1980's to overcome the shortcomings of the traditional literature review. These include that traditional reviews are rarely explicit about how studies are selected, assessed and included in the review and that they are particularly prone to bias, especially that of the reviewer. Systematic reviews try to address these issues by working with a well-defined protocol, documenting the review process, and by collecting all the available evidence (Davies *et al.*, 1998). We propose to use the systematic review process to review case reports and case series of naturally occurring NE and to formulate an objective, evidence-based case definition of the disease. Such a case definition can be an important tool in aiding diagnosis in the field, in reviewing results from intervention studies aiming to reproduce NE, and in guiding future observational and experimental studies.

Objectives

The objective of this paper is to use the systematic review process to develop a case definition of NE, following Cochrane Collaboration guidelines (Alderson *et al.*, 2004) . To our knowledge, this is the first time this process has been used in this way. This case definition will be used in a systematic review of intervention studies aiming to reproduce NE.

Criteria for considering studies for this review

The inclusion criteria for papers for this review are based on the types of study, the types of participants and the types of outcome measures.

Types of studies

The types of included studies are case reports and case series describing naturally occurring disease.

Types of participants

Participants considered for inclusion in this review are any type of domestic chicken (*Gallus gallus*). Excluded are any other types of poultry or wild birds.

Types of outcome measures

The outcome measures are the description of the clinical signs, gross pathological signs and histo-pathological signs of naturally occurring NE, all included in a single publication.

Search strategy for identification of studies

Three electronic databases were searched (Medline:1958 – 2001, Web of Science:1981 – 2001 and CAB abstracts:1993 – 2001) using the following search stategy: *(Poultry OR broiler* OR chicken* OR fowl* OR avian OR bird* OR flock*) AND (Necroti* enteritis OR Clostridium perfringens).* In addition, Index Veterinarius, a paper-based predecessor of CAB abstracts was hand-searched from 1961-1993 looking under the following headings: *avian*, birds, broiler, chick*, Clostridium perfringens, digestive system diseases, enteritis, flock, fowl, necrosis, poultry, poultry diseases.* The proceedings of the Western Poultry Disease Conference were hand-searched from 1961 to 2001. Two research institutes (National Veterinary Institute, Oslo, Norway; Swedish University of Agricultural Sciences, Uppsala, Sweden) that were known to have conducted research on NE in the past were formally contacted and asked for unpublished reports. Informal requests for reports on NE were made during conferences and scientific meetings.

Methods of the review

Foreign language papers were presented to native speakers, preferably with a science background. The translators were provided with a short questionnaire designed to determine if the paper contained information on NE in chickens and the type of study design (case report, case series, or intervention study). The data from this questionnaire were used to assess if the paper met the first two inclusion criteria (types of studies and types of participants). If it did, the paper was translated in full. Two independent reviewers read each paper and made a decision on whether it should be included or excluded on the basis of all the defined inclusion criteria. Disagreements were resolved by discussion. Data collection consisted of listing the clinical, gross and histo-pathological signs mentioned in each of the included papers. In addition, age at which NE occurred and mortality as a result of the outbreak were recorded. The signs were grouped into two categories, clinical and pathological. The latter included gross and histo-pathological signs. The two reviewers and a third veterinarian independently identified synonyms used in the clinical descriptions. Discussion and consensus resolved disagreements. A similar process was carried out for the gross and histo-pathological signs but in this case two veterinary pathologists working together supplemented the work of the two reviewers. Disagreements were again resolved by discussion and consensus. Clinical and pathological signs were then scored according to the number of times they appeared in separate publications. Signs mentioned in more than half of the papers contributed towards a case definition for NE. The sign which was mentioned most frequently within the synonymous group was used in the case definition.

The methodological quality of the included studies was scored independently by the two reviewers, on the basis of nine criteria (Table 2. 1). These criteria assessed the papers as to their completeness in describing the cases. Equal weight was assigned to each criterion and fulfilment of one criterion was awarded with one point, leading to a maximum possible score of nine.

Table 2. 1: Criteria used to assess the scientific quality of included case reports and case series in a systematic review of NE.

Criterion
Description of number of cases
Description of time (i.e. year, month, season)
Description of type of chicken (i.e. broiler, layer, breeder)
Description of the size of the flock in which the outbreak occurred
Description of the age of the chickens at the time of the outbreak
Description of the mortality because of the outbreak
Description of the duration of the disease
Description of the treatment
Description of additional diagnosis (i.e. bacterial isolation & identification, toxin identification, experimental reproduction, electron microscopy)

Description of studies

Overall, 21 papers were included and 24 papers were excluded from this review.

Included studies

Author, type of study, country where the study was carried out, language in which the paper was written, type of participants and the quality score of each included study are listed in Table 2. 2. Eight different languages were used in the included studies and they were conducted in sixteen different countries. Just over half of the included papers (12 or 57%) were written in English. Twelve papers described the disease in broilers, eight in layers or layer pullets and three in broiler breeders.

Excluded studies

Excluded papers are those which on the basis of their title, abstract, or translator questionnaire were deemed eligible for inclusion in the review but after inspection did not fulfil the inclusion criteria. The characteristics of these studies, including the reason for their exclusion, are given in Table 2. 3. The majority of these papers (16 or 67%) were written in a language other than English, using eight different languages and they were carried out in 16 different countries. Ten of the papers described the disease in broilers versus five papers describing NE in layers and one paper describing it in breeders. Notably, quite a number of these studies (10 papers) did not specify in what type of chicken the disease occurred. The most frequent reason for exclusion was incomplete outcome measures (17 papers). Of these, three papers did not describe the clinical signs (no's 31, 35, 39), two papers did not describe the pathology (no's 28, 44) and fifteen papers did not describe the histo-pathology of the disease (no's 22, 23, 24, 26, 28, 29, 32, 34, 35, 36, 38, 40, 41, 44, 45). Seven papers were excluded because they were not considered to be case reports or case series. One of these (no 37) was based on surveillance data and did not describe any of the outcome measures. The other six papers were either literature reviews (no's 25, 27, 42) or it was made insufficiently clear if the results were based on self-observed, well-defined cases (no's 30, 33, 43).

Table 2. 2: Included studies in a systematic review of case reports and case series describing NE.

No	Author	Type of study	Country	Language	Participants	Quality score
1	Baldassi *et al* (1995)	Case report	Brasil	English	Broilers	7
2	Bartalos *et al* (1976)	Case series	Hungary	Hungarian	Broilers, layers	5
3	Bernier *et al* (1974)	Case series	Canada	French	Broilers	8
4	Broussard *et al* (1986)	Case report	USA	English	Layer pullets	8
5	Chakraborty *et al* (1984)	Case series	India	English	Pullets & layers	6
6	Cisar (1977)	Case report	Czech Republic	Czech	Broiler breeders	5
7	Dewan & Das (1989)	Case report	Bangladesh	English	Layer pullets	5
8	Hemboldt & Bryant (1971)	Case series	USA	English	Broilers, layers	7
9	Jantosovic *et al* (1992)	Case report	Slovakia	Slovak	Broilers	6
10	Kaldhusdal (1995)	Case series	Norway, Sweden	English	Broilers	3
11	Kralj *et al* (1979)	Case series	Croatia	Serbo-Croatian	Broilers	8
12	Kwatra & Chaudhury (1976)	Case report	India	English	Layers	4
13	Murakami *et al* (1989)	Case report	Japan	Japanese	Broilers	9
14	Nairn & Bamford (1967)	Case series	Australia	English	Broilers, broiler breeders	7
15	Oda *et al* (1977)	Case report	Japan	Japanese	Layers	7
16	Parish (1961a)	Case report	UK	English	Cockerels	7
17	Payne (1987)	Case report	USA	English	Layers	5
18	Li & Zhou (1997)	Case report	China	Chinese	Chickens	7
19	Rahamathulla (1994)	Case series	Nigeria	English	Broiler breeders, broilers	7
20	Tsai & Tung (1981)	Case report	Taiwan	English	Broilers	9
21	Zhang *et al* (1986)	Case series	China	Chinese	Broilers, pullets	5

Methodological quality of included studies

By including only those studies that described the clinical, pathological, and histopathological signs of the disease, a basic standard of methodological quality was set. The additional quality score, which assessed the included studies as to their completeness in describing the case(s), is shown in Table 2. 2. The average score given was 6.4, ranging from 3 to 9.

Table 2. 3: Excluded studies from a systematic review of case reports and case series describing NE.

No.	Author	Type of study	Country	Language	Participants	Reason for exclusion
22	Bains (1968)	Case report	Australia	English	Broilers	No histo-pathology described
23	Bennetts (1930)	Case report	Australia	English	Pullets	No histo-pathology described
24	Cygan & Wawrzkiewiczowa (1966)	Case series	Poland	Polish	Broilers, pigs	No histo-pathology described
25	Cygan (1974)	Review	Poland	Polish	Chickens	Type of study
26	Cygan & Nowak (1974)	Case series	Poland	Polish	Chickens	No histo-pathology described
27	Cygan (1987)	Review	Poland	Polish	Chickens	Type of study
28	Dosoky (1990)	Case report	Egypt	English	Layers	No gross pathology and histo-pathology described
29	Duben (1968)	Case series	Czech Republic	Czech	Chickens, ducks, turkeys	No histo-pathology described
30	Gardiner (1967)	Review	Australia	English	Chickens	Type of study
31	Glavits *et al* (1989)	Case series	Hungary	Hungarian	Chickens	No clinical signs described
32	Jylling & Mørch (1969)	Case series	Denmark	Danish	Broilers	No histo-pathology described
33	Kakuk (1974)	Review	Hungary	Hungarian	Broilers, pullets, turkeys, geese	Type of study
34	Köhler *et al* (1974)	Case series	Germany	German	Broilers, pullets, layers	No histo-pathology described
35	Kosovac *et al* (1976)	Case series	Serbia	Serbo-Croatian	Chickens: all categories	No clinical signs & histo-pathology described
36	Kovarik & Lojda (1997)	Case series	Czech Republic	Czech	Chickens	No histo-pathology described
37	Kralj *et al* (1979)	Survey	Croatia	Serbo-Croatian	Breeders, broilers, layers	Type of study

Table 2. 3 (continued)

No.	Author	Type of study	Country	Language	Participants	Reason for exclusion
38	Lakshmana Char *et al* (1986)	Case report	India	English	Layers	No histo-pathology described
39	Long *et al* (1974)	Case series	Canada	English	Broilers	No clinical signs described
40	Machaj & Machaj (1978)	Case series	Poland	Polish	Broilers	No histo-pathology described
41	Minarik & Dyml (1973)	Case series	Czech Republic	Czech	Broilers	No histo-pathology described
42	Popovic & Latinovic (1981)	Review	Bosnia	Serbo-Croatian	Chickens	Type of study
43	Sesaciu & Bross (1978)	Review	Romania	Romanian	Chickens	Type of study
44	Siegel *et al* (1993)	Case report	USA	English	Layers	No gross pathology and histo-pathology described
45	Wijewanta & Senevirtna (1971)	Case report; experimental study	Indonesia	English	Chickens	No histo-pathology described

Results

Clinical signs, gross pathological signs and histo-pathological signs that were mentioned in more than half of the included papers are listed in Table 2. 4, Table 2. 5, and Table 2. 6 respectively. The signs used in the case definition are underlined and all the synonyms are included in each row of the table.

Table 2. 4: Clinical signs described in more than half of the included case reports and case series in a systematic review of NE.

Clinical signs	Papers	No. of times mentioned
Sudden increase in mortality Synonyms: rapid death; sudden death; death within hours; usually found dead	2,3,4,8,10,11,13,14,17,20	11
Depression Synonyms: quiet; down; somnolent; dull; apathy; drowsy; lethargic; dispirited; listless	1,3,6,7,8,9,10,11,13,14,18, 19,20,21	18
Loss of appetite Synonyms: decreased feed consumption; anorexia; poor appetite; decreased appetite; refusal to eat	2,6,9,10,13,15,16,18,20,21	11

Table 2. 5: Pathological signs described in more than half of the included case reports and case series in a systematic review of NE.

Pathological signs	Papers	No. of times mentioned
Distended intestines Synonyms: enlarged; dilated; ballooning; widened; expanded	2, 3, 4, 5, 8, 12, 16, 17, 19, 20, 21	11
Intestinal surface covered with pseudomembrane Synonyms: intestines filled with necrotic tissue; intestinal surface covered with fibrino-necrotic membrane; intestinal surface covered with thick hard core of granular debris lying free or adherent to the mucosa; intestinal surface covered with cheesy necrotic layers; intestinal surface having dirty Turkish towel appearance	2, 3, 4, 5, 8, 9, 10, 11, 12, 15, 16, 17, 18, 20	16
Lesions present in small intestine	2, 3, 4, 6, 8, 9, 11, 14, 16, 18, 20, 21	12

Table 2. 6: Histopathological signs described in more than half of the included case reports and case series in a systematic review of NE.

Histopathological signs	Papers	No. of times mentioned
Fibrino-necrotic enteritis Synonyms: necrotic and diphtheritic enteritis; necrotic enteritis; disintegrating villosities forming a membrane covering the mucous membrane; disintegrating villosities filled intestine with epithelial cells in fibrinoid structure; eosinophilic exudates in lumen of intestine comprised of dead or dying mucosal epithelium trapped in fibrin; like above but infiltrated with granulocytes, erythrocytes, plasma cells; presence of diphtheritic membrane; mucous membrane covered with eosinophilic membrane comprising debris of necrotic epithelial cells, and fibrin; diphtheritic membrane tightly adhered to the mucosa; fibrinous pseudomembranes fixed on necrosis; disappearance of the epithelium of the villosities; mucous membrane devoid of villi; infarction from the middle to the tips of villi of the duodenum; infarcted parts showing fibrinous exudates, cluster of gram positive bacilli and small amount of gram negative bacilli; necrosis of the villosities; fibrinoid necrosis of villosity extremities; necrosis of tips of villi; focal necrosis comprising few epithelial and stromal cells apically on villi; lesions characterized by necrosis with cellular debris; necrosis of villi; necrosis of villus stroma; denudation of necrotic villi; villi partially or completely denuded of epithelium; shedding of villi into lumen; necrosis and desquamation of epithelium; necrosis and degeneration of epithelial cells lining villi; cellular debris (columnar cells, goblet cells) in lumen; large sheets of disintegrating cells deposited into lumen; lumen filled with fused core of granular debris, with groups of shed degenerate epithelial cells and erythrocytes on the periphery; lumen contained desquamated epithelium mixed with blood clots; coagulation necrosis of villi, fusing into amorphous mass with fibrin deposits and some erythrocytes; epithelial cells that retained cytoplasm deeply and homogeneously stained by eosin; coagulation necrosis; mucosa devoid of villi tips; sloughing of intestinal epithelium; necrosis extending into the lamina propria	1, 3, 4, 6, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20	41
Presence of basophilic rod-like bacteria Synonyms: large numbers of thick Gram positive rods in debris of sloughed tissue; Gram-positive rods detected in large numbers; dark basophilic foci representing bacterial colonies present in necrotic areas; numerous colonies of bacteria present; numerous clumps of Gram-positive rods noted in necrotic tissue	1, 3, 4, 5, 6, 8, 9, 10, 12, 16, 17, 20	13

Age at which NE occurred and mortality as a result of the NE outbreak as reported in the included studies are shown in Table 2. 7. Age and mortality figures were not included in the case definition.

Table 2. 7: Age at which NE occurred and resulting flock mortality described in included case reports and case series in a systematic review of NE.

No	Author	Participants	Age	Mortality
1	Baldassi *et al* (1995)	Broilers	Average age 29 days	10%
2	Bartalos *et al* (1976)	Broilers, layers	Not specified	Did not exceed 5-10%
3	Bernier *et al* (1974)	Broilers	10 days – 20 weeks (75% between 2-4 weeks)	0.2-2% (daily mortality)
4	Broussard *et al* (1986)	Layer pullets	12 weeks	0.02%
5	Chakraborty *et al* (1984)	Pullets & layers	3-6 months	30-90%
6	Cisar (1977)	Broiler breeders	Not specified	Not specified.
7	Dewan & Das (1989)	Pullets	4 months	Not specified
8	Hemboldt & Bryant (1971)	Broilers, layers	Average age 50 days (range 14-78)	Rarely exceeded 1% (daily mortality)
9	Jantosovic *et al* (1992)	Broilers	24 days	5.3-25.6%
10	Kaldhusdal (1995)	Broilers	2-5 weeks	Not specified.
11	Kralj *et al* (1979)	Broilers	11-70 days	0.18-15.20%
12	Kwatra & Chaudhury (1976)	Layers	9 months	Not specified.
13	Murakami *et al* (1989)	Broilers	25-32 days	3.10%
14	Nairn & Bamford (1967)	Broilers, broiler breeders	2-7 weeks in broilers, 13 weeks in broiler breeders	Rarely exceeded 5%
15	Oda *et al* (1977)	Layers	6-17 months	Not specified
16	Parish (1961a)	Cockerels	6-7 weeks	53%
17	Payne (1987)	Layers	40 weeks	Not specified
18	Li & Zhou (1997)	Chickens	Not specified	15.5%
19	Rahamathulla (1994)	Broilers, Broiler breeders	6-8 days (broilers) 23 weeks (broiler breeders)	Up to 10% (broilers) 2.8% (broiler breeders)
20	Tsai & Tung (1981)	Broilers	45 days	5%
21	Zhang *et al* (1986)	Broilers, pullets	56 days	2-3%

On the basis of these signs the following case definition was constructed:

Necrotic enteritis of chickens is a disease characterized ***clinically*** *by depression, loss of appetite and a sudden increase in mortality,* ***pathologically*** *by distended intestines and a pseudomembrane covering the intestinal mucosa of the duodenum, jejunum and ileum and* ***histo-pathologically*** *by a fibrino-necrotic enteritis and the presence of basophilic rod-like bacteria.*

Discussion

In this study we have demonstrated a novel use of the systematic review process in the development of a case definition. This case definition establishes a criterion for a systematic review of experimental studies of NE and a validation standard for future putative experimental models of the disease.

Traditional literature reviews are vulnerable to several forms of bias, such as publication, language, and reviewer bias. Systematic reviews aim to minimise these forms of bias. Empirical evidence demonstrates that studies with significant or favourable results are more likely to be published or cited than those with non-significant or unfavourable results (McAuley *et al.*, 2000; Song *et al.*, 2000). In order to avoid this publication bias an attempt should be made to identify, retrieve, and include all reports, both published, unpublished, peer reviewed and non-peer reviewed, that meet the predefined inclusion criteria. In this study, a search was undertaken for unpublished reports on the world wide web, in a database of the Western Poultry Disease Conference and by contacting two research institutes. This resulted in the identification and retrieval of one unpublished report that met the inclusion criteria (no 17). To what extent publication bias has an important role to play in case reports and case series describing natural disease is uncertain.

The importance of including foreign language papers to avoid language bias has been demonstrated in systematic reviews of randomised controlled trials and meta-analyses (Grégoire *et al.*, 1995; Egger *et al.*, 1997). Nine reports (43%) out of the 21 papers that met the inclusion criteria were written in a language other than English, suggesting the importance of including CAB abstracts and Index Veterinarius, databases that cover a wide range of foreign language journals, in our search strategy. However, translation of these papers into English presented a number of practical difficulties. These included identifying translators with the necessary scientific knowledge to understand the context of the paper, increased review costs, and a complication of the already difficult semantic process of summarising clinical and pathological descriptions. By using a preliminary questionnaire to identify foreign language papers which did not meet the inclusion criteria, translation costs were kept to a minimum. Defined terms can assist in ensuring standardisation of reporting. This is well developed in pathology, where terms such as necrosis are used to describe clearly defined pathological signs. In contrast, clinical descriptions can be much less well defined and can present major constraints across language barriers. In this review, we used expert opinion to summarise the clinical and pathological signs but there is a need to develop more objective methods to identify synonyms. To what extent online data sources such as UMLS[1] or SNOMED[2] can be helpful in this respect remains to be explored.

By using a set of pre-defined criteria on the basis of which papers were included or excluded from the review and by using a standard data extraction protocol, carried out by two independent reviewers, we attempted to avoid reviewer bias. Although the adoption of strict inclusion criteria aids objectivity and and lends focus to the review, it can also lead to incomplete reporting. For example, the review only included case reports and case series, which typically describe first occurrences of the disease in a specific country or region. As such, the review was less likely to include descriptions of endemic disease. However, other literature reviews present no evidence for different manifestations of endemic or epidemic states of NE (Ficken & Wages, 1997). Similarly, by only including papers with descriptions of the clinical, gross and histo-pathological signs of NE we greatly restricted the number of papers eligible for inclusion in the review. There is a danger that by setting such rigorous inclusion criteria, papers that contain valuable information are lost from the review process. The widely quoted papers by Glavits *et al.* (1989) and Long *et al.* (1974) were excluded because they failed to give a description of the clinical signs of the

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[1] Unified Medical Language System. U.S. Department of Health and Human Services, National Institutes of Health, National Library of Medicine.
[2] Systematized Nomenclature of Medicine. College of American Pathologists, Northfield, IL, USA.

disease, yet the descriptions of the pathology and histo-pathology in these papers were detailed. For reasons of consistency it was decided not to make an exception for these papers but to retain the inclusion criteria as they had been decided upon prior to the commencement of the review.

However, not all our inclusion criteria were restrictive to such an extent. For example, our case definition included descriptions of NE in all domestic chickens instead of restricting the inclusion of papers to descriptions in one particular type of chicken such as broilers or layers. Although the condition is more commonly seen in broilers than in layers, we did not find evidence that the disease presents differently in these types of chicken. Defining this inclusion criterion rather broadly was in our opinion therefore justified.

Because the first description of NE dates back to 1961, our literature search required hand-searching of those years which were not covered in the electronic databases used. This resulted in inconsistencies in the search criteria. Citation searches of retrieved publications, revealed that a report of a disease with similar pathological signs to NE was published as early as 1930 (Bennetts, 1930). This report was not cited in the putative original description of the disease and therefore it is possible that other such reports might not have been picked up with our search strategy. Similar observations have been made for other syndromes such as Crohn's disease which was first described and named in 1932 (Crohn *et al.*, 1932) but was already reported as early as 1913 (Dalziel, 1913).

No immediate judgement was made about whether or not the description of the clinical signs, pathological signs and histo-pathological signs in the included papers agreed with our prior knowledge of NE. By including only those signs that were mentioned in more than half of the papers, we are assured of a case definition of the disease as it is most commonly seen. Signs that were caused by extraordinary circumstances or were part of a different disease process would not become part of the case definition. For example, Murakami *et al.* (1989) reported a dual infection of *Clostridium perfringens* and *Escherichia coli* in broiler chicks. The pathological changes of the respiratory organs and serous membranes reported in this study were most likely a result of the *E. coli* infection. These types of changes were not reported in any other paper and therefore did not become part of the case definition. Similarly, in the case report of Dewan & Das (1989), the authors report quite extensive pathological changes in the proventriculus which are not seen in any of the other studies. Whether or not these are rare manifestations of NE or are caused by other underlying disorders is not clear, but they were sufficiently infrequent to be excluded from our case definition.

Although our case definition was not formally validated, feedback from poultry veterinarians active in the field suggested that it was reasonably accurate. It is a feature of systematic reviews that they should be regularly updated, incorporating the most recent studies. Future updates of this review will provide an indication of the validity and repeatability of the present case definition. Comparisons of this case definition with the definitions of other common gastro-intestinal disorders of chickens indicate that it is sufficiently discriminatory. In the field, diseases that must be differentiated from NE are ulcerative enteritis, associated with *Clostridium colinum* infection, and coccidiosis caused by *Eimeria brunetti* (Ficken & Wages, 1997). Ulcerative enteritis is characterised by necrosis and ulceration in the small intestine and caeca, and areas of necrosis in the liver (Berkhoff, 1997). Involvement of caeca and liver is not part of the case definition for NE. *E. brunetti* infection produces a coagulation necrosis in the lower small intestine but a characteristic pseudo-membrane, as found with NE, does not feature in the definition of this disease. The presence of schizonts and absence of a large numbers of basophilic rod-like bacteria on histopathological examination further helps to differentiate this disease from NE (McDougald & Reid, 1997).

However, there is clearly a need to improve the method of combining signs to maximise the sensitivity of the case definition. The use of factor analysis or principal components analysis in which the combinations of signs in each report are taken into consideration may enhance this (Nisenbaum et al., 2004). In the scoring method used in the current study, signs which were fairly commonly observed but not described in the majority of papers received no mention. This was the case for example for diarrhoea (observed in 10/21 papers), ruffled feathers (10/21 papers), good bodily condition (8/21 papers), friable intestines (8/21 papers), and gas-filled intestines (8/21 papers). The presence or absence of coccidial parasites deserves special mention because these are considered to be an important risk factor in experimental studies (Katitch *et al.*, 1966; Al-Sheikhly & Al-Saieg, 1980). Eight studies found evidence of coccidial involvement whereas five other studies did not detect the presence of this parasite. The remaining studies did not mention the presence or absence of coccidia. On the basis of this review it can be concluded that coccidia, although often present, do not seem to be a necessary factor in the pathogenesis of NE.

Conclusions

Implications for practice

This review demonstrates that any flock presented with a sudden increase in mortality should have NE in the differential diagnosis. Although the disease has been reported most frequently in broilers, it can occur in any type of chicken, both in pullets and in adult birds, and both in birds housed on the ground and in cages. Total flock mortality is usually low, but was reported as high as 90%. Neither diarrhoea nor the presence of coccidia are consistent features of the disease. The provided case definition can be used as a source of reference for diagnoses in the field.

Implications for research

This systematic review highlights the need for improved standardisation of clinical and pathological recording, and the semantic challenges which translation of non-English language papers adds to this. The outcome measures of this review set minimum standards for future case reports or case series describing this disease. The case definition stands as a criterion for the systematic review of interventions trying to reproduce NE or for future experimental models of the disease.

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Chapter 3: A systematic review of the experimental reproduction of necrotic enteritis in chickens

Summary

Background

The study of the pathogenesis and risk factors of necrotic enteritis has been undermined by the fact that the disease is difficult to reproduce under experimental conditions. Disease models have been inconsistent and poorly reproducible. There is a need to review these experimental models in order to objectively evaluate what is currently known about the pathogenesis and putative risk factors of this disease.

Objectives

To identify risk factors for necrotic enteritis from experimental studies, using an evidence-based case definition of the disease as an objective diagnostic standard.

Search strategy

Three electronic databases were searched (Medline:1958 – 2004, Web of Science:1981 – 2004 and CAB abstracts:1993 – 2001) using the following search strategy: (Poultry OR broiler* OR chicken* OR fowl* OR avian OR bird* OR flock*) AND (Necroti* enteritis OR *Clostridium perfringens*). Index Veterinarius was hand-searched from 1961-1993 looking under the following headings: avian*, birds, broiler, chick*, *Clostridium perfringens*, digestive system diseases, enteritis, flock, fowl, necrosis, poultry, poultry diseases. The proceedings of the Western Poultry Disease Conference were hand-searched from 1951 to 2001 and research institutes were contacted for unpublished reports.

Selection criteria

All experimental studies which either, successfully or unsuccessfully, attempt to reproduce necrotic enteritis in chickens or carry out such procedures from which necrotic enteritis could possibly result or which accidentally reproduce necrotic enteritis where it was not within the paper's objective to do so. Disease diagnosis must be based on the clinical signs, pathology and histology and the study design should include a negative control group.

Data collection

All papers eligible for this review were entered into specialised software (Review Manager 4.2.7) Foreign language papers were fully translated. Two independent reviewers included or excluded the papers on the basis of the above defined inclusion criteria. Data was collected from papers which met the inclusion criteria, including type of interventions used, number of experimental animals and the number of experimental animals who developed necrotic enteritis or necrotic enteritis associated mortality. Papers which used similar intervention strategies were collectively analysed using Mantel-Haenszel methods for combining trials. Data from intervention trials which could not be pooled were, if sufficient information was available, individually analysed using chi-squared tests or Fischer exact tests.

Main results

Direct oral gavage of *C. perfringens* did not produce necrotic enteritis associated mortality and only one out of 158 experimental animals developed lesions characteristic of the disease. In contrast, administering *C. perfringens* via the feed favoured the occurrence of disease associated mortality (OR 17.92; 95% C.I. 7.05-45.58) as did preceding the *C. perfringens* challenge by an oral dose of the coccidian parasite *Eimeria acervulina* (OR 6.66; 95% C.I. 3.52-12.61). Concurrent challenges of other species of coccidia did not significantly increase the risk of disease. On the basis of one included paper, rations based on hammer-milled wheat increased the risk for necrotic enteritis compared to diets consisting of maize or roller-milled wheat. There is a need to repeat these experiments in order to confirm the role of wheat in the occurrence of necrotic enteritis.

Conclusions

In spite of stringent inclusion criteria, all papers failed to satisfy nine quality criteria. In order to facilitate the conduct of future systematic reviews and to raise the quality of experimental studies, the design of these studies should be standardised according to objectively determined criteria.

Background

A wide range of experimental studies attempting to reproduce necrotic enteritis (NE) and to study its putative risk factors have been carried out since the first description of the disease in 1961(Parish, 1961a,b,c), Although *Clostridium perfringens* type A and C have been associated with the disease, reproduction of NE with these agents has been notoriously difficult, clearly underlining the multi-factorial nature of this disorder. The lack of experimental models that are consistent and reproducible has complicated efforts to determine predisposing factors and as a consequence these are ill-defined and inconsistent. There is a need to assess these models and to combine and evaluate the existing information. We propose to use the Cochrane Collaboration systematic review process for this purpose.

In human medicine, causal relationships between putative risk factors and disease are most often studied in observational studies or in controlled trials. Cochrane Collaboration systematic reviews were specifically designed to summarise the results of these controlled trials (Dickersin & Manheimer, 1998). In contrast in veterinary medicine, the most common method to examine causal relationships is in laboratory based experimental studies. Although recent years have seen the use of the systematic review process for study designs other than the controlled trials, including in the field of veterinary medicine (Constable *et al.*, 1997; Hirst *et al.*, 2002; Martin-Curran & MacLehose, 2004), to our knowledge it has not yet been used to review laboratory based experimental studies.

Numerous case reports and case series describing naturally occurring disease, have provided a substrate for an objective evidence-based case definition of NE, based on the clinical, pathological and histo-pathological signs (Chapter 2). This case definition will provide a diagnostic standard for the evaluation of the experimental models.

Objectives

In this paper, we use the systematic review process as specified by the Cochrane Collaboration guidelines (Alderson *et al.*, 2004) to critically review studies which aim to experimentally produce NE. The main objective of this review is to identify risk factors for NE from experimental studies, using objective diagnostic criteria.

Criteria for considering studies for this review

The inclusion criteria for papers for this review are based on the types of study and the types of participants.

Types of study

All experimental studies which either, successfully or unsuccessfully, attempt to reproduce NE or carry out such procedures from which NE could possibly result (i.e. inoculation with *C. perfringens*) or accidentally reproduce NE where it was not within the paper's objective to do so, were considered for inclusion in this review. In order to fulfil the inclusion criteria, disease diagnosis had to be based on the clinical signs, pathology and histology and the study design had to include a negative control group.

Types of participants

Domestic chickens.

Outcome measures

The included studies were assessed as to whether or not NE in accordance with the following case definition was produced: A disease characterized clinically by depression, loss of appetite and a sudden increase in mortality, pathologically by distended intestines and a pseudomembrane covering the intestinal mucosa of the duodenum, jejunum and ileum and histo-pathologically by a fibrino-necrotic enteritis and the presence of basophilic rod-like bacteria (Chapter 2).

Search strategy for identification of studies

Three electronic databases were searched (Medline:1958 – 2004, Web of Science:1981 – 2004 and CAB abstracts:1993 – 2001) using the following search stategy: *(*Poultry *OR* broiler* *OR* chicken* *OR* fowl* *OR* avian *OR* bird* *OR* flock*)*

AND (Necroti enteritis OR Clostridium perfringens).* In addition Index Veterinarius was hand-searched from 1961-1993 looking under the following headings: avian*, birds, broiler, chick*, *clostridium perfringens,* digestive system diseases, enteritis, flock, fowl, necrosis, poultry, poultry diseases. The proceedings of the Western Poultry Disease Conference were hand-searched from 1951 to 2001. Two research institutes (National Veterinary Institute, Oslo, Norway; Swedish University of Agricultural Sciences, Uppsala, Sweden) that were known to have conducted research on NE in the past were contacted and asked for unpublished reports.

Methods of the review

All papers eligible for this review, on the basis of their title and/or abstract, were entered into specialised software (Review Manager 4.2.7; The Cochrane Collaboration). Foreign language papers were presented to native speakers, preferably with a science background, and fully translated. Two independent reviewers included or excluded the papers on the basis of the above defined inclusion criteria. Disagreements were resolved by discussion.

Data were collected from papers which met the inclusion criteria, such as the type of interventions which were used, the number of experimental animals in each treatment group, and the number of experimental animals which developed NE or NE associated mortality. Papers which used similar intervention strategies were collectively analysed with Mantel-Haenszel methods for combining trials, using either NE or NE associated mortality as the outcome variable. Data from interventions which could not be pooled were, if sufficient information was available, individually analysed using chi-squared tests or Fisher exact tests.

Methodological quality of included studies

Included studies were assessed as to their scientific quality according to the criteria in Table 3. 1.

Table 3. 1: Criteria used to assess the scientific quality of the included studies in a systematic review of the experimental reproduction of NE.

Criterion
Were power calculations carried out to determine experimental group size?
Were treatments logical across treatment groups (i.e. split plot design)?
Were results scored blind (i.e. without knowledge of treatment group)?
Were results presented accurately (e.g. no discrepancies between text and tables/graphs)?
Were results presented in detail before being summarized?
Was statistical analysis carried out?
Was the appropriate statistical test described and used?
Were appropriate conclusions drawn from the results?
Does the abstract accurately convey the contents of the paper?

Description of studies

A total of 86 papers were eligible for inclusion in this review.

Included studies

On the basis of the inclusion criteria, 19 papers were included in our review (Table 3. 2). Thirteen of these papers were written in English, four in German and two in French. The majority of these studies attempted to reproduce NE through the administration of *C. perfringens*, using five different inoculation routes. Two papers relied solely on a diet with putative risk factors as a method of NE reproduction. The majority of these papers performed the experiments with normal chickens, except for two studies which used specific pathogen free (SPF) chickens only. The set of experiments described in these two papers will be considered separately. Two papers (Vissiennon *et al.*, 1994a,b), although published separately, were part of the same study. The first paper described the clinical signs and mortality whereas the second paper discussed the pathological and histo-pathological signs. In order to meet the inclusion criteria these papers need to be considered together, however for consistency each paper is seen as a separate publication and its scientific quality is scored separately.

Table 3. 2: Included studies in a systematic review of the experimental reproduction of NE.

Author	Method of reproduction	Investigated risk factor	NE produced?	Score
Al-Sheikhly & Truscott (1977a)	Intra-duodenal inoculation with *C.perfringens*	*C. perfringens* broth culture	Yes	5
Al-Sheikhly & Truscott (1977b)	Intra-duodenal inoculation with *C.perfringens*	*C. perfringens* toxin	Yes	5
Al-Sheikhly & Al-Saieg (1980)	Inoculation with *C.perfringens* via feed	*E. acervulina, E. necatrix*	Yes	2
Baba *et al* (1992)	Inoculation with *C.perfringens* via feed	*E. brunetti*; zinc	Yes	2
Balauca (1976)	Oral inoculation with *C. perfringens*; SPF chickens	*E. acervulina, E. necatrix, E. mitis*	Yes	3
Balauca *et al* (1976)	Oral inoculation with *C. perfringens*; SPF chickens	*E. acervulina, E. necatrix, E. mitis*; floor vs wire husbandry; age	Yes	4
Bernier *et al* (1977)	IV inoculation with *C.perfringens*; Oral inoculation with *C.perfringens*	 Opium; Sodium bicarbonate	Yes Yes	4
Branton *et al* (1987)	Spontaneous	Wheat vs maize diet; hammer vs roller mill	Yes	6
Kaldhusdal & Hofshagen (1992)	Spontaneous	Barley vs maize diet; avoparcin	No	6
Kaldhusdal *et al* (1999)	Inoculation with *C.perfringens* via feed	Pre-challenge antibiotics; challenge strain; length of challenge	Yes	5
Katich *et al* (1965)	Inoculation with *C.perfringens* via feed	Temperature & humidity; type of ration; opium	No	1
Long & Truscott (1976)	Oral inoculation with *C.perfringens*; Inoculation with *C.perfringens* via feed	 Starch; anaerobic vs aerobic incubation of inoculate; length of incubation; challenge strain; duration of challenge; treatment with penicillin or chloramphenicol	No Yes	2
Nairn & Bamford (1967)	Oral inoculation with *C.perfringens*	Diet	Yes	4
Parish (1961c)	Oral inoculation with *C.perfringens*	Opium, sodium bicarbonate & calcium carbonate; duration of challenge; age	Yes	4
Shane *et al* (1985)	Inoculation with *C.perfringens* via feed	*E. acervulina*	Yes	6
Vissiennon *et al* (1994a)	Oral inoculation with *C.perfringens*; Intra-duodenal inoculation with *C.perfringens*		No	3
Vissiennon *et al* (1994b)	Intra-duodenal inoculation with *C.perfringens*		No	6

Table 3. 2 (continued)

Author	Method of reproduction	Investigated risk factor	NE produced?	Score
Vissiennon *et al* (2000)	Intra-duodenal inoculation with *C.perfringens*	Treatment with avilamycin, tylosin, narasin, monensin	No	2
Williams *et al* (2003)	Intra-cloacal inoculation with *C.perfringens*	*E. maxima*; coccidial vaccination	Yes	2

Inoculation route of C. perfringens

Direct oral inoculation

Six studies tried to reproduce NE by oral inoculation with *C. perfringens*. These studies either used a broth culture of *C. perfringens* (Parish, 1961c; Nairn & Bamford, 1967; Bernier *et al.*, 1977), or resuspended *C .perfringens* cells (Long & Truscott, 1976; Vissiennon *et al.*, 1994a,b).

Oral inoculation via feed

Five studies compared chickens fed with *C. perfringens* inoculated feed to chickens fed identical but non-inoculated feed (Long & Truscott, 1976; Al-Sheikhly & Al-Saieg, 1980; Shane *et al.*, 1985; Baba *et al.*, 1992; Kaldhusdal *et al.*, 1999).
The effects of the number of days that infected feed was offered were examined by Kaldhusdal *et al* (1999) and Long & Truscott (1976). These last authors also looked at factors such as anaerobic and aerobic incubation of the inoculated feed and the length of the incubation period.

Intra-duodenal inoculation

Five papers attempted to reproduce NE by inoculating directly into the duodenum, either with *C. perfringens* broth culture (Al-Sheikhly & Truscott, 1977a), vegetative cells (Vissiennon *et al.*, 1994a,b; Vissiennon *et al.*, 2000), toxin (Al-Sheikhly & Truscott, 1977b), or a combination of vegetative cells, toxin and spores (Vissiennon *et al.*, 1994a,b).

Intra-cloacal inoculation

Williams *et al* (2003) introduced *C. perfringens* cells through the cloaca into the ileum.

Intra-venous inoculation

Bernier *et al* (1977) attempted to reproduce NE by injecting broth culture of *C. perfringens* intra-venously.

Other risk factors

Feed

Six studies looked at feed as a whole or various feed components as risk factors for the increased occurrence of NE. Diets high in cereals, such as wheat or barley, were compared with maize diets as to their ability to elicit a spontaneous NE outbreak (Branton *et al.*, 1987; Kaldhusdal & Hofshagen, 1992) and the effects of using a hammer or a roller mill to grind these grains were investigated (Branton *et al.*, 1987). Katich *et al* (1965), using feed inoculated with *C. perfringens* broth, compared a diet high in concentrates either to a diet based on 'green feed' or to a diet based on severe feed restriction.

Other feed components which were looked at were the effects of high levels of zinc (Baba *et al.*, 1992) and various levels of starch in the feed (Long & Truscott, 1976). Nairn & Bamford (Nairn & Bamford, 1967), using direct oral inoculation, compared two non-specified commercial broiler diets, one of which had been associated with a previous NE outbreak.

One study (Williams *et al.*, 2003), used feed with high levels of components considered to be putative risk factors such as wheat and fish meal. These feed components were purely used to elicit the disease and as such were not evaluated by using appropriate control groups, which were fed diets which lacked or had low levels of the particular component. This study therefore cannot be used to evaluate the significance of these feed factors.

Coccidia

Using *C. perfringens* inoculated feed, three studies measured the effects of additional coccidial infection on the occurrence of NE by challenging the birds with oocysts of *E. acervulina* (Al-Sheikhly & Al-Saieg, 1980; Shane *et al.*, 1985), *E. necatrix* (Al-Sheikhly & Al-Saieg, 1980), or *E. brunetti* (Baba *et al.*, 1992).

Williams *et al* (2003) tested the effects of challenge with *E. maxima* oocysts prior to intra-cloacal inoculation of *C. perfringens*.

Alteration of gut conditions

Three studies investigated the effects of changing gut physiology with substances such as opium (Parish, 1961c; Katitch *et al.*, 1965; Bernier *et al.*, 1977), sodium bicarbonate (Parish, 1961c; Bernier *et al.*, 1977) and calcium carbonate (Parish, 1961c), either by themselves or in combination. Parish (1961c) also varied the number of days these substances were given.

Antimicrobials

Three studies looked at the preventive or curative effects of growth promoters such as avoparcin (Kaldhusdal & Hofshagen, 1992), zinc bacitracin (Kaldhusdal *et al.*, 1999) or avilamycin (Vissiennon *et al.*, 2000), ionophore anticoocidials such as narasin (Kaldhusdal *et al.*, 1999; Vissiennon *et al.*, 2000) and monensin (Vissiennon *et al.*, 2000), or prescription antibiotics such as tylosin (Vissiennon *et al.*, 2000).

Other

Other factors which were investigated as to their effect on the reproduction of NE included age (Parish, 1961c), challenge strain (Long & Truscott, 1976; Kaldhusdal *et al.*, 1999), temperature and relative humidity in the shed (Katitch *et al.*, 1965) and vaccination with a coccidial vaccine (Williams *et al.*, 2003).

Experiments with SPF chickens

Two studies used SPF chickens in their efforts to reproduce NE (Balauca, 1976; Balauca *et al.*, 1976). They orally inoculated SPF chickens with a mixture of resuspended *C. perfringens* cells and toxin with or without spores. They looked at the effects of a preceding challenge with a mixture of *E. acervulina*, *E. necatrix* and *E. mitis* oocysts, floor versus wire cage husbandry and age of the chicken.

Excluded studies

Sixty-seven studies were excluded from this review for failure to include a negative control group (20 studies) or because there was no evidence that diagnosis was based on clinical signs (4 studies), pathology (6 studies), or histology (47 studies). In addition, 13 studies, usually conference abstracts, provided insufficient detail about study design and/or results to be included in our review. Three studies did not intentionally set out to reproduce NE, nor did they unintentionally reproduce the disease.

Five different methods of *C.perfringens* inoculation were employed by 52 studies whereas six studies raised the experimental animals on litter previously utilised by a flock with a NE outbreak as a method of disease reproduction. Eight studies relied on spontaneous development of the disease, using feed with putative risk factors or studying flocks on farms with histories of NE occurrence.

Table 3. 3: Excluded studies from a systematic review of the experimental reproduction of NE.

Author	Method of reproduction	Investigated risk factor	Reason for exclusion
Al-Sheikhly & Truscott (1977c)	Intra-duodenal inoculation with *C.perfringens*	*C. perfringens* cells and toxin	No negative control group; no histology
Annett *et al* (2002)	In-vitro proliferation of *C. perfringens*	Barley, wheat or maize diets	Did not aim to reproduce NE
Baba *et al* (1988)	Oral inoculation with *C.perfringens*	*E .tenella*	No clinical signs; no histology
Baba *et al* (1997)	Oral inoculation with *C.perfringens*	*E. necatrix*	No histology
Balauca (1978)	Oral inoculation with *C.perfringens*	*E. acervulina, E. necatrix, E.mitis* floor vs wire husbandry; age; conventional vs SPF chickens	Experimental design and results unclear
Bartalos *et al* (1976)	Oral inoculation with *C.perfringens*; IV inoculation with *C.perfringens*	-	Insufficient detail
Bernier *et al* *(1974)*	IV inoculation with *C.perfringens*	*C .perfringens* toxin type	No pathology; no histology
Bolder *et al* (1999)	Oral inoculation with *C.perfringens*	Flavophospholipol, salinomycin sodium	No negative control group; no histology
Branton *et al* (1997)	Inoculation with *C.perfringens* via feed; oral inoculation with *E.acervulina*	Maize, wheat, pectin, guar gum and ground pine shavings in the feed	No histology used to diagnose NE in dead birds
Brennan *et al* (1996)	Inoculation with *C.perfringens* via feed	Zinc bacitracin, bacitracin methylene disalicylate	No histology; insufficient detail (abstract)
Brennan & Cheng (1997)	Inoculation with *C.perfringens* via feed	Lasalocid	No negative control group; no histology; insufficient detail (abstract)
Brennan *et al* (2001a)	Inoculation with *C.perfringens* via feed; High protein & fish meal starter	Narasin	No histology

Table3. 3 (continued)

Author	Method of reproduction	Investigated risk factor	Reason for exclusion
Brennan *et al* (2001b)	Inoculation with *C.perfringens* via feed; High protein & fish meal starter	Tylosin	No negative control group; no histology
Brennan (2001)	Inoculation with *C.perfringens* via feed; High protein & fish meal starter	Tylosin, narasin, bacitracin methylene disalicylate	Seminar paper; no histology; same data as Brennan *et al*(2001a), Brennan *et al*(2001b), Brennan *et al*(2003)
Brennan *et al* (2003)	Inoculation with *C.perfringens* via feed; High protein & fish meal starter	Bacitracin methylene disalicylate, narasin	No histology
Collier *et al* (2003)	Oral inoculation with *C.perfringens* and *E.acervulina*; wheat & barley diet	Tylosin; pectin	No negative control group; no clinical signs; no histology
Cowen *et al* *(1987)*	Inoculation with *C.perfringens* via feed; raised on NE infectious litter; turkey starter & fish meal		No histology
Craven *et al* (1999)	Oral inoculation with *C.perfringens*; inoculation with *C.perfringens* via drink water	Competitive exclusion product (CE); rye or maize feed	No clinical signs, no pathology, no histology
Cygan & Nowak (1974)	Oral inoculation with *C.perfringens*	Opium, calcium carbonate, sodium bicarbonate	Participants are quail; no negative control group; no histology
Das *et al* *(1997)*	Oral inoculation with *C.perfringens*	Fish meal; strain type; dose size; penicillin, tetracycline, metronidazole, virginiamycin	No pathology, no histology
El-Seedy (1990)	Inoculation with *C.perfringens* via feed	Strain type	No histology
Elwinger *et al* (1992a)	Spontaneous	CE; narasin; wheat; animal versus vegetable protein	No histology
Elwinger *et al* (1992b)	Spontaneous	Narasin	No histology of intestine
Elwinger *et al* (1994)	Spontaneous	Diet composition; lasalocid, maduramicin, monensin, narasin, halofuginone	No histology of intestine
Elwinger *et al* (1998)	Spontaneous	Avoparcin, avilamycin, maduramicin, monensin, narasin	No histology
Fukata *et al* (1988)	Oral inoculation with *C.perfringens*	Germ free chickens; *C.perfringens* broth, supernatant or vegetative cells	No negative control group (Exp.1); no negative control group, pathology, histology (Exp. 2+3)
Fukata *et al* *(1991)*	Oral inoculation with *C.perfringens*	Germ free or mono-flora chickens; *C.perfringens* broth, supernatant or vegetative cells	No negative control group; no histology
George *et al* (1982)	Oral inoculation with *C.perfringens*; fish meal	Virginiamycin	No histology

Table 3. 3 (continued)

Author	Method of reproduction	Investigated risk factor	Reason for exclusion
Hamdy *et al* (1983a)	Raised on NE infectious litter	Lincomycin	No negative control group; no histology
Hamdy *et al* (1983b)	Raised on NE infectious litter	Lincomycin	No negative control group; no histology
Hofacre *et al* (1998a)	Oral inoculation with *C.perfringens* and *E.acervulina*; fish meal	CE; probiotic	No histology
Hofacre *et al* (1998b)	Oral inoculation with *C.perfringens* and *E.acervulina*; fish meal	Virginiamycin, bacitracin methylene disalicylate, CE	No histology
Hofacre *et al* (1999)	Oral inoculation with *C.perfringens* and *E.acervulina*; fish meal	Virginiamycin, bacitracin methylene disalicylate, CE, probiotic, bambermycin (flavomycin)	No histology; data partially the same as Hofacre *et al* (1998a) and Hofacre *et al* (1998b)
Hofacre *et al* (2003)	Oral inoculation with *C.perfringens*, E.*acervulina* and *E.maxima*; fish meal	Bacitracin methylene disalycilate; mannan-oligosaccharide; lactic acid bacteria; CE; fructose oligosaccharide; propionic acid	No histology
Jansson *et al* (1990)	Inoculation with *C.perfringens* via drinking water	Diet (i.e barley, protein); virginiamycin; enzymes	No histology
Jianzhong *et al* (1997)	Oral inoculation with *C.perfringens*	Lincomycin; tylosin	No histology
Kaldhusdal *et al* (2001)	Spontaneous	CE	No negative control group
Katitch *et al* (1964)	Oral inoculation with *C.perfringens*; subcutaneous injection with *C.perfringens*	Strain type; opium; crushed glass	No negative control group
Katitch *et al* (1966)	Oral inoculation with *C.perfringens*	*E. tenella* and/or *E. necatrix*; *C.perfringens* broth, vegetative cells or toxin	No negative control group
Kling & Quarles (1995)	Oral inoculation with *C.perfringens*; fish meal	Bambermycin; virginiamycin; bacitracin methylene disalycilate; lincomycin	No negative control group; no histology insufficient detail (abstract)
Kozitch *et al* (1966)	-	-	Review article of data published by Katich *et al* (1964; 1965; 1966)
Litta (1999)	-	-	Review article
Lovland *et al* (2003)	Spontaneous	-	No pathology or histology described
Lovland *et al* (2004)	Spontaneous	Maternal immunization with *C.perfringens*	No histology
Maxey & Page (1977)	Raised on NE infectious litter	Lincomycin	No negative control group; no histology
McDougald & Reid (1971)	Spontaneous	Coccidiostatic and growth promoting agents	Insufficient detail

Table 3. 3 (continued)

Author	Method of reproduction	Investigated risk factor	Reason for exclusion
Miller (1983)	-	-	Abstract; review of primary data
Mørch (1973)	Spontaneous	Zinc bacitracin; oleandomycine; nifurazolidone	No negative control group; no histology
Mørch (1982)	Inoculation with *C.perfringens* via feed or drinking water	Virginiamycin, avoparcin, nitrovin	No negative control group
Niilo (1974)	Injection of *C. perfringens* in ligated intestinal loops	-	Does not aim to reproduce NE
Niilo (1976)	IV inoculation with *C.perfringens*	-	Does not aim to reproduce NE
Prescott *et al* (1978)	Inoculation with *C.perfringens* via feed; turkey starter & fish meal	Zinc bacitracin	No histology
Prescott (1979)	Inoculation with *C.perfringens* via feed; turkey starter & fish meal	Zinc bacitracin; avoparcin	No negative control group; no histology
Riddell & Kong (1991)	See Riddell & Kong (1992)	See Riddell & Kong (1992)	Abstract; data published in Riddell & Kong (1992)
Riddell & Kong (1992)	Inoculation with *C.perfringens* via feed	Wheat, rye, barley, oats, pentosanase, pectin & guar gum, glucose	No negative control group; no histology
Roney & Fitz-Coy (1999)	Oral inoculation with *C.perfringens* and *Eimeria* (species not specified)	Bacitracin methylene disalycilate	No negative control group; no clinical signs, no pathology, no histology; insufficient detail (abstract)
Shane *et al* (1981)	Inoculation with *C.perfringens* via feed	*E.acervulina*	Insufficient detail (abstract); data published in Shane *et al* (1985)
Skinner & Hunter (1999)	Inoculation with *C.perfringens* via feed	Semduramicin, bacitracin methylene disalycilate	Insufficient detail (abstract)
Skinner & Brennan (1999)	Inoculation with *C.perfringens* via feed	Narasin; bacitracin methylene disalycilate	Insufficient detail (abstract)
Skinner *et al* (1999)	Inoculation with *C.perfringens* via feed	-	Insufficient detail (abstract)
Trammell & Iscrigg (1975)	Raised on NE infectious litter	Zinc bacitracin	Abstract - data published in Wicker *et al* (1977)
Truscott & Al-Sheikhly (1977)	Inoculation with *C.perfringens* via feed; Intra-duodenal inoculation with *C.perfringens*	Fish meal; lincomycin; strain type	No histology
Vissiennon *et al* (1996)	Intra-duodenal inoculation with *C.perfringens*		No histology of the intestine

Table 3. 3 (continued)

Author	Method of reproduction	Investigated risk factor	Reason for exclusion
Wang & Davidson (1992)	Inoculation with *C.perfringens* via feed; oral inoculation with *E.acervulina*	Maduramicin, monensin, salinomycin, zinc bacitracin, virginiamycin, avoparcin	No histology
Wicker *et al* (1977)	Raised on NE infectious litter	Zinc bacitracin	No histology
Wijewanta & Senevirtna (1971)	Oral inoculation with *C.perfringens*; contaminated fish meal	-	No histology
Wijewanta (1971)	Oral inoculation with *C.perfringens*; intra-muscular inoculation with *C.perfringens*	Strain type	No histology

Results

Direct oral inoculation

None of the studies attempting to reproduce NE by orally inoculating *C. perfringens*, succeeded in producing NE associated mortality (Parish, 1961c; Nairn & Bamford, 1967; Long & Truscott, 1976; Bernier *et al.*, 1977) (Figure 3. 1) and only one out of 158 experimental animals used in these studies developed lesions resembling NE (Bernier *et al.*, 1977) (Figure 3. 2). Duration of challenge seemed to have little effect because although the lesions in the affected experimental animal were produced after administration of *C. perfringens* for 5 days, the attempts by Parish (1961c) were unsuccessful even after a seven day challenge.

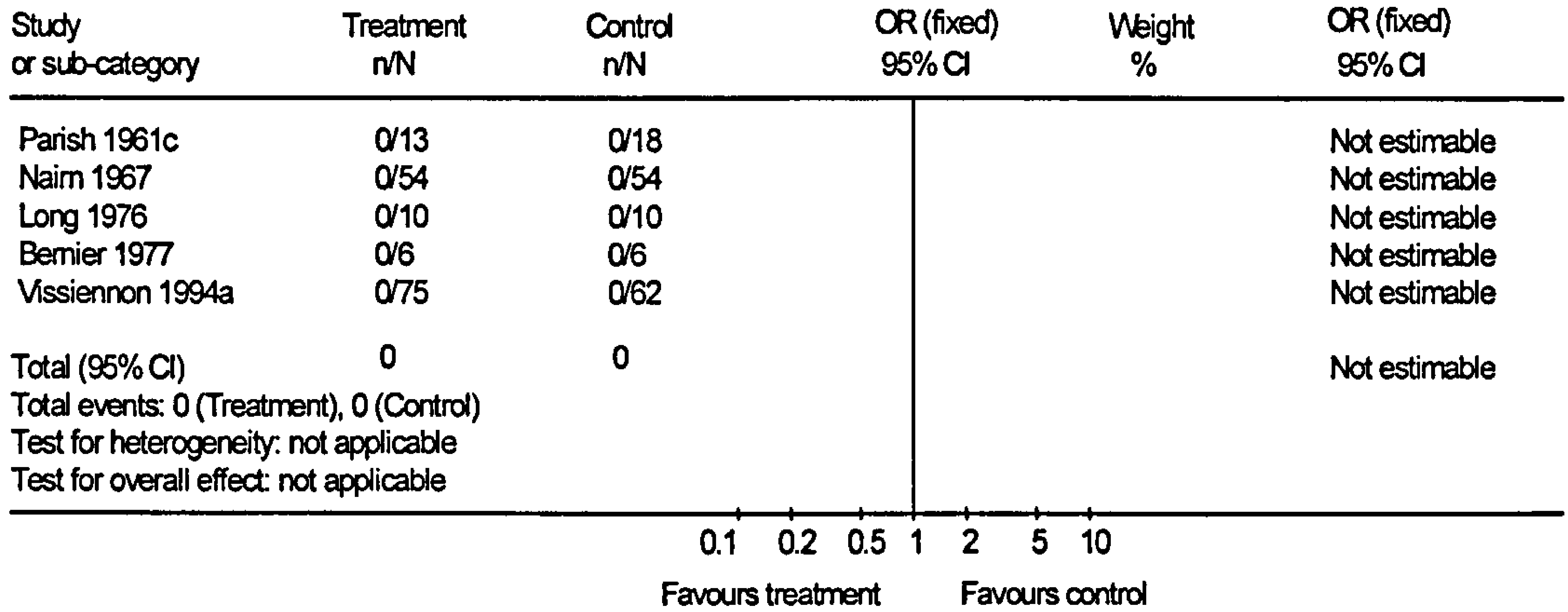

Figure 3. 1: Meta-analysis of studies comparing chickens orally inoculated with *C. perfringens* with non-inoculated control chickens, using necrotic enteritis associated mortality as an outcome.

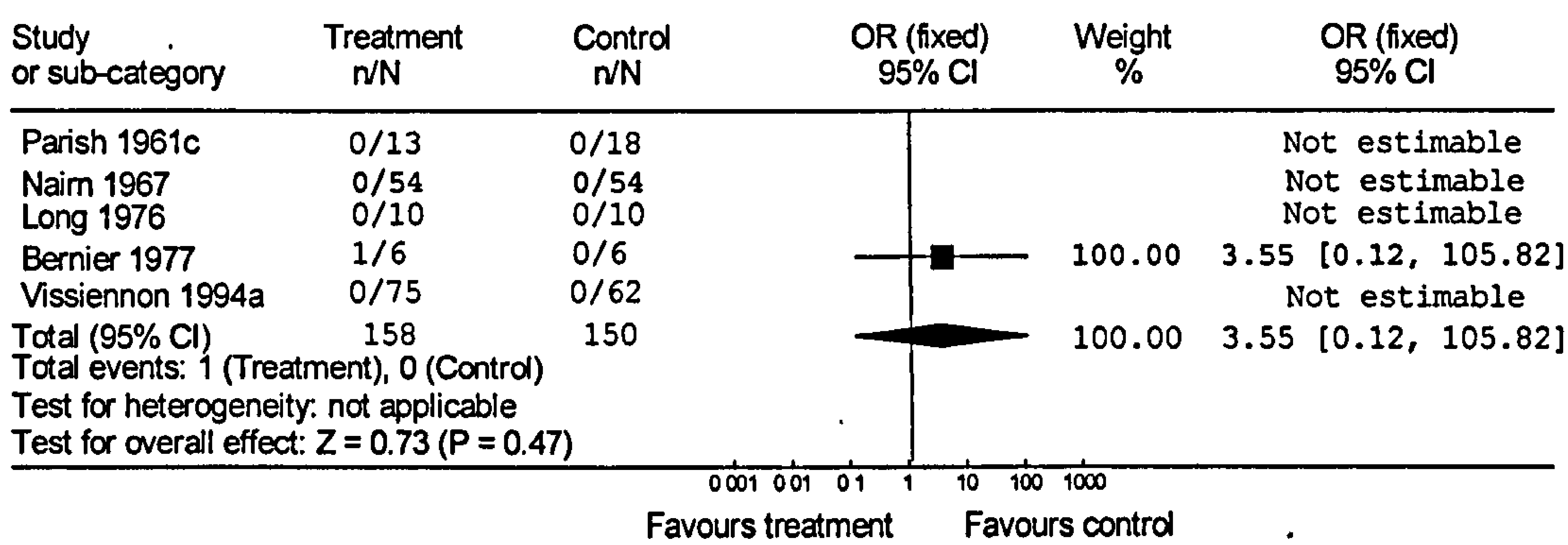

Figure 3. 2: Meta-analysis of studies comparing chickens orally inoculated with *C. perfringens* with non-inoculated control chickens, using necrotic enteritis as an outcome.

Oral inoculation via the feed

Of the five studies (Long & Truscott, 1976; Al-Sheikhly & Al-Saieg, 1980; Shane *et al.*, 1985; Baba *et al.*, 1992; Kaldhusdal *et al.*, 1999) that used *C. perfringens* inoculated feed as a method to reproduce NE, NE associated mortality was observed in a total of 100 out 1000 (10.0%) experimental animals whereas five out of 2539 (0.20%) control animals, being fed non-inoculated feed, died of NE (OR 17.92; 95% C.I. 7.05-45.58) (Figure 3. 3). Only one study did not succeed in producing NE associated lesions or mortality unless the feed was supplemented with zinc (Baba *et al.*, 1992). Most of the studies challenged with *C.perfringens* inoculated feed for five days. Two studies investigated the duration of challenge. Kaldhusdal *et al* (1999) looked at the effect of challenging for one or four days and found that NE associated lesions were produced in four out of 62 birds (6.5%) when challenged for one day and in five out of 61 animals (8.2%) when challenged for four days. This increase in proportion of diseased animals is not statistically significant. Equally, Long & Truscott (1976) found a non-significant increase in NE associated mortality when challenging five days (17/100;17%) as compared to providing inoculated feed for one day (11/92;12%) or two days (10/92;11%).

Study or sub-category	Treatment n/N	Control n/N	OR (fixed) 95% CI	Weight %	OR (fixed) 95% CI
Long 1976	61/290	0/29		20.17	15.81 [0.95, 262.43]
Al-Sheikhly 1980	8/50	0/50		11.75	20.20 [1.13, 360.28]
Shane 1985	7/80	0/40		17.00	8.27 [0.46, 148.47]
Baba 1992	0/20	0/20			Not estimable
Kaldhusdal 1999	24/560	5/2400		51.08	21.45 [8.15, 56.47]
Total (95% CI)	1000	2539		100.00	17.92 [7.05, 45.58]

Total events: 100 (Treatment), 5 (Control)
Test for heterogeneity: Chi² = 0.42, df = 3 (P = 0.94), I² = 0%
Test for overall effect: Z = 6.06 (P < 0.00001)

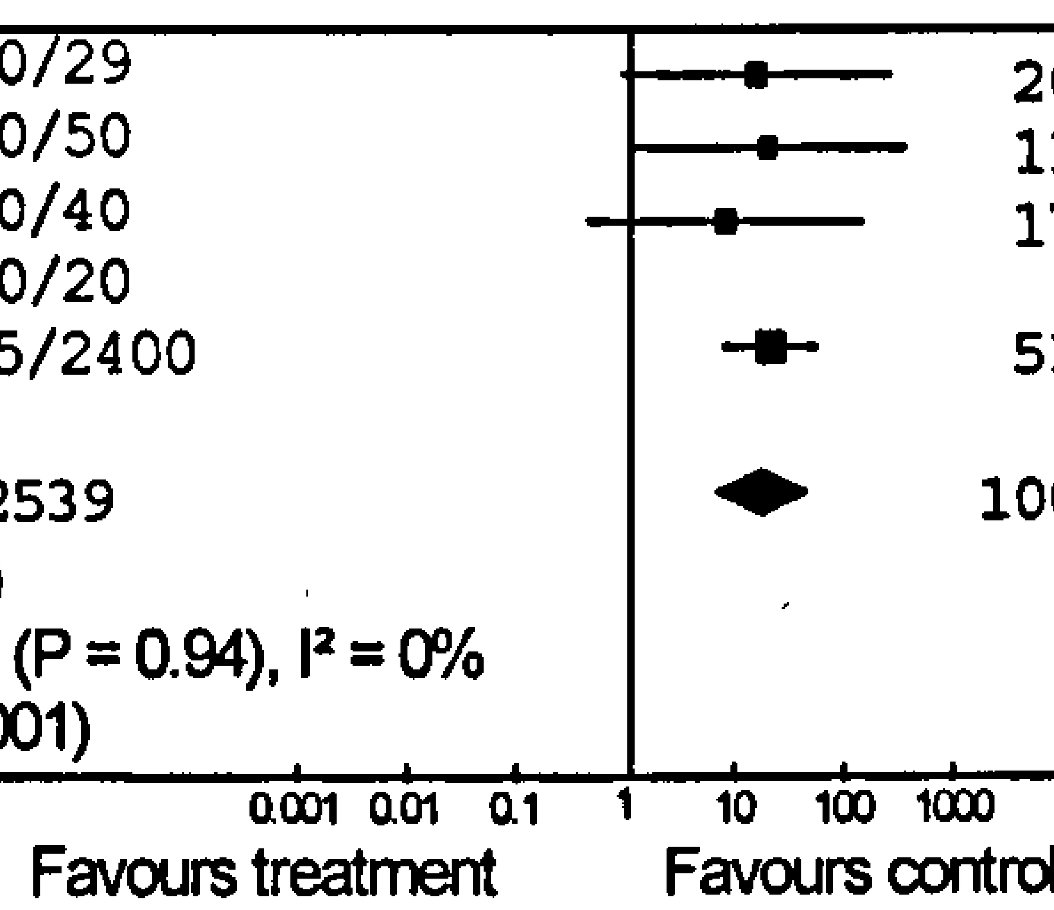

Figure 3. 3: Meta-analysis of studies comparing chickens inoculated with *C. perfringens* via the feed with non-inoculated control chickens, using necrotic enteritis associated mortality as an outcome.

Intra-duodenal inoculation

Placing *C. perfringens* material directly into the duodenum via a surgically implanted catheter, has been used, with differing results, as a method to reproduce NE. Extensive necrosis of the intestinal mucosa in two out of six birds was reported as early as five hours after inoculation of *C. perfringens* broth. By 12 hours all of the inoculated birds (n=6) had necrotic enteritis on post-mortem examination. No death due to NE was reported within the 12 hour experimental period (Al-Sheikhly & Truscott, 1977a). *C. perfringens* toxin produced NE three hours after intra-duodenal after inoculation. No natural deaths occurred within the 20 hour observation period (Al-Sheikhly & Truscott, 1977b).

In contrast, no intestinal lesions characteristic of NE were found after inoculation of vegetative cells of an enterotoxin negative *C. perfringens* type A strain, although several experimental animals died (Vissiennon *et al.*, 1994a,b; 2000). No mortality occurred amongst two day old chicks and neither did deaths occur when *C. perfringens* toxin was inoculated (Vissiennon *et al.*, 1994a). Application of *C. perfringens* spores only resulted in death if it was accompanied by the administration of atropine for five days (Vissiennon *et al.*, 1994a). However, none of the mortality was associated with NE.

Intra-cloacal inoculation

By inoculating vegetative cells of *C. perfringens* directly into the ileum, via the cloaca, Wiliams *et al* (2003) were able to produce NE lesions in 25 of 30 (83.3%) examined birds whereas six out of 13 (46.2%) un-inoculated control birds also developed lesions (p=0.02). Lesions were scored, according to the scoring system developed by Truscott & Al-Sheikhly (1977), until eight days after inoculation and daily mean scores were totalled for each treatment. The authors reported that the total mean scores were significantly higher for *C. perfringens* inoculated birds as opposed to un-inoculated control birds (10.8 versus 3.8) but individual bird scores were not specified. Hence, there is no indication of range of values and standard deviations. No NE associated mortality was observed.

Intra-venous inoculation

A minimum of 0.4ml of a *C. perfringens* culture injected intra-venously was sufficient to elicit death in three out of five experimental animals (Bernier *et al.*, 1977). Macroscopic and microscopic lesions in the dead birds were identical to those observed in NE cases in the field. One hundred percent NE associated mortality was achieved with the injection of 1ml of culture.

Other risk factors

Feed

Diets high in cereals such as wheat and barley were associated with an increased occurrence of NE lesions (Branton *et al.*, 1987; Kaldhusdal & Hofshagen, 1992) but differences in experimental diets, study design and outcome prevent the results of these studies from being combined. In an experiment designed to investigate milling procedures, high mortality (28.9%) occurred in broilers fed a diet based on hammer mill-ground wheat whereas mortality in broilers fed a maize based diet, also ground by hammer mill, was 2.9%. The use of a roller mill to grind the wheat based diets reduced mortality to 18.1%. When the grain components were approximately 50% wheat and 50% maize, mortality was 12.6% for hammer mill-ground wheat diets and 3.4% for roller mill-ground wheat diets (Branton *et al.*, 1987).

Kaldhusdal & Hofshagen (1992) used two experimental diets with high inclusion levels of either barley or maize. Both diets also contained oats and wheat, albeit inclusion levels were reduced in the maize diet. No clinical signs characteristic of NE or any NE associated mortality were observed. Post-mortem investigation revealed small necrotic lesions in the small intestine of six out of 48 birds fed the barley diet and in one out 48 birds fed the maize diet.

Nairn & Bamford (1967) fed experimental chickens a commercial feed which had been associated with an outbreak of NE and challenged the birds orally with *C. perfringens* broth. They were able to reproduce NE and cause mortality in three out of 54 animals (5.6%) whereas 54 non-challenged birds fed the suspected feed remained healthy ($p=0.12$). They were unable to ascertain the exact composition of the feed.

Katich *et al* (1965) did not observe any mortality when experimental chicks were fed a diet consisting of 75% 'green food' and 25% concentrates mixed with a broth culture of *C. perfringens*, nor did any deaths occur when chicks had unlimited access to concentrates mixed with broth culture. When experimental animals received half of their normal ration of concentrates, inoculated with *C. perfringens* broth, and were kept at 6-10 °C and 85-90% humidity, two out of 50 animals died. The 48 remaining birds were dosed with opium tincture after which 15 more chicks died within five days. *C. perfringens* was isolated from all dead birds but post-mortem pathology did not fully resemble our case definition.

Baba *et al* (1992), using a NE model which consisted of oral administration with *Eimeria brunetti* oocysts prior to *C. perfringens* inoculation via the feed, found NE associated lesions in four out 20 birds which received zinc supplemented feed (1000ppm) whereas no NE lesions were observed in the twenty chickens which received non-supplemented feed (p=0.05). In a subsequent experiment, three out of ten experimental animals receiving zinc supplemented feed died with NE associated lesions whereas all ten birds remained alive in the non-supplemented group (p=0.11). However, the pathological signs of the dead birds did not fulfil our case definition. Long & Truscott (1976), inoculating birds with *C. perfringens* via the feed, found that the highest NE associated mortality was produced with 2% starch in the feed (four out of ten) compared to 0% starch (one out of ten), 1% starch (zero out of ten) or 5% starch (zero out of ten) (p=0.02).

Coccidia

Four Eimeria species were evaluated for their predisposing effect on an experimental NE infection. When challenging birds with *C. perfringens* inoculated feed for four (Al-Sheikhly & Al-Saieg, 1980) or five days (Shane *et al.*, 1985), the risk of NE associated mortality increased when preceded by an oral challenge with oocysts of *E. acervulina* (OR 6.66; 95% C.I. 3.52-12.61) (Figure 3. 4).

Study or sub-category	Treatment n/N	Control n/N	OR (fixed) 95% CI	Weight %	OR (fixed) 95% CI
Al-Sheikhly 1980	32/60	8/50		49.76	6.00 [2.41, 14.91]
Shane 1985	33/80	7/80		50.24	7.32 [2.99, 17.91]
Total (95% CI)	140	130		100.00	6.66 [3.52, 12.61]

Total events: 65 (Treatment), 15 (Control)
Test for heterogeneity: Chi² = 0.09, df = 1 (P = 0.76), I² = 0%
Test for overall effect: Z = 5.83 (P < 0.00001)

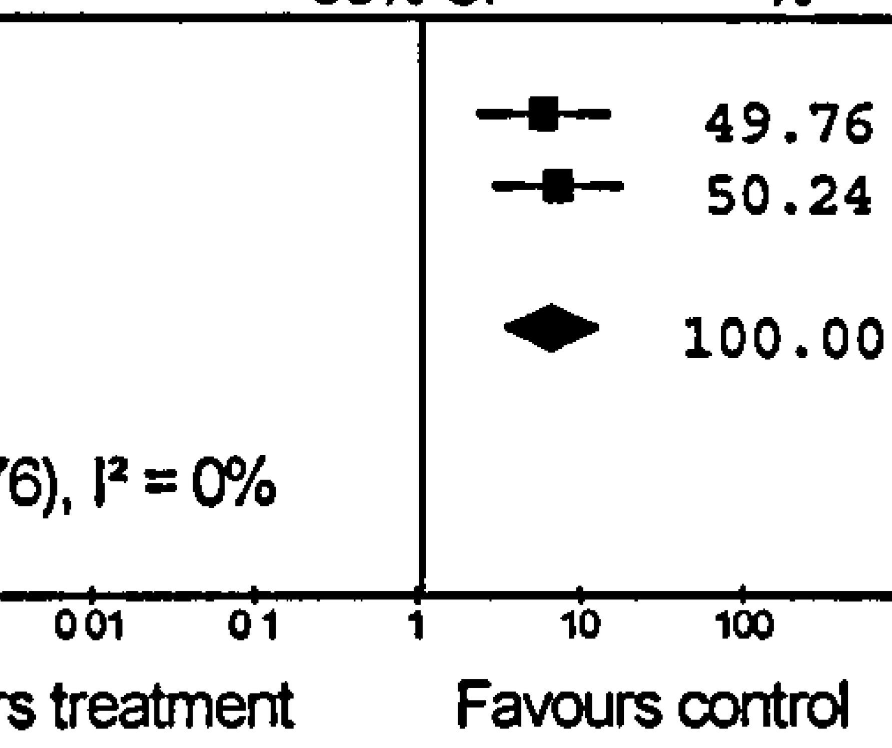

Figure 3. 4: Meta-analysis of studies comparing chickens challenged with E. acervulina and *C. perfringens* (treatment) to chickens challenged with *C. perfringens* alone (control), using NE associated mortality as an outcome.

Similarly, the proportion of deaths due to NE was increased when *C. perfringens* challenge was preceded with an infection with *E. necatrix* but this increase was not statistically significant (p=0.12) (Al-Sheikhly & Al-Saieg, 1980).

No NE associated pathology or mortality was observed when *C. perfringens* challenge via the feed was preceded by infection with *E. brunetti* (Baba *et al.*, 1992) unless accompanied by high levels of zinc in the feed (1000ppm), after which 20% of experimental animals developed lesions characteristic of NE (p=0.05).
Williams *et al* (2003) managed to produce NE lesions in 83% of birds examined (n=30) after intra-cloacal inoculation with *C. perfringens* and in 80% of birds (n=30) when inoculation was preceded by an oral challenge with *E. maxima* oocysts. However, mean daily lesions were not significantly higher for the birds receiving the combined coccidial-clostridial challenge compared to those receiving only *C. perfringens* except for on the eighth day after inoculation (which was also the last day of sampling) when all five sampled birds that had received a combined challenge had diffuse intestinal necrosis (lesion score 4) as compared to a mean lesion score of 1.6 in the group that received only *C. perfringens*.

Altering gut conditions

A combination of buffering agents such as sodium bicarbonate and peristalsis inhibitors such as opium have been used in attempt to create favourable conditions for the production of NE. By dosing the birds with a mixture of chalk, sodium bicarbonate and opium for seven days and challenging with *C. perfringens* on the second day, Parish (1961c) was able to produce classical signs of NE in two out five chickens (40.0%). When the same mixture was administered for only two days, no classical NE lesions developed. In a similar experiment, Bernier *et al* (1977) produced necrotic enteric lesions in all six experimental chickens after administration of sodium bicarbonate and opium for three days and challenge with *C. perfringens* on the third day. Four of the chickens died (66.7%). No NE was produced with sodium bicarbonate alone and only one out of six chickens (16.7%) died of necrotic enteritis when opium was administered by itself before *C. perfringens* challenge.

Katich *et al* (1965) did not observe significant mortality in birds inoculated with *C. perfringens* via the feed until they were treated with opium tincture when 15 of 48 birds died. However, the recorded pathological lesions were not consistent with the case definition of NE as formulated in chapter 2.

Antimicrobials

Long & Truscott (1976) tested the preventive effect of penicillin and chloramphenicol on NE by providing experimental animals either with 100,000 I.U. penicillin/litre or 110 mg chloramphenicol/litre in their water from eight hours prior to infection with *C. perfringens* via the feed until six days after the last batch of infected feed. None of the chickens that received penicillin died (n=190 in two separate trials) as compared to a mortality of 19% (n=190) in the control group ($p<0.001$). Twelve percent mortality (n=100) was observed in the group which received chloramphenicol whereas 25% (n=100) of the control birds died ($p=0.02$). The authors noted that the mortality was delayed in the chickens receiving chloramphenicol. The first deaths in this group did not occur until 48 hours after those in control birds.

Kaldhusdal & Hofshagen (1992), comparing diets high in barley to diets high in maize, did not find birds with intestinal necrotic lesions if the feed had been supplemented with avoparcin whereas six birds fed the unsupplemented barley feed (n=48) and one bird on the unsupplemented maize feed (n=48) developed intestinal lesions. However, lesions were small and circumscribed and although histologically necrotic in nature, macroscopically did not fit the description of field-type NE. This prompted the authors to name this condition sub-clinical NE.

Kaldhusdal *et al* (1999) tested the effects of supplementing feed with narasin and zinc bacitracin during the two weeks preceding a *C. perfringens* challenge. Although the onset of lesions was later in the group that received antibacterial compounds, the proportion of birds with lesions was actually higher (albeit not statistically significantly) in this group at the end of the study i.e. 20 days post-challenge (8/74 versus 4/80, p=0.18).

The effects of feed supplemented with narasin were also tested by Vissiennon *et al* (2000) but in contrast to the previous study, the supplemented feed was provided from birth until the end of the experiment. The authors also looked at the prophylactic effects of feed supplemented with avilamycin, monensin and the therapeutic effects of tylosin tartrate. Intra-duodenal inoculation of *C. perfringens* failed to elicit NE in these studies although mortality rates ranged from 16% to 36% in the non-medicated group in three separate trials. Medication with narasin, avilamycin and monensin delayed the onset of clinical signs for two to four days and reduced mortality to 0%-8%. Tylosin tartrate, which was administered via the drinking water after the first mortality also reduced total mortality to 0%-8%.

Other

Other factors which were investigated as to their role in NE pathogenesis included the strain of *C. perfringens*, the age of the experimental animal, temperature and humidity within the experimental set-up and anti-coccidial vaccination. Both Long & Truscott (1976) as Kaldhusdal *et al* (1999) found significant strain differences with regard to the amount of NE specific mortality which was produced although differences in challenge dose prevented Kaldhusdal *et al* (1999) from making meaningful comparisons. In addition, Long & Truscott (1976) suggested that strain virulence may be increased by repeated bird passage and re-isolation although the differences in reported mortalities were not always statistically significant.

Parish (1961c) found an effect of age. He could not reproduce NE in adult birds of five months of age after pre-treatment with opium and mineral salts, whereas 40% (2/5) of younger birds (age not specified) died of NE.

Katich *et al* (1965) suggested that low temperatures (6-10°C) and high relative humidities (85%-90%) contributed to increased NE associated mortality compared to a control group raised at normal temperatures (24-28°C) and normal relative humidities (40%-45%). The experimental group differed in so many other aspects from the control group (i.e. halved rations, administration of opium) that it is difficult to substantiate this conclusion.

Chickens vaccinated with an anticoccidial vaccine containing live, attenuated oocysts of *E. acervulina*, *E. maxima*, *E. mitis* and *E. tenella*, and challenged orally with *E. maxima* oocysts and *C. perfringens* via the cloaca had significantly less total daily mean lesions than similarly challenged animals who were not vaccinated (Williams *et al.*, 2003)

Experiments using SPF chickens

A series of experiments have been carried out with SPF chickens to reproduce NE but no comparisons were made with conventional chickens within these experiments (Balauca, 1976; Balauca *et al.*, 1976). Using SPF chickens, the authors looked at the effects of duration of challenge, wire cage versus floor husbandry, age of the experimental animal, and pre-challenge with coccidia. Treatments were often inconsistent across experimental groups making comparison difficult.

Balauca (1976) found that one oral dose of *C. perfringens* broth containing spores, vegetative cells and toxin was not sufficient to reproduce NE in seven day old SPF chickens kept in wire cages. When this challenge was preceded by four days with an oral dose of a mixture of three coccidial species (*E. acervulina*, *E. necatrix*, *E. mitis*), 48.5% (n=33) of the chicks died of NE and mortality was further increased when the *C. perfringens* broth was given for seven consecutive days directly after coccidia administration (62.5%; n=32)

When seven day old SPF chicks were kept on the ground and challenged for six days with *C. perfringens* spores and vegetative cells, NE associated mortality reached 40% (n=35) and increased to 60% (n=35) when the challenge dose included toxin (Balauca *et al.*, 1976). When a single dose of *C. perfringens* spores and vegetative cells was administered together with the same mixture of coccidial oocysts as described previously, NE associated mortality was 52.0% (n=25) in floor-kept SPF chickens but this time no mortality occurred in cage kept SPF chickens (n=25). When SPF chickens were challenged twice with *C. perfringens* and coccidia (on the first and the 10th day), a significantly higher mortality occurred in the cage-kept birds (32.0%; n=25) as compared to the floor-kept chickens (8.0%; n=25) (Balauca *et al.*, 1976).

Eight-week-old SPF chickens appeared to be less susceptible than seven day old SPF chicks. Challenging twice over a five-day period or challenging five times on alternate days produced a NE associated mortality of 4.7% in floor-kept birds (n=43; separate mortality figures for each challenge regime were not provided) and no mortality was observed in cage-kept chickens challenged with the latter regime (Balauca *et al.*, 1976). Preceding the clostridial challenge with an oral dose of the coccidal mixture did not cause mortality in either floor-kept or cage-kept birds when challenging twice over a five-day period. When floor-kept chickens were challenged five times on alternate days, with the coccidial mixture being administered on the first day, 32.0% (n=50) died of NE. This regime was not tried on cage kept birds.

Discussion

In contrast to the relative scarcity of observational field studies, a reasonable number of experimental studies designed to elucidate the pathogenesis and risk factors for NE have been published. Despite this wealth of information, only a small proportion of studies were useful for this review, largely due to the fact that the majority of papers lacked the essential component that in our view any paper attempting to reproduce a disease should contain; adequate diagnosis. In the same way that a complete description of naturally occurring NE should contain the clinical, pathological and histo-pathological signs, confirmation of experimentally produced disease should similarly be based upon these aspects. The most common reason to exclude a study from this review was because the paper failed to describe the histopathological features of the produced disease.

Despite these rather stringent inclusion criteria, the quality of the included papers according to the measures listed in Table 3. 1, was disappointing. None of the papers included calculations to determine the minimum number of experimental animals needed in each treatment group in order to have sufficient statistical power to demonstrate an effect. As a consequence, some experiments failed to produce statistically significant results. Experimental designs of the included papers were often inconsistent in that experimental groups differed with regard to more than one studied factor or because of a failure to include adequate control groups. None of the studies indicated that they scored the results blind, i.e. that they recorded the results within each experimental group without knowledge of the treatment that that group received. More than half of papers did not use a statistical test to analyse their results and of the eight studies that did, five failed to describe the statistical test, used an inappropriate test or analysed only parts of the results.

Attempts were made to combine study results which used similar inoculation routes and risk factors in order to come to a pooled measure of effect. Apart from different inoculation strategies, studies differed in various other aspects such as type of *C. perfringens* challenge strain, duration of challenge, age, breed and type of the experimental animals, housing, husbandry methods and dietary aspects. The time period over which the studies had been carried out ranged from 1961 to 2003 and arguably this time difference, with its corresponding changes in experimental material and conditions, also contributed to study heterogeneity. Although some of these factors, such as age and type of the chickens (i.e. conventional or SPF), were taken into consideration while combining the study results, most were not. Although this heterogeneity might well influence the size of the combined effect, it is unlikely to change the direction of the effect. The graphical output of Review Manager includes a chi-squared statistic to assess whether observed differences in results between studies are compatible with chance alone, where a low p-value provides evidence for heterogeneity. Also, a measure of inconsistency (I^2) is calculated which describes the percentage of variability in effect estimates that is due to heterogeneity rather than sampling error (Alderson *et al.*, 2004). None of these measures were significant in our meta-analysis.

Included studies also varied in terms of outcome. Whereas sudden death was an important sign in our case definition (Chapter 2), spontaneous mortality was not always an outcome. In some instances, this was because birds were euthanased before natural death occurred, but in others death simply did not occur in spite of intestinal lesions being consistent with those described in our case definition. In contrast, mortality on its own, without intestinal lesions suggestive of NE, also occurred. Finally, a mild form of NE was described, in which small necrotic lesions were present but the overall clinical, pathological and histo-pathological signs did not fulfil our case definition.

Results of the included studies demonstrate that experimental reproduction of NE is difficult and inconsistent. Direct oral inoculation of *C. perfringens* resulted in one case of NE among 158 experimental animals and no mortality whereas administering *C. perfringens* via the feed gave rise to NE associated mortalities ranging from 0% to 21%. The reasons for this difference are unknown but could be associated with a protective effect of the feed against the low gastric pH which is unfavourable for the development of *C. perfringens*. Indeed, Al-Sheikhly & Truscott (1977a) attributed their ability to elicit field-type NE lesions in 100% of the birds after intra-duodenal inoculation of *C. perfringens* broth to the by-passing of the low pH of the gizzard. However, Vissiennon *et al* (Vissiennon *et al.*, 1994a,b; 2000) were unable to produce NE through intra-duodenal inoculation although reportedly using an enterotoxin-negative, alpha-toxin producing strain. The clinical disease described in their papers perhaps more closely resembles that as reported for infections with enterotoxin producing *C. perfringens* (Niilo, 1976; 1980).

Two other methods for the experimental reproduction of NE which have been included in this review, intra-venous inoculation (Bernier *et al.*, 1977) and intra-cloacal inoculation (Williams *et al.*, 2003), have not been used widely. In an earlier experiment (Bernier *et al.*, 1974), 100% mortality (n=5) was reported after intra-venous inoculation of 0.7ml *C. perfringens* culture but no pathology was described. Bartalos *et al* (1976) inoculated an unspecified number of chickens with supernatant of a *C. perfringens* broth culture by intra-venous injection. This resulted in one death on the second day after injection but no pathology characteristic of NE was observed. Niilo (1976) injected enterotoxin of *C. perfringens* type A intra-venously, again resulting in mortality but no NE associated lesions. Although the experiments of Bernier *et al* (1977) satisfy Koch's postulates, the exact mechanism of disease causation through this method has not been elucidated and it does little to clarify NE pathogenesis under natural circumstances.

The use of intra-cloacal inoculation to produce NE (Williams *et al.*, 2003) has, to our knowledge, not been described elsewhere. The authors assert that theirs could be a suitable repeatable challenge model but arguably a NE model that fails to induce mortality in its experimental subjects eight days post-challenge does not completely represent naturally occurring NE where sudden death is a constant feature.

Coccidiosis has long been implicated as a risk factor for NE outbreaks in the field and experimental studies seem to confirm this in the case of *E. acervulina* (OR 6.66; 95% C.I. 3.52-12.61)(Al-Sheikhly & Al-Saieg, 1980; Shane *et al.*, 1985). The results of experiments using *E. necatrix* as a predisposing factor showed an increased, albeit statistically insignificant, risk (Al-Sheikhly & Al-Saieg, 1980). *E. brunetti* did not increase the risk for experimentally produced NE unless it was accompanied by high levels of zinc in the feed (Baba *et al.*, 1992). The authors attributed these findings to the ability of zinc to protect alpha-toxin from degradation by trypsin but more current insights suggest that it can be explained by the fact that alpha-toxin is a zinc-metalloenzyme (Titball *et al.*, 1999). Experiments with *E. maxima*, like those with *E. necatrix* and *E. brunetti*, failed to provide conclusive evidence that it has a significant role to play in the pathogenesis of NE. Although the highest average lesion score was eventually achieved in those birds which were challenged with both *E. maxima* and *C. perfringens*, the proportion of birds with lesions was actually higher in those which only received *C. perfringens* (Williams *et al.*, 2003).

There exists some evidence that timing of the administration of the coccidial oocysts in relation to when the clostridial dose is given is important, as it is hypothesized that the mucosal insult caused by the coccidial infection leads to the establishment and proliferation of *C. perfringens* at the damaged site (Al-Sheikhly & Truscott, 1977c).

Shane *et al* (1985) recorded the highest incidence of NE lesions and NE associated mortality when an *E. acervulina* challenge preceded the clostridial challenge by two days and slightly lower incidences were achieved when coccidia and clostridia were given simultaneously. No statistically significant increase in NE occurrence was observed when *E. acervulina* oocysts were given four days prior to the *C. perfringens* inoculation compared to giving *C. perfringens* alone. Similarly, Al-Sheikhly & Al-Saieg (1980) found a significant increase in NE associated mortality when experimental animals were dosed with *E. acervulina* two days before *C. perfringens* challenge whereas no statistical significant increase was observed when *E. necatrix* was given four days prior to the clostridial challenge. In contrast, experiments using SPF chickens found that when a coccidial mixture of *E. acervulina*, *E. necatrix* and *E. mitis* was administered four days prior to a clostridial challenge, NE associated mortality in seven-day old cage-kept birds was significantly increased compared to a clostridial challenge by itself (Balauca, 1976) but when *C. perfringens* and the coccidial mixture were administered simultaneously no mortality occurred (Balauca *et al.*, 1976). Differences in the number of challenge days could possibly account for these contrasting experimental results; whereas chickens were challenged with *C. perfringens* on only one day in the studies by Balauca (1976) and Balauca *et al* (1976), Shane *et al* (1985) challenged with *C. perfringens* for four days and Al-Sheikhly & Al-Saieg (1980) for five days.

Feed components are regularly implicated as a cause for NE although this review has demonstrated that the evidence from well-designed experimental studies is scarce. A diet based on wheat is generally considered to be a large risk factor for the disease and one of the included papers in this review (Branton *et al.*, 1987) is frequently cited as evidence for this. Although the results of that study were a chance finding, as the experiment was designed to determine methods of incorporating wheat into broiler diets, significant differences in NE associated mortality were found between experimental groups fed diets based on maize or wheat, and diets based on roller mill-ground wheat or hammer mill-ground wheat. Similarly by chance, Kaldhusdal & Hofshagen (1992) found an association between diets high in barley and intestinal necrotic lesions, but the clinical and pathological signs of this disease do not fit our case definition. There clearly exists a need to repeat these types of experiments under more controlled conditions or to gather good dietary data from natural outbreaks of disease. Indeed two papers have specifically attempted to do so (Riddell & Kong, 1992; Branton *et al.*, 1997) but neither of them fulfilled the inclusion criteria for this review.

Another dietary ingredient, which is routinely associated with an increased risk for NE, is fish meal. This is often based on a field observation which linked rations containing 13-19% fish meal with an increased occurrence of gizzard erosion (Johnson & Pinedo, 1971). Coincidentally, the authors noted that three of the five observed flocks also displayed NE. Few experimental studies have been conducted to test the association between fish meal and NE (Kubena *et al.*, 1976; Truscott & Al-Sheikhly, 1977) and none were eligible for inclusion in this review. Yet a large number of papers use experimental NE models which incorporate dietary fish meal (Wijewanta & Senevirtna, 1971; Prescott *et al.*, 1978; Prescott, 1979; George *et al.*, 1982; Cowen *et al.*, 1987; Kling & Quarles, 1995; Das *et al.*, 1997; Hofacre *et al.*, 1998a; Hofacre *et al.*, 1998b; Hofacre *et al.*, 1999; Brennan *et al.*, 2001a; Brennan *et al.*, 2001b; Brennan *et al.*, 2003; Hofacre *et al.*, 2003; Williams *et al.*, 2003) but all of them lack adequate control groups to test the actual role of fish meal in the pathogenesis of NE.

Both Parish (1961c) and Bernier *et al* (1977), by dosing with opium and sodium bicarbonate, make it plausible that decreased intestinal motility and increased intestinal pH increase the risk of NE, possibly by favouring the establishment and multiplication of *C. perfringens*. Shane *et al* (1985), investigating the role of coccidiosis in the pathogenesis of NE, demonstrated an increased intestinal passage time five days after oral inoculation with *E. acervulina* oocysts, coinciding with an increased occurrence of NE when the birds were also dosed with *C. perfringens*. However, they also found a decreased pH in the duodenum and jejunum, attributed to damage to the mucosa by sexual stages of *E. acervulina*, followed by loss of serum protein into the intestinal lumen and subsequent degradation of protein into amino acids by proteolytic enzymes.

Several studies demonstrated an effect of age (Parish, 1961c; Balauca *et al.*, 1976) in that it was more difficult to reproduce NE in older birds. To what extent this is associated with age-dependent resistance and/or acquired immunity is unclear. In contrast, Vissiennon *et al* (1994a) were unable to elicit disease in two day old chicks after intra-duodenal inoculation of *C. perfringens*. It has been suggested that aerobic conditions in the gut of the new-borne chick preclude clostridial infections at this age and that the anaerobic conditions necessary for the establishment of *C. perfringens* do not occur until several days after birth.

The prophylactic treatment efficacy of penicillin was demonstrated by Long & Truscott (1976) whereas chloramphenicol, although significantly reducing NE associated mortality, did not completely prevent it. Other studies on antimicrobial treatment included in this review were more difficult to assess because of the difficulties encountered in reproducing NE. Both Kaldhusdal & Hofshagen (1992) and Vissiennon *et al* (2000) produced disease which did not agree with our case definition and as such the prophylactic effects on NE of the anti-microbial compounds which these studies tested, cannot be determined. Kaldhusdal *et al* (1999), evaluating the preventive effects of narasin and zinc bacitracin administered prior to *C. perfringens* challenge, failed to demonstrate a significant effect. However, their experimental model was inconsistent in producing NE and suffered from obvious wild-type infections in the control groups.

Conclusions

Implications for disease control

Coccidiosis has been implicated as an important risk factor for NE in observational studies. This review has confirmed that in the case of *E. acervulina*, yet for other coccidial species (*E. necatrix*, *E. brunetti*, *E. maxima*) the evidence is inconclusive. Similarly, wheat was a strong risk factor in one experimental study but this needs to be confirmed in future controlled trials as does the role of the milling process. Fish meal is also often associated with the occurrence of NE but this seems to be based on few experimental studies, none of which could be included in this review.

Implications for research

Research into the pathogenesis and risk factors of NE has been complicated by a lack of suitable experimental models. On the basis of this review, a model consisting of the inoculation of *C. perfringens* via feed, preceded by an oral challenge with *E. acervulina* combines reasonable consistent disease production with relative simplicity and non-invasiveness. Other models which have been utilised are deficient in one or both of these aspects. There is a need to standardise these experimental studies in order to achieve a basic scientific quality of the individual papers and to facilitate the conduct of future systematic reviews.

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Chapter 4. The epidemiology of necrotic enteritis on broiler farms in the UK[1]

Summary

In order to determine the prevalence and risk factors for necrotic enteritis in broilers, a cross-sectional survey was conducted among 857 farms, rearing broilers for nine UK poultry companies. The main data collection tool was a postal questionnaire directed at farm managers. Additional information on disease occurrence on the farm was collected from veterinary post-mortem reports. The response rate to the questionnaire was 75%, ranging from 54% to 90% within companies. During 2001, 32.8% of the respondents indicated that they had had a case of necrotic enteritis (95% C.I. 29.1 – 36.8) in at least one crop. The disease was most often reported during the months October to February. Farm manager reported point prevalence (necrotic enteritis occurrence in the most recently cleared crop) was 12.3% (95% C.I. 9.8 – 15.2). Multilevel logistic regression with poultry company as random effect was performed using the occurrence of necrotic enteritis in the farm's most recently cleared crop as dependent variable. Strong associations were found between the outcome variable and the occurrence of wet litter (O.R. 2.39; 95% C.I. 1.27-4.52; p=0.007) and coccidiosis (O.R. 4.68; 95% C.I. 1.74-12.55; p=0.002). In addition, the use of ammonia as a disinfectant for coccidial oocysts appeared to be an independent risk factor (O.R. 3.44; 95% C.I. 1.53-7.71; p=0.003). Finally, the positive association between the use of plasterboard walls in poultry houses and the occurrence of necrotic enteritis might point to an important role of cleaning and disinfection in the epidemiology of this disease (O.R. 3.72; 1.38-10.00; p=0.009).

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[1] Avian Pathology (accepted for publication)

Introduction

Necrotic enteritis (NE) is an acute, often fatal disease of chickens, characterised by depression, loss of appetite and sudden death. Since its first description in 1961, (Parish, 1961a,b,c), it has subsequently been reported from many different regions in the world including Australia (Gardiner, 1967; Nairn & Bamford, 1967), North America (Bernier & Filion, 1971; Helmboldt & Bryant, 1971), South America (Baldassi *et al.*, 1995), Europe (Jylling & Mørch, 1969; Köhler *et al.*, 1974), Asia (Kwatra & Chaudhury, 1976; Oda *et al.*, 1977; Tsai & Tung, 1981) and Africa (Dosoky, 1990; Rahamathulla *et al.*, 1994).

Until recently, NE has been controlled rather effectively by the presence of anti-microbial growth promoters in the feed. Concerns about increased antibiotic resistance in human pathogens have led to restrictions in the use of these compounds in animal feed. Consequently, it has been a common perception in many countries that NE is a re-emerging disease although few studies have been done to determine the prevalence or incidence of the disease, in the past or at present. Frequency measures that have been obtained, were derived by evaluating consignments of chickens submitted to diagnostic laboratories (Long, 1973; Bernier *et al.*, 1974; Köhler *et al.*, 1977; Kralj *et al.*, 1979; Kaldhusdal & Skjerve, 1996) and as such did not study representative samples of the total population at risk.

Much work has been carried out to elucidate the pathogenesis and risk factors of NE, usually by experimental studies aimed at reproducing the disease. However, reproduction of NE is difficult and inconsistent (Chapter 3) and as a consequence these studies are often inconclusive and risk factors remain ill-defined. Moreover, there exists a need to study the determinants of NE in the field as findings from earlier studies may not be applicable to modern husbandry systems. In this paper, the results of a cross-sectional study are described which had as objectives to estimate the prevalence of NE in a representative sample of broiler farms in the UK and to determine the risk factors associated with its occurrence under natural conditions.

Materials and Methods

Study population

The eleven major poultry companies producing broilers in the UK were approached through their company veterinarian and asked if they were willing to participate in a study on NE. Nine companies expressed an interest and meetings were arranged with the agricultural managers and veterinarians of these companies to discuss study objectives, study design, and confidentiality issues. All nine companies agreed to participate, comprising a total study population of 942 broiler producing farms.

Sample size

Total sample size was obtained by stratified random sampling based on an expected prevalence of necrotic enteritis of 5% with 95% confidence limits and 1% precision. The number of farms per company, calculated sample size and final sample size are shown in Table 4. 1. The final sample size was different from the calculated sample size either because some of the selected farms had ceased operations by the time our survey started or because some selected farms with multiple sites appeared as separate sampling units on the sampling list but only one questionnaire was sent to the farm as a whole.

Table 4. 1: Sampling frame and sample size for a cross-sectional study of NE.

Company	No. of farms per company	Calculated sample size	Final sample size
1	31	31	29
2	38	38	38
3	121	114	109
4	291	252	252
5	86	83	82
6	94	90	90
7	109	103	97
8	93	89	85
9	79	76	75
Total	**942**	**876**	**857**

Data collection

Questionnaire

Data were collected through a postal questionnaire directed at farm managers. Questionnaire design was accomplished with the help of the agricultural managers and veterinarians of participating companies and researchers with experience in poultry health questionnaire design. To avoid any bias associated with focussing the questionnaire on necrotic enteritis, it was left out of the title and was not the first topic in the questionnaire. The questionnaire contained eight sections dealing with disease occurrence on the farm during the year 2001 and in particular in the last crop to be reared and finished on that farm prior to the farm-manager responding to the questionnaire. Data were collected on the characteristics of the farm (size, age, intensity of production), the crop (size, breed, performance), climate control, hygiene and biosecurity, feeders and feeding practices, water and drinkers, and disease management. In total there were 206 questions of which 108 (52%) were closed, 59 (29%) were open-ended and 39 (19%) were semi-open questions (a close-ended question with the addition of a category 'other – please specify').

The questionnaire was pre-tested by sending it to the farm managers and area managers of 26 farms, which were not part of the sample population, selected at random from the seven largest companies. On the basis of the responses received to the pilot questionnaire, it was judged that for most questions the farm managers were better informed than the area managers. Copies of the final questionnaire with covering letters were sent to the farm managers of the 857 selected farms during the first week of February 2002. In order to maximise response rates, a reminder card was sent four weeks after the questionnaire was posted and a second questionnaire was mailed to all non-respondents eight weeks after the first one. The final deadline for the receipt of questionnaires was set at 20 May 2002. A copy of the cover letter, questionnaire, reminder card and reminder letter can be found in Appendices 1 to 4, respectively.

Post-mortem reports

In order to validate the disease occurrence on the farm, veterinary practitioners were sent lists of the farms that had returned their questionnaire with the corresponding placement dates of the farms' most recently cleared crop. They were asked to submit post-mortem or other veterinary examination reports that were carried out on the crop during the specified time period.

Non-response

Farm managers whom had not returned their questionnaire by the end of May were contacted by telephone and inquired as to their reasons for not responding. In addition, they were asked the size of their most recently cleared crop and the number of houses on the farm. This latter information was used to determine any indication of non-response bias. A copy of this brief telephone questionnaire can be found in Appendix 5.

Statistical analysis.

The questionnaire data were entered by a commercial company into a Microsoft Access database and checked and corrected. The statistical packages that were used for data analysis were EpiInfo Version 6 (CDC, Atlanta, Georgia, USA) and Stata 7 (Stata Corporation, College Station, Texas, USA). The farm was the unit of study and the outcome variable was NE in the farm's last cleared crop.

Associations between independent dichotomous variables and the outcome variable were analysed with 2x2 contingency tables and tested for significance with the chi-squared test or Fisher's exact test. Independent categorical variables were analysed in univariate logistic regression models, by creating dummy variables. Continuous variables were categorized according to their quartile values and analysed as categorical variables. In addition,. the best fitting forms of the continuous variables were assessed in a univariate logistic regression model, using the following fractional polynomials: -2, -1, -0.5, 0, 0.5, 1, 2, 3 (Royston *et al.*, 1999).

Variables with a statistical significance of $p \leq 0.25$ in the univariate analysis were tested in a multilevel logistic regression model with poultry company as a random effect. The model was built manually, starting with variables with the lowest p-values in the univariate analysis. Variables with more than 10% missing values were not included to avoid numerical instability of the model. Variables that were highly correlated (correlation coefficient >0.7) were not included in the model together to avoid problems with collinearity. Whenever this created a conflict, the variable that produced the best model fit (lowest deviance) was kept in the model. Variables with a term-wise Wald test p-value ≤0.05 or variables that significantly improved the model fit (likelihood ratio chi squared statistic p≤0.05) were retained in the model. Confounding was assessed by examining the estimates of the coefficients of the individual variables in the model. If these estimates changed considerably (>25%) after entry or removal of a variable, this variable was considered a confounder and was retained in the model. Interaction terms were tested for all variables left in the final model and for those that seemed biologically plausible.

In the output of its random effects models, Stata includes a variable rho which represents the proportion of the total variance attributed to the random effect. Using likelihood ratio tests (LRT), the null hypothesis of rho=0 can be tested.

Stata uses Gauss-Hermite quadrature with 12 quadrature points as default to compute the log likelihood in its random effects models. To assess the soundness of the quadrature approximation, the model was re-estimated using two different numbers of quadrature points (8 and 16) and the log likelihood and coefficient estimates for the original model and the two re-estimated models were compared. A relative difference of less than 10^{-2} (1%) between the estimated coefficients was taken as evidence that the number of quadrature points did not significantly affect the outcome[1].

Because model diagnostic tests are not available for random effects models, model fit was assessed by constructing a standard non-hierarchical logistic regression model with the variables that remained in the final random effects model and determining the Pearson chi-squared statistic, Hosmer-Lemeshow chi-squared statistic, and the area under the receiver operating characteristic (ROC) curve.

[1] Stata Reference Manual, Release 6, Volume 3 P-St, pp116-121, Stata Press, College Station, Texas.

Results

Questionnaire response

Response rate

During the specified survey period (8 February '02 – 20 May '02), 640 completed questionnaires (75%) were returned. Response rates within individual companies ranged from 56% to 90%. Questionnaires that came from non-conventional farms (i.e. organic, free-range) were excluded from the analysis and this left 603 completed questionnaires, giving a useable response rate of 70%.

Non-response

Five questionnaires were received after the deadline date of the 20th of May and these were neither included in the response rate nor in the analysis. Telephone numbers were obtained for the remaining 212 farms that had not returned their questionnaire and 124 (59%) were successfully contacted. Of these, 4 (3%) declined to answer questions over the phone. Twelve (9.7%) farm managers indicated they never received a questionnaire whereas fifteen (12.1%) maintained they had completed and returned it. 'Being too busy' was the most common reason for not returning the questionnaire (n=52; 41.9%), followed by 'finding the questionnaire too long and complicated' (n=12; 9.7%) and 'losing or misplacing the questionnaire' (n=7; 5.6%).

The average size of the most recently cleared crop of the non-responding farms was 63690 broilers and significantly smaller ($z=6.36$, $p<0.001$) than the average crop size of the responding farms (103332 broilers). Similarly, average number of houses on the non-responding farms was significantly less ($z=5.40$, $p<0.001$) compared to the number of houses on farms that returned a questionnaire (3.5 and 5.1, respectively).

Descriptive epidemiology

In 2001, 32.8% of the respondents indicated that they had had a case of NE (95% C.I. 29.1 – 36.8) in at least one crop. The median number of crops affected was 2 (I.Q.R. 1 –3). The monthly frequency distribution of farm manager reported NE is shown in Figure 4. 1. NE occurred significantly more frequently during the months October to March than during the months April to September ($z=10.1$, $p<0.001$).

Farm manager reported point prevalence (NE occurrence in the most recently cleared crop) was 12.3% (95% C.I. 9.8 – 15.2), and the frequency distribution by poultry company is shown in Figure 4. 2. NE occurred at a median age of 26 days (I.Q.R. 20–30) and was reported as early as 10 days of age and as late as 49 days.

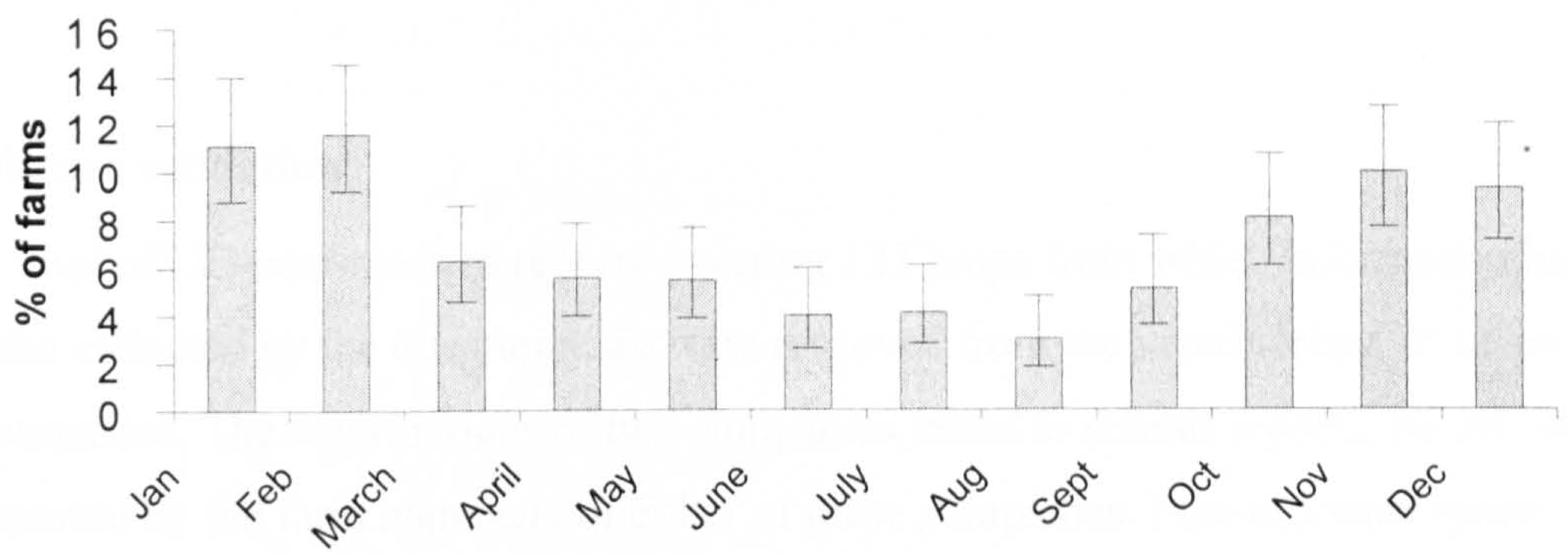

Figure 4. 1: Monthly distribution of farmer reported necrotic enteritis cases during 2001 (error bars represent 95% confidence intervals).

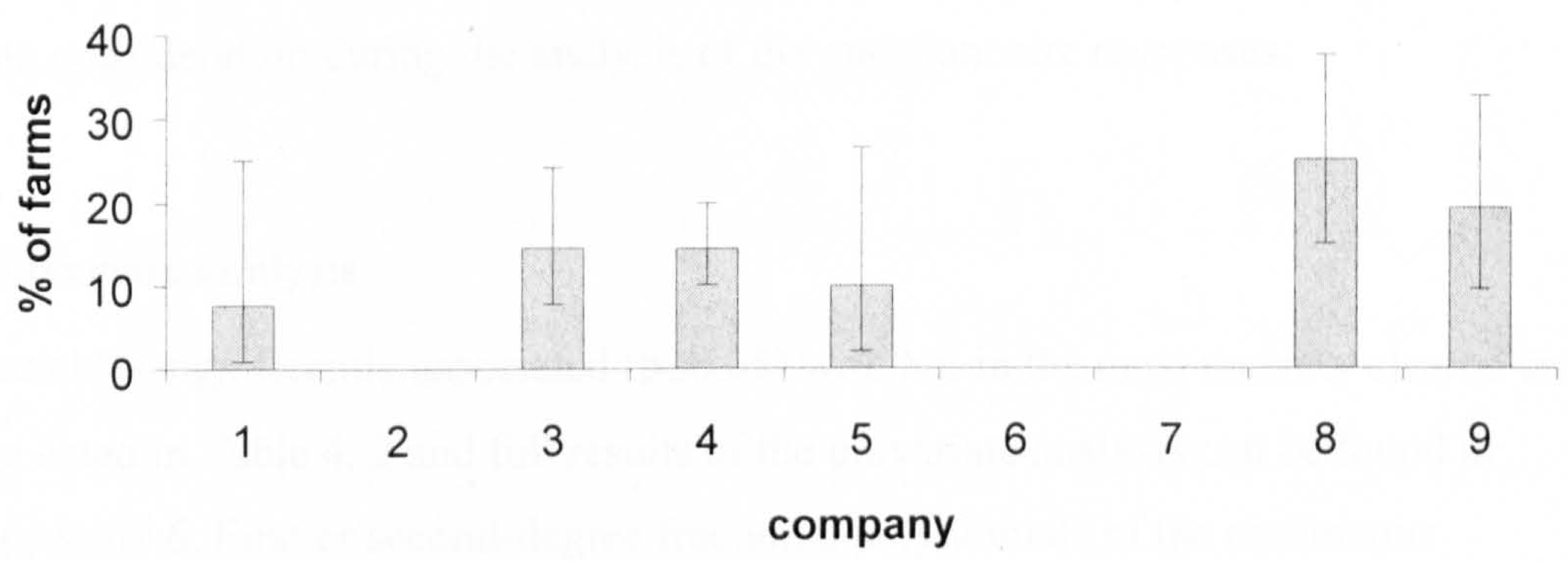

Figure 4. 2: Prevalence of farmer reported necrotic enteritis cases in nine UK broiler companies (error bars represent 95% confidence intervals).

In the majority of the cases of NE, the veterinarian was involved in making a diagnosis (56.8%; 95% C.I. 44.7 – 68.2). In 43.2% of the cases he did this by himself, or otherwise in collaboration with the farm manager (8.1%), the area manager (1.4%) or both (4.1%).

Almost all cases of NE in the last flock (90.5%; 95% C.I. 81.5 – 96.1) were treated therapeutically; the use amoxycillin or tylosin were the only reported treatments. Amoxycillin was most frequently used (87.9% of all treated cases).

Disease validation

A total of 183 post-mortem reports covering 123 crops from which information had been collected by the questionnaire were retrieved from the veterinarians of seven companies. The veterinarians of two companies failed to submit reports. No NE was reported by the farm managers of either of these companies. Post-mortem reports were available for 33 of the 74 crops with farm manager reported NE but only 19 of these were carried out at a similar time (within five days) during the grow-out period as when NE had reportedly occurred. Eleven of these post-mortem examinations confirmed NE whereas eight reports made no mention of the disease. Five post-mortem reports carried a diagnosis of NE where the farm manager had not reported this in the questionnaire. The results of the disease validation exercise was not taken into consideration during the analysis of the questionnaire responses.

Univariate analysis

Variables significantly associated ($p \leq 0.05$) with NE in the most recently cleared crop are listed in Table 4. 2 and full results of the univariate analysis can be found in Appendix 6. First or second-degree fractional polynomials of the continuous variables are shown if these significantly decreased the deviance ($p \leq 0.05$) of the univariate logistic regression model. On the basis of these variables, a causal web was constructed which was used to guide the building of a multivariable model and to generate hypotheses (Figure 4. 3).

Univariate analysis showed strong associations with disease, with both wet litter and coccidiosis increasing the odds of NE occurrence. Similarly, there were positive associations with increased mortality and curative antibiotics use. Preventive antibiotics did not decrease the odds of NE.

There was some evidence that increased farm size increased the likelihood of NE occurrence in that an increased number of chickens placed in the last crop, an increased number of hatcheries supplying the chicks, and an increased number of feed lorries coming onto the farm all increased the odds of NE.

A wide variety of variables related to hygiene and biosecurity were associated with NE occurrence. These included preventive effects of having separate farm clothing, separate farm boots, and hand-washing facilities available in each shed as well as having a turn-around time (i.e. the time period between the last chickens leaving the farm for slaughter and new chicks coming unto the farm) of greater than 14 days, which might allow for more effective cleaning and disinfection schedules. Cleaning and disinfection carried out by the farm manager as opposed to contract cleaners decreased the odds for NE whereas cleaning carried out with a dry brush or compressed air compared to water and detergent increased the odds of NE as did using ammonia as a disinfectant. Other associations between biosecurity variables and NE were less apparent such as the preventive effects of having cattle on the farm and spreading cow manure on fields on and adjoining the farm.

Stress may be involved in the occurrence of NE in that associations were found between NE and the type of lighting in the shed and feed equipment failures. Finally, one certain type of broiler breed was at an increased risk for NE.

Table 4. 2: Univariate associations (p≤0.05) between the occurrence of necrotic enteritis and explanatory variables.

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Disease					
Wet litter in the last crop					
	Yes	54/277 (19.5)	2.82	1.56–5.13	0.002
	No	19/240 (7.9)	Ref.	-	-
Coccidiosis in the last crop					
	Yes	12/25 (48.0)	6.63	2.66-16.51	<0.001
	No	59/483 (12.2)	Ref.	-	-
Environment					
Use of light bulbs					
	Yes	13/144 (9.0)	0.52	0.26-1.02	0.04
	No	60/375 (16.0)	Ref.	-	-
Light other than light bulbs, fluorescent tubes or low-energy bulbs					
	Yes	3/6 (50.0)	6.34	0.83-48.03	0.01
	No	70/514 (13.6)	Ref.	-	-
Number of houses on the farm (*continuous*)		-	1.03	0.96-1.10	0.43
First degree fractional polynomial: -2		-	0.99	0.97-1.00	0.05
Age of the newest house (*continuous*)			1.01	0.99-1.03	0.51
Second degree fractional polynomial:	0.5	-	0.02	0.001-0.22	0.002
	0.5	-	5.82	1.96-17.29	0.002
Husbandry					
Breed A used during the last crop					
	Yes	69/426 (16.2)	3.29	1.28-10.74	0.009
	No	5/90 (5.6)	Ref.	-	-
Chickens were grown separated by sex					
	Yes	53/329 (16.1)	1.84	0.97-3.53	0.05
	No	15/158 (9.5)	Ref.	-	-
Number of chicks placed in the last crop					0.11[1]
	8300-46500	11/131 (8.4)	Ref.	-	-
	47000-90000	19/124 (15.3)	1.97	0.90-4.34	0.09
	90200-144000	21/124 (16.9)	2.22	1.02-4.83	0.04
	144200-582260	23/131 (17.6)	2.32	1.08-4.99	0.03
Number of chickens placed in the last crop (*continuous, per 10000*)		-	1.03	1.00-1.05	0.05
Number of hatcheries that supplied the chicks for the last crop (*continuous*)		-	1.46	1.05-2.05	0.03
Age at slaughter (days) of last crop (*continuous*)		-	1.04	1.00-1.09	0.05
Hygiene & Biosecurity					
Who cleaned the houses					0.07[1]
	Contract cleaner	57/329 (17.3)	Ref.	-	-
	Company cleaner	4/36 (11.1)	0.60	0.20-1.75	0.35
	Farm manager	11/120 (9.2)	0.48	0.24-0.95	0.04
How were the houses cleaned					0.12[1]
	With water and detergent	63/431 (14.6)	Ref.	-	-
	With water	6/45 (13.3)	0.90	0.37-2.21	0.82
	Dry brush and/or compressed air	3/6 (50.0)	5.84	1.15-29.59	0.03
Who disinfected the houses					0.04[1]
	Contract cleaner	57/325 (17.5)	Ref.	-	-
	Company cleaner	4/42 (9.5)	0.49	0.17-1.44	0.20
	Farm manager	12/129 (9.3)	0.48	0.25-0.93	0.03

Table 4. 2 (continued)

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Hygiene & Biosecurity					
Length of turn-around (days) before last crop					0.02[1]
	1-6	22/129 (17.1)	Ref.	-	-
	7-9	19/145 (13.1)	0.73	0.38-1.43	0.36
	10-12	23/118 (19.5)	1.18	0.62-2.25	0.62
	14-62	9/125 (7.2)	0.38	0.17-0.86	0.02
Length of turn-around (days) before last crop (*continuous*)		-	0.97	0.93-1.02	0.25
Second degree fractional polynomial:	3	-	0.75	0.60-0.94	0.01
	3	-	1.18	1.04-1.34	0.009
Use of ammonia as a disinfectant before the last crop					
	Yes	14/40 (35.0)	3.60	1.66-7.74	<0.001
	No	56/430 (13.0)	Ref.	-	-
Were bacterial counts taken from inside house					
	Yes	18/211 (8.5)	0.41	0.22-0.76	0.002
	No	52/283 (18.4)	Ref.	-	-
Are hand wash facilities available in each house					
	Yes	17/182 (9.3)	0.47	0.25-0.88	0.01
	No	55/308 (17.9)	Ref.	-	-
Are shower facilities available					
	Yes	17/73 (23.3)	2.09	1.08-4.02	0.02
	No	57/449 (12.7)	Ref.	-	-
Is separate clothing available for each house					
	Yes	10/151 (6.6)	0.32	0.15-0.67	<0.001
	No	60/327 (18.3)	Ref.	-	-
Are farm boots available for each house					
	Yes	24/240 (10.0)	0.49	0.28-0.88	0.01
	No	40/218 (18.3)	Ref.	-	-
Are there cattle on the farm					
	Yes	11/128 (8.6)	0.51	0.24-1.04	0.04
	No	61/389 (15.7)	Ref.	-	-
Litter disposal other than on the field, deep stacked, power plant or mushroom composted					
	Yes	8/27 (29.6)	2.73	1.15-6.49	0.02
	No	66/494 (13.4)	Ref.	-	-
Is manure spread on fields of the farm					
	Yes	16/167 (9.6)	0.54	0.29-1.01	0.04
	No	56/342 (16.4)	Ref.	-	-
Is cow manure spread on fields of the farm					
	Yes	12/142 (8.5)	0.47	0.23-0.95	0.02
	No	59/361 (16.3)	Ref.	-	-
Is human sludge spread on fields of the farm					
	Yes	2/4 (50.0)	6.19	0.44-86.76	0.04
	No	69/499 (13.8)	Ref.	-	-
Is cow manure spread on fields adjoining the farm					
	Yes	26/244 (10.7)	0.55	0.31-0.97	0.03
	No	42/237 (17.7)	Ref.	-	-
Lorries other than feed, hatchery, collection, gas, oil, straw/shavings lorries coming onto the farm					
	Yes	23/111 (20.7)	1.84	1.01-3.31	0.03
	No	47/377 (12.5)	Ref.	-	-

Table 4. 2 (continued)

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Feed and feeding					
Were there any feeding equipment failures during the last crop					
	Yes	35/191 (18.3)	1.70	1.00-2.90	0.04
	No	38/326 (11.7)	Ref.	-	-
Whole wheat was blended in the mill					
	Yes	17/150 (11.3)	0.50	0.26-0.94	0.02
	No	52/256 (20.3)	Ref.	-	-
Number of feed lorries coming on to the farm during the last crop					0.02[1]
	1-10	8/119 (6.7)	Ref.	-	-
	11-17	19/126 (15.1)	2.46	1.03-5.87	0.04
	18-26	21/114 (18.4)	3.13	1.33-7.40	0.009
	27-96	23/127 (18.1)	3.07	1.31-7.16	0.01
Number of feed lorries entering farm during last crop (*continuous*)		-	1.02	1.00-1.03	0.03
First degree fractional polynomial: -1		-	0.41	-0.21-0.82	0.01
Prevention/treatment					
Were preventive antibiotics used					
	Yes	45/268 (16.8)	1.81	1.00-3.28	0.03
	No	21/209 (10.0)	Ref.	-	-
Were curative antibiotics used					
	Yes	61/193 (31.6)	22.27	9.34-64.66	<0.001
	No	6/197 (2.0)	Ref.	-	-
Were competitive exclusion products (CE) used					
	Yes	2/56 (3.6)	0.19	0.03-0.83	0.01
	No	65/398 (16.3)	Ref.	-	-
Flock performance					
Total mortality (%) during the last crop					0.009[1]
	0.20-2.63	8/120 (6.7)	Ref.	-	-
	2.65-3.44	21/122 (17.2)	2.91	1.23-6.86	0.02
	3.45-4.24	18/121 (14.9)	2.45	1.02-5.87	0.05
	4.25-11.80	24/113 (21.2)	3.78	1.62-8.81	0.002
Total mortality (%) during last crop (*continuous*)		-	1.26	1.07-1.48	0.005

1. Likelihood ratio test statistic

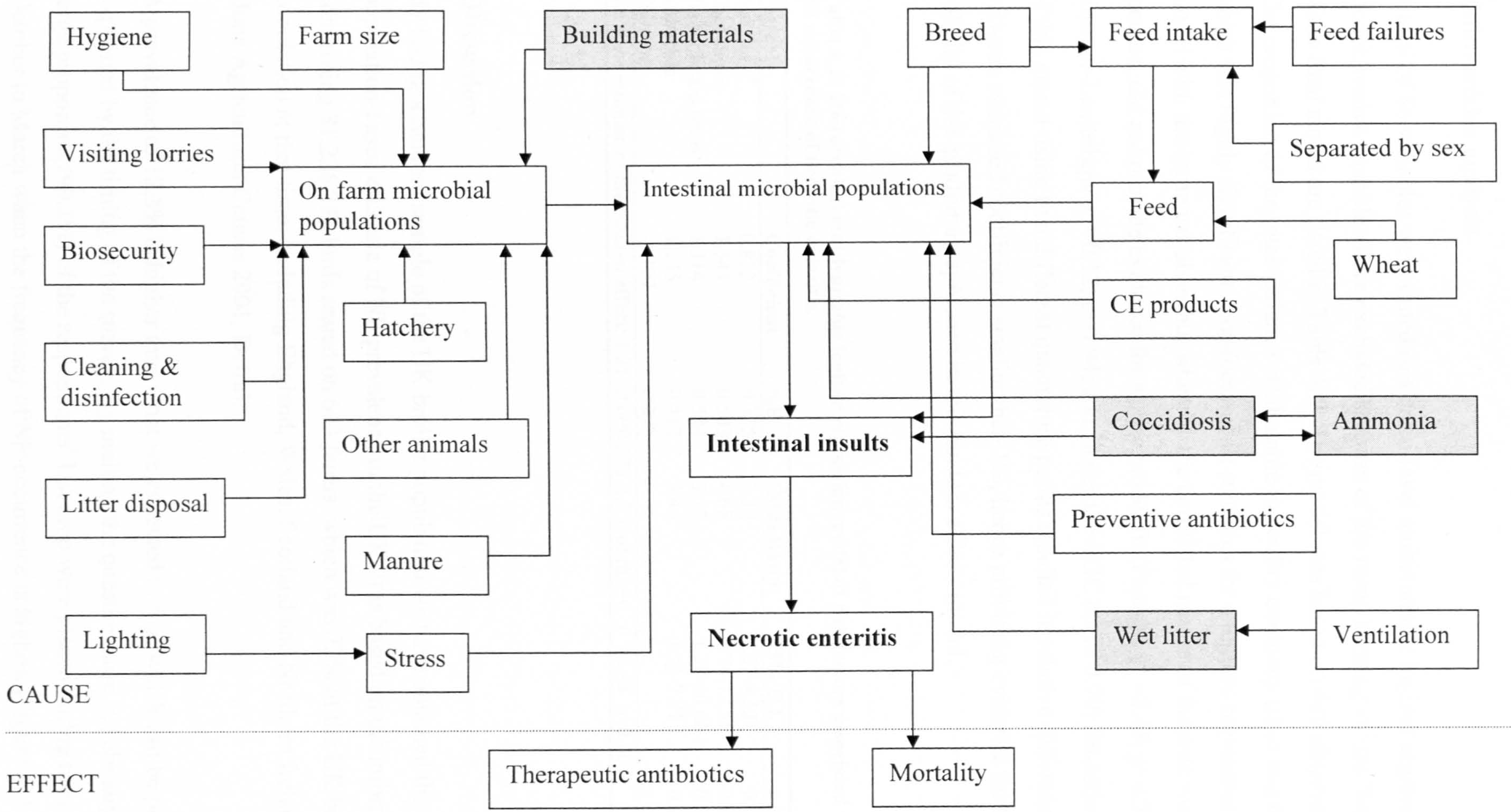

Figure 4. 3 Hypothetical causal pathway of necrotic enteritis, based on univariate analysis (p<0.05). Variables in grey boxes, remained significant in the final multivariable model.

Multivariable analysis

A total of 86 variables were tested in a multi-level multivariable logistic regression model; twelve variables were excluded because of too many missing values. Results of the final model are listed in Table 4. 3. The model was based on 449 observations. The proportion of the total variance attributable to poultry company (rho) was 0.27 which was highly significant. Goodness-of-fit statistics for a logistic regression model with the same variables but whereby the correlated nature of the data was ignored, did not provide evidence for a poor model fit (Pearson χ^2 =2.45, p=0.78; Hosmer-Lemeshow χ^2=0.12, p=0.94; area under the ROC curve 0.70) Re-estimation of this model using two different quadrature points resulted in relative differences between estimated coefficients smaller than 1%, hence providing evidence for the validity of the quadrature approach in this random effects model.

Table 4. 3: Hierarchical multivariable logistic regression model of risk factors associated with the occurrence of necrotic enteritis.

Variable	Coefficient	S.E.	Odds Ratio	95% C.I.	p-value
Wet litter	0.872	0.325	2.39	1.27-4.52	0.007
Coccidiosis	1.544	0.503	4.68	1.74-12.55	0.002
Plaster board walls	1.314	0.504	3.72	1.38-10.00	0.009
Ammonia	1.235	0.412	3.44	1.53-7.71	0.003
Constant	-3.197	0.375	-	-	-
Variance estimate of the random effect: 1.21 rho=0.270 LRT of rho=0 χ^2 =8.48 p=0.002					

Discussion

By taking a random sample of the UK broiler population we have obtained the first population-based estimate of NE prevalence in the UK. It is based on the information concerning 81,226,231 birds reared on 603 farms, which was 72% of the UK broiler population at that time, including England, Wales, Scotland and Northern Ireland (June Agricultural Census 2001, Defra).

A prevalence of 12.3% is higher than what we expected to find which can be partly explained by the timing of the survey. By mailing the questionnaire in February, a large proportion (99.1%) of the respondents' last crop were placed during the months October to March when the frequency of NE occurrence is highest (Figure 4. 1). Had the questionnaire been send out in one of the summer months, we would expect to have observed a lower prevalence.

Few population-based measures of NE occurrence exist in the literature. Long (1973) reported a prevalence of 7.7% based on the number of broiler consignments diagnosed with NE submitted to three diagnostic laboratories over a three year period. The total broiler population in the areas served by the three laboratories was unknown and the denominator was taken as the total number of poultry consignments, including non-broiler type chickens, submitted to the three laboratories over the three year period. Similarly, 8.57% of all broiler consignments submitted to a German diagnostic centre over a three year period were diagnosed with NE but also in this study, the number of birds in the catchment area was not specified (Köhler *et al.*, 1977). In contrast to these two studies, Kaldhusdal and Skjerve (1996) attempted to derive a measure of frequency of occurrence with a population-based denominator. They retrospectively analysed data from the Central Veterinary Laboratory in Norway over a twenty-year period for broiler consignments diagnosed with NE and approximated the number of birds in the study region based on the number of hatched birds during this period and figures from regional processing plants. In this way, they calculated quarterly cumulative incidences which varied from zero to 34.8% during the specified period. An advantage of collecting information from veterinary laboratories in order to obtain a measure of occurrence is that diagnostic data are likely to be fairly accurate and usually available over long periods of time. A major disadvantage is that the total population at risk is often not considered because it is unknown or needs to be approximated. In addition, not all cases of NE will be submitted to the laboratory as some farmers may seek diagnosis and treatment elsewhere, and it is not always possible to distinguish between consignments that are from one and the same outbreak or from different outbreaks.

In our survey we relied on the farm manager's ability to report NE which is vulnerable to misclassification. Validation of this information was incomplete. Post-mortem reports were obtained of 20% of the 603 responding farms and only 26% (19/74) of flocks with reported NE had a post-mortem report which could be used for validation. Of these 19 post-mortem reports, more than half (58%) were consistent with the farm manager's questionnaire response of having had NE in his last flock. It appears that a large proportion of disease diagnosis on broiler farms is not accompanied by a post-mortem report and as such these cannot be solely relied upon for disease validation in future studies. It also suggests that if our disease information had been exclusively collected from veterinary laboratories, the observed prevalence would have been much lower. This has important implications for disease surveillance in the poultry industry.

The seasonal distribution of NE as found in this survey is in agreement with the study from Norway (Kaldhusdal & Skjerve, 1996), where NE occurred more often during the months October to March than during the months April-September. In contrast, studies from Canada reported NE to be most frequent during the months July, August, September and October in Ontario (Long, 1973) and from May to November in Quebec (Bernier *et al.*, 1974). In Germany, NE was also observed most frequently during the summer months. Differences in seasonal patterns of occurrence might be explained by geographical differences but could also be due to different management and husbandry systems or different genetic make-up of the broiler bird.

The occurrence of NE varied widely between companies, with three out of nine companies not reporting NE. This was reflected by the large proportion of variation residing at company level, identified by a significant intra-class correlation coefficient in the final multi-level model. It underlines the importance of such hierarchical models in a highly centralised population such as that seen in the poultry industry. Model diagnostic tests are not available for multilevel models in Stata but re-estimation of the final model with different quadrature points demonstrated its numerical stability.

Our model demonstrates strong associations between NE and wet litter and NE and coccidiosis. It is unclear if the occurrence of NE leads to wet litter and coccidiosis or if they can be considered true risk factors for the disease. Farmer reported median age of occurrences of 24 and 25 days for wet litter and coccidiosis respectively compared with 26 days for NE suggests the latter as it is consistent with one of the criteria of causality that cause should precede the outcome (Evans, 1978). Furthermore, a systematic review of case reports and case series describing naturally occurring NE (Chapter 2), demonstrated that diarrhoea is not a consistently reported clinical sign and therefore a majority of NE cases may not lead to wet litter.

Wet litter is a common phenomenon on UK broiler farms with a reported prevalence of 56% (Chapter 5). The term is used when the bedding in poultry houses reaches its saturation threshold, loses its friability and forms a wet cap on the surface (Pattison, 1987). Although there are a wide variety of causes for wet litter, including climate conditions in the poultry house and leaking drinkers and roofs, in recent years it has been increasingly associated with a non-specific enteritis. This condition has also been termed 'dysbacteriosis' because of an apparent overgrowth of certain bacterial populations in the gut. Although ill-defined, it is characterised by the production of large volumes of abnormally wet droppings and maldigestion (Pattison, 2002). A disturbance of the intestinal mobility and pH has been demonstrated to be conducive for the production of NE in experimental studies (Parish, 1961c; Bernier *et al.*, 1977) and presumably, digestive disorders such as 'dysbacteriosis' could have similar effects. This needs further investigation.

The role of coccidial infections in the occurrence of NE has been surmised from field observations (Helmboldt & Bryant, 1971; Kralj *et al.*, 1979) and documented in experimental studies (Al-Sheikhly & Al-Saieg, 1980; Shane *et al.*, 1985) for *Eimeria acervulina*. However, the evidence for other coccidial species has been inconclusive (Chapter 3). Clinical coccidiosis was reported by 5.8% of the farm managers in their last crop in this study with a little over a third (37.1%) indicating that *Eimeria tenella* was the causative agent, while the implicating species in the other cases was unknown. Baba *et al* (1988) inoculated broilers with *C. perfringens* in the presence and absence of an *E. tenella* challenge and found significantly higher caecal *C. perfringens* counts in birds infected with this coccidial species although no NE was observed. They concluded that increased numbers of *C. perfringens* excreted into the poultry environment during *E. tenella* infections may predispose towards NE outbreaks. The exact mechanism of the predisposing role of coccidiosis in the pathogenesis of NE remains unclear. Al-Sheikhly & Truscott (1977) hypothesised that minor intestinal damage, such as can be caused by coccidiosis, in combination with sufficient numbers of *C. perfringens* are prerequisites for NE to occur. Williams (2005) pointed out however that coccidial lesions and NE lesions are often found in

(2005) pointed out however that coccidial lesions and NE lesions are often found in different locations in the gut. Several studies have demonstrated that coccidial infections induce intestinal stasis (Colnago *et al.*, 1982; Shane *et al.*, 1985) and an increased intestinal acidity (Ruff & Reid, 1975; Shane *et al.*, 1985). The proposed mechanism for these patho-physiological changes is that damage to the intestinal mucosa by the sexual stages of the coccidia may facilitate loss of protein into the lumen and that subsequent degradation of protein into amino acids leads to increased acidity of the intestinal contents. A lowered pH affects the permeability of the cell membranes of smooth muscle and will suppress intestinal motility. Shane *et al* (1985) suggested that intestinal stasis favours the proliferation of *C. perfringens* and subsequent accumulation of toxin, resulting in NE. However, evidence exists that a lowered pH inhibits *C. perfringens* proliferation *in vitro* (Kmet *et al.*, 1993), and it is unknown how this offsets the effects of intestinal stasis. Although our study has quantified the association between farmer observed coccidiosis and NE in the field, further work is needed to unravel the pathogenesis and to elucidate the role of the individual coccidial species.

Ammonia is often used as a disinfectant in broiler houses, specifically for coccidial oocysts. Reportedly, 8.6% of the farms in this survey used this disinfectant preceding their last crop and its use was significantly associated with the occurrence of NE. There was no significant association between the use of ammonia and clinical coccidiosis in the last crop (OR 1.72; 95% C.I. 0.55-5.04; p=0.28) and no evidence of confounding in the multivariable model. It is conceivable that farms with a history of NE outbreaks are more thorough in their disinfection procedures and are more likely to use ammonia. Similarly, farms with recurring coccidiosis problems are also more likely to use ammonia, and we hypothesise that the disinfectant will prevent the occurrence of clinical coccidiosis in the subsequent crop but any lingering oocysts would produce subclinical disease which would act as a precursor for NE.

The presence of plaster-board walls as a risk factor in our final model might be an indication that hygiene and disinfection have an important part to play in the prevention of NE. This porous type of material, used by 6% of farms in our survey, could be more difficult to clean and disinfect and therefore present a biosecurity hazard. Alternatively, the variable might be a proxy for another common factor that was not identified by our study.

Equally revealing as the risk factors that remained in our final model, are those variables which showed no significant associations with the occurrence of NE in this study but which are often implicated in other studies. For example, the inclusion of wheat in broiler diets was shown to be a strong risk factor for NE in an experimental study (Branton *et al.*, 1987) but the addition of whole wheat to the feed in our survey was not significantly associated with the occurrence of NE (OR 1.63; 95% C.I. 0.70-4.42; p=0.24). However, this variable did not measure the total content of wheat in the diet but only the proportion that was added as whole wheat. More detailed analyses of feed rations would be required to determine the role of wheat and other feed components as determinants of NE. Efforts to do this in this study were unsuccessful, partly because of the high commercial value and extreme sensitive nature of broiler feed information and also because of the large degree of homogeneity of broiler rations within companies.

Preventive measures such as the prophylactic use of antibiotics and the use of competitive exclusion products (CE) failed to remain significant factors in the final multivariable model. Fifty-one percent of farm managers reported having used at least one prescription-only antibiotic for preventive reasons in their most recently cleared flock The antibiotic most often used was lincomycin-spectinomycin (34% of all instances of antibiotic use for a preventive reason) followed by tylosin (29% of all instances). Several experimental studies claim beneficial effects of these antimicrobial compounds against NE (Maxey & Page, 1977; Hamdy *et al.*, 1983; Brennan *et al.*, 2001; Collier *et al.*, 2003) but the results of our survey could not demonstrate a significant protective effect. Equally, CE use has been shown to be effective in experimental studies (Elwinger *et al.*, 1992; Hofacre *et al.*, 1998), but the 11% of farm managers who reported its use in our survey, were not at a significantly lower risk of NE occurrence in their last crop.

Increasing restrictions in the use of antibiotics in animal production will require alternative strategies for the control of animal diseases. Studies as the one described in this paper will be indispensable to evaluate existing strategies and to generate ideas for future control methods. By working with the major broiler companies in the UK on a topical issue, we achieved a large degree of cooperation and compliance, which is important in any observational study. However, by leaving out independent growers and smaller companies, selection bias cannot be completely ruled out. In addition, rudimentary analysis of non-responding farms, seem to indicate that these, on average, are farms with fewer chickens. This might have over-estimated our frequency estimate as univariate analysis demonstrates a positive association between farm size and NE occurrence. By maximising our response rate we hope to have kept this non-response bias to a minimum. Also, future studies will need to devise methods to secure case ascertainment, as our survey demonstrates that to rely on veterinary post-mortem reports alone is insufficient.

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Chapter 5: Prevalence of wet litter and associated risk factors in broiler flocks in the UK[1]

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Summary

A cross-sectional survey was carried out among 857 broiler farms in the UK to determine the prevalence and risk factors for wet litter. Information was collected by postal questionnaire directed at farm managers. The response rate was 75%. Wet litter was reported by 75% (95% C.I. 71.3-78.3) of the respondents in at least one flock during the year 2001 and more than half of the farmers reported that they had an outbreak of wet litter in their most recently reared flock (56.1%; 95% C.I. 52.0-60.0). Wet litter occurred more often during the winter months and farms using side ventilation systems were at an increased risk (OR 1.74; 95% C.I. 1.09-2.76). Multivariable analysis was carried out using two different definitions of wet litter as outcome variables. These were all cases of wet litter and wet litter with disease as the reported cause. Consistent risk factors for both outcomes were coccidiosis, feed equipment failures and the availability of separate farm clothing for each house. Wet litter with an implicated disease aetiology was reported by 18.9% (95% C.I. 15.9-22.3) of the farmers in their last flock and was associated with the use of hand sanitizers and house walls made of concrete.

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Introduction

Wet litter is the term used when the material covering the floors of poultry houses, usually consisting of wood shavings or chopped straw, reaches its saturation threshold and is unable to hold more moisture. It loses friability and a wet cap may form on the litter surface (Pattison, 1987). Wet litter can have important implications for the health, welfare and productivity of intensively reared broiler flocks. It has been shown to be associated with the occurrence of foot-pad, breast and hock lesions (Greene *et al.*, 1985; Martland, 1985; McIlroy *et al.*, 1987; Ekstrand *et al.*, 1997; Menzies *et al.*, 1998) and recently, increased litter moisture levels have been demonstrated to be correlated with higher faecal corticosteroid concentrations, suggesting that these flocks are subjected to higher levels of stress (Dawkins *et al.*, 2004).

Various risk factors have been identified for the occurrence of wet litter, including clinical disease in which diarrhoea is an important sign (Neill *et al.*, 1984; Collins *et al.*, 1989), feed components (McIlroy *et al.*, 1987; Tucker & Walker, 1992), season (McIlroy *et al.*, 1987; Bruce *et al.*, 1990), stocking density (McIlroy *et al.*, 1987; Tucker & Walker, 1992), average age at slaughter (McIlroy *et al.*, 1987; Bruce *et al.*, 1990), sex (McIlroy *et al.*, 1987), drinker design (Bray & Lynn, 1986; Tucker & Walker, 1992), litter depth (Ekstrand *et al.*, 1997), and house temperature and relative humidity (Payne, 1967; Tucker & Walker, 1992).

Relatively little information is available about the prevalence of wet litter. Representative data on disease prevalence is an useful prelude to longitudinal studies and the study described here was part of a larger study to estimate the prevalence of enteric diseases in broilers and to inform future epidemiological study design.

Materials and Methods

Study design

The eleven major poultry companies producing broilers in the UK were approached through their company veterinarian and asked to participate in a study on necrotic enteritis (NE). Nine companies agreed, comprising a sampling frame of 942 company and contract farms. The smallest company contained 31 farms whereas the largest company owned or contracted 291 farms. A random sample of farms, stratified by company, was selected. Sample size was based on an expected prevalence of NE of 5% with 95% confidence limits and 1% precision and consisted of 857 farms.

Data were collected by a postal questionnaire directed at farm managers. It consisted of 206 questions and addressed the following topics:

- Occurrence of wet litter in the farm's most recently cleared flock at the time of the survey and in the flocks reared during the year 2001.
- Disease occurrence in the farm's most recently cleared flock and in the flocks reared during the year 2001, including NE, coccidosis, respiratory diseases and immuno-suppressive diseases.
- Farm characteristics including total number, age, and building materials of the houses, distance between them, total number of flocks reared per year, number of people working on the farm, membership of a quality assurance scheme and number of years of farming experience rearing broilers.
- Characteristics of the most recently cleared flock including total number of chicks at placement, breed, number of parent flocks and hatcheries that supplied the chicks, type of litter used, rearing separated by sex, 'thinning', age and weight at clearance, maximum stocking density, feed conversion, total mortality and EPEF (European Production Efficiency Factor).
- Climate control including type of ventilation, heating, cooling and lighting.
- Hygiene and biosecurity issues including cleaning and disinfection of the houses, availability and use of hand washing facilities, hand sanitizers, shower facilities, separate farm clothing and boots, boot dips, plastic over-boots, barrier systems, rodent and other pest control, domestic animals on the

farm and on adjacent fields, poultry farms in the neighbourhood, disposal of litter, spread of manure on farm fields, and the number of people entering the houses and lorries coming onto the farm.

- Feed and water supply including types of feeders and drinkers, number of birds per feeder and drinker, feed and water regimens, percentage of whole wheat added, water source and water sanitizing practices.
- Disease prevention and management including vaccinations and preventive and therapeutic antibiotic use.

The questionnaire was pre-tested by sending it to the farm managers and area managers of 26 farms, which were not part of the sample population, selected at random from the seven largest companies. After correction, questionnaires were posted to the farm managers of the 857 selected farms during the first week of February 2002. To maximise response rates, a reminder card and second copy of the questionnaire were mailed to all non-respondents four and eight weeks respectively after the initial mailing. A copy of the cover letter, questionnaire, reminder card and reminder letter can be found in Appendices 1 to 4 respectively. The final deadline for receipt of questionnaires was 20 May 2002.

Statistical analysis

The questionnaire data was entered into a Microsoft Access database by a commercial company and checked and corrected. The unit of study was the farm's most recently reared flock whereby a flock was considered to be the total number of chickens on the farm. In the questionnaire, farmers were asked about the occurrence and causes of wet litter and two dependent variables were created from these data. These were all cases of wet litter and cases of wet litter reported to be associated with disease. Separate analyses were performed for each dependent variable. Independent dichotomous variables were analysed using 2x2 contingency tables and tested for significance with the chi-squared test or the Fisher's exact test when expected values were less than five. Independent categorical variables were analysed in a univariate logistic regression model, by creating dummy variables. Continuous variables were also analysed using univariate logistic regression and their best fitting form were assessed using the following fractional polynomials: -2, -1, -0.5, 0, 0.5, 1, 2, 3 (Royston *et al.*, 1999). In addition, continuous variables were categorized according to their quartile values to explore the shape of their relationship with the dependent variable.

Variables with a statistical significance of $p \leq 0.25$ in the univariate analysis were tested in a multilevel logistic regression model with poultry company as random effect. Two separate models were developed for the two dependent variables, i.e. all wet litter cases and wet litter cases with a reported disease aetiology. The models were built manually, starting with variables with the lowest p-values in the univariate analysis. Variables with more than 10% missing values were not included to avoid numerical instability of the model. Other variables that were not included were those variables that represented a possible effect rather than a cause, such as the use of antibiotics and EPEF, and the variable necrotic enteritis (NE). Previous analyses using NE as an outcome variable had demonstrated strong associations with wet litter.

Variables with a term-wise Wald test p-value of ≤ 0.05 or variables that significantly improved the model fit (likelihood ratio chi squared statistic $p \leq 0.05$) were retained in the model. Interaction terms were tested for all variables left in the final model and for those that seemed biologically plausible. To estimate the proportion of variation attributable to poultry company, the intra-cluster correlation coefficient (ICC) was calculated using a latent variable approach (Goldstein *et al.*, 2002). This approach assumes the binary outcome arises from an underlying continuous distribution and that the level 1 variance (poultry company) is on the logit scale and is $\pi^2/3$.

The predictive accuracies of the final multivariable models were assessed by k-fold cross-validation (Kohavi, 1995) with k=10. The observations in the data set were randomly divided into ten equal subsets and one of these was used as a 'test' set. The partition was performed separately among the cases and the non-cases and the corresponding subsets were joined, so that each subset had the same proportion of cases as the real data set. The remaining nine subsets were combined to form a "training" set. The training set was then used to build a model, using regularized logistic regression (Genkin *et al.*, 2004) which was evaluated on the test set. This was repeated using each of the nine remaining subsets in turn as the test set. This allowed a prediction for each available observation, an estimate of the predictive performance of the model built on the whole data, and ten estimates of model coefficients. This procedure was repeated nine more times with a different partition into ten subsets every time, giving 100 estimates of the coefficients, and ten estimates of the predictive accuracy of the model trained on all observations. Mean coefficients and standard deviations were calculated from the coefficients of these 100 models and the mean predictive accuracy was determined. For the model of wet litter cases caused by disease, the number of observations with wet litter was twice as small as the number of observations without wet litter. Because this created a bias in the predictive model, wet litter cases in the training set were reweighted by a factor of 2 in order to improve the predictive accuracy of the model. Reweighting was not necessary for the general wet litter model because the number of observations with wet litter were similar to the number of observations without wet litter.

The software packages that were used were EpiInfo Version 6 (CDC, Atlanta, Georgia, USA) for the descriptive statistics and univariate analysis, Stata 7 (Stata Corporation, College Station, Texas, USA) for the univariate and multivariable logistic regression and BBR package (http://www.stat.rutgers.edu/~madigan/BBR/) for regularized logistic regression. The code for the cross-validation was written in shell script.

Results

Response rate

During the specified survey period, 639 questionnaires (75%) were returned. Questionnaires that came from non-conventional farms (i.e. organic, free-range) were excluded and this left 603 completed questionnaires, giving a useable response rate of 70%.

Descriptive epidemiology

Seventy-five percent of the respondents (n=452; 95% confidence intervals (C.I.) 71.3-78.3) indicated they had wet litter during 2001 in a median number of three flocks (I.Q.R. 2-5). The monthly distribution of farmer reported wet litter is shown in Figure 5. 1. More than half of the farmers responded that they had wet litter in their most recently cleared flock (n=338; 56.1%, 95% C.I. 52.0-60.0) occurring at a median age of 24 days (I.Q.R. 20-28).

The prevalence of wet litter on farms within the different companies ranged from 33.3% (95% C.I. 17.3-52.8) to 83.3% (95% C.I. 65.3-94.4) for all cases and from 1.9% (95% C.I. 0-10.1) to 42.7% (95% C.I. 31.8-54.1) for wet litter associated with disease (Figure 5. 2).

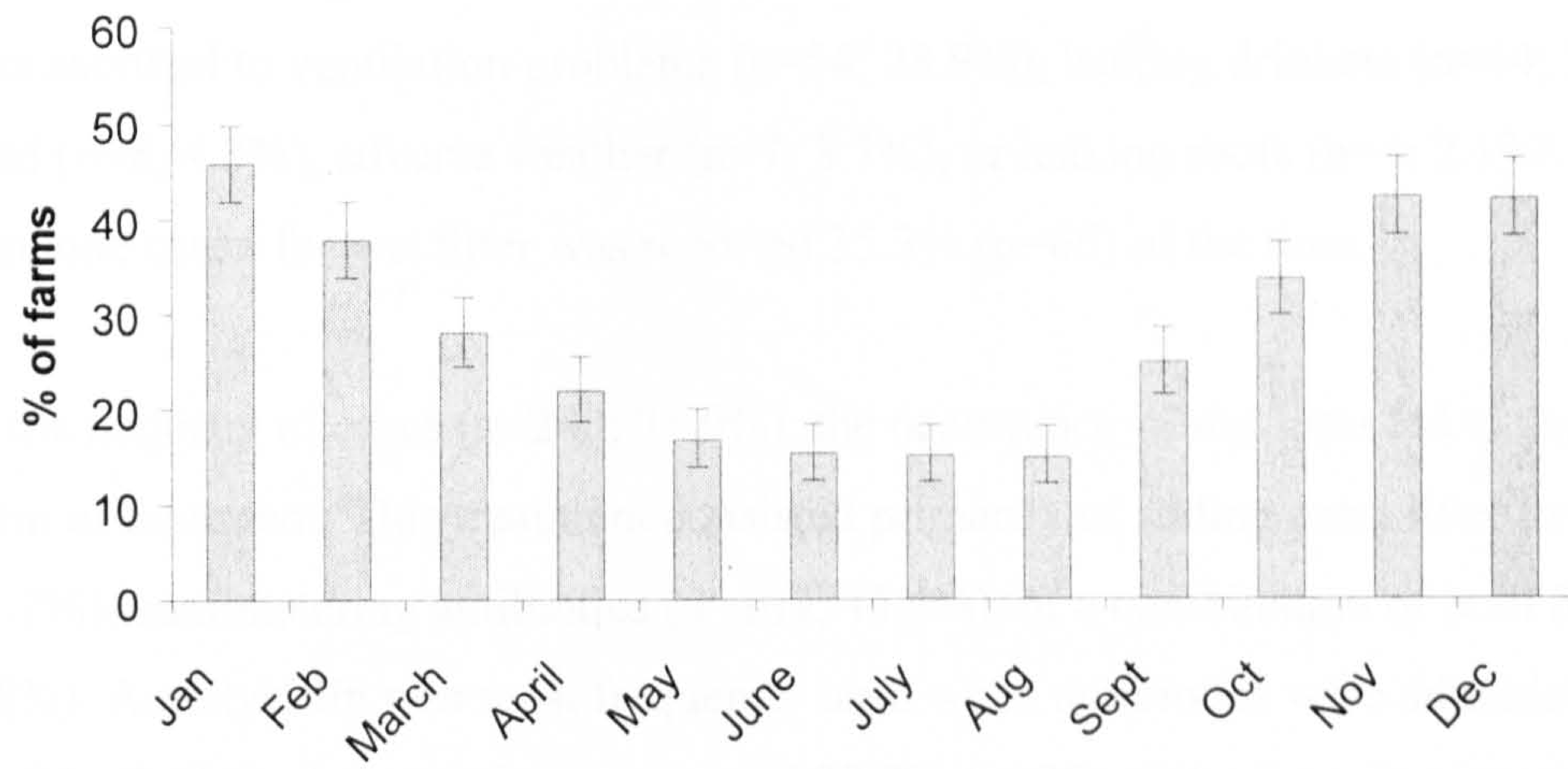

Figure 5. 1: Monthly distribution of farmer reported wet litter cases during 2001 (error bars represent 95% confidence intervals).

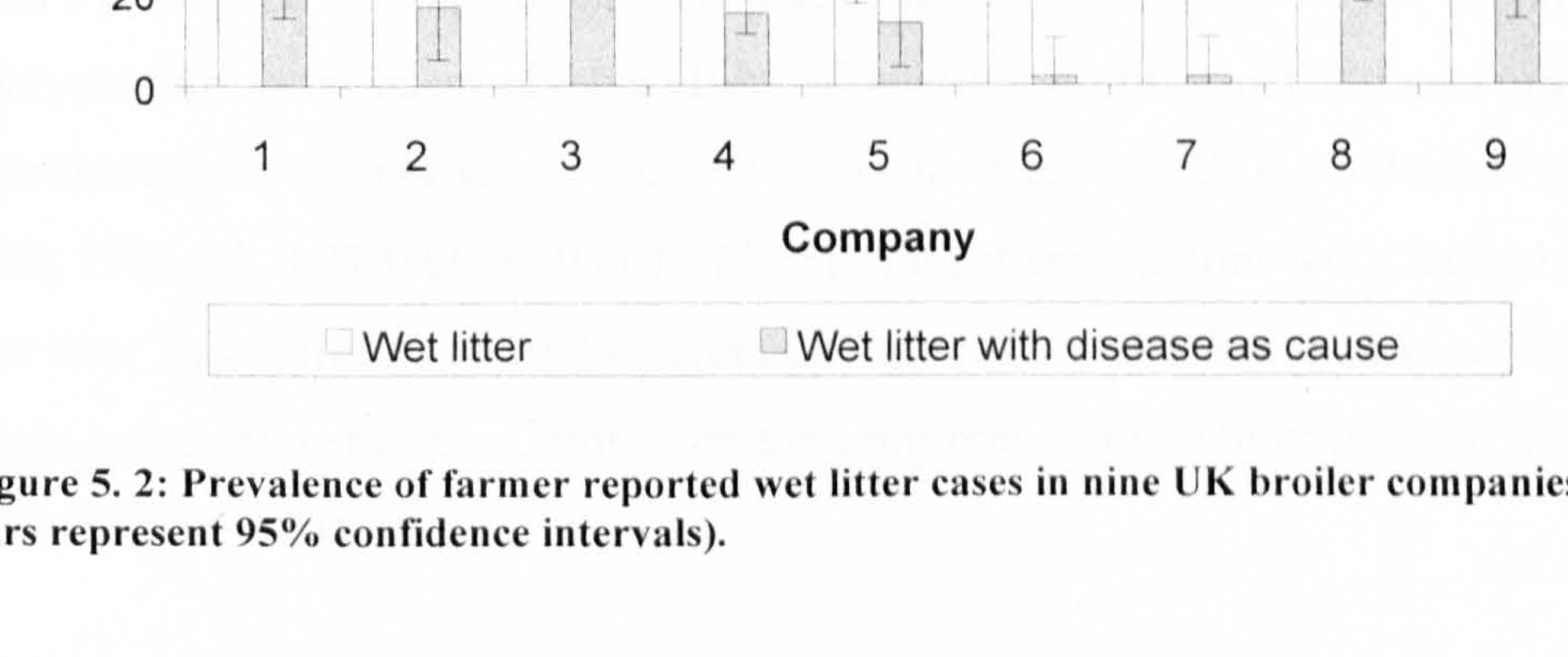

Figure 5. 2: Prevalence of farmer reported wet litter cases in nine UK broiler companies (error bars represent 95% confidence intervals).

Just over half of the farmers reporting wet litter in their most recently cleared flock indicated that a cause for the wet litter was identified (n=187; 55.3%). Of these, some type of disease process was thought to be a underlying cause in 61.0% (n=114) of the cases which included the following disorders by themselves or in combination: non-specific enteritis (n=76; 40.6%), coccidiosis (n=23; 12.3%), a viral infection (n=21; 11.2%), 'dysbacteriosis' (n=9; 4.8%) or a bacterial infection (n=2; 1.1%). In half of these cases associated with disease (n=57; 50.0%), a veterinarian was involved in making the diagnosis of the cause of wet litter. In the instances that causes other than disease were thought to be underlying the occurrence of wet litter (n=91; 48.4%), it was ascribed to ventilation problems (n=54; 28.9%), leaking drinkers (n=44; 23.5%), feed (n=8; 4.3%), adverse weather (n=7; 3.7%), or leaking roofs (n=4; 2.1%). More than one cause for wet litter was reported 35.3% (n=66) of the time.

In the majority of cases (n=240; 71.0%), the occurrence of wet litter led to some form of treatment. This treatment consisted primarily of adding extra litter (n=100; 41.7%), administering antibiotics (n=109; 45.4%), or a combination of both (n=9; 3.8%). Amoxycillin was most frequently used when antibiotics were administered (n=75; 63.6%) whereas tylosin was used 23.7% (n=28) of the time. In nine cases (7.6%) a combination of these two antibiotics was used whereas in six cases (5.1%) other types of antibiotics were used.

Univariate analysis

Factors significantly associated ($p \leq 0.05$) with the occurrence of all cases of wet litter and with those with a disease aetiology are listed in Table 5. 1. Full univariate results can be found in Appendix 7& 8. Only those fractional polynomials are listed that significantly improved the fit of the univariate logistic regression model ($p \leq 0.05$). On the basis of the univariate analysis, a causal web was constructed using variables with a p value of less than or equal to 0.05 (Figure 5. 3). Results of the univariate analysis demonstrated an adverse effect of wet litter on flock performance, in that the occurrence of wet litter was associated with a decrease in EPEF (Odds Ratio (OR) 0.98; 95% C.I. 0.97-0.99, $p<0.001$). No significant associations were found between wet litter and mortality or feed conversion ratio (FCR). We found no beneficial effect of the use of preventive antibiotics on the occurrence of wet litter (OR 1.63; 95% C.I. 0.98-2.72; $p=0.05$)

Table 5. 1: Univariate associations ($p \leq 0.05$) between the occurrence of wet litter (all cases and cases with a reported disease aetiology) and explanatory variables.

	Wet litter – all causes			Wet litter - disease		
Variable	**OR**	**95% C.I.**	**p-value**	**OR**	**95% C.I.**	**p-value**
Disease						
Necrotic enteritis in the last flock	2.82	1.56-5.13	<0.001	7.68	4.15-14.20	<0.001
Coccidiosis in the last flock	9.17	2.77-30.33	<0.001	28.67	6.85-255.94	<0.001
Respiratory diseases in the last flock				3.02	0.98-9.39	0.03
Environment						
Wooden walls	1.60	1.14-2.25	<0.007	1.59	0.97-2.61	0.05
Breeze block walls	1.38	1.00-1.92	0.05			
Side ventilation	1.66	1.18-2.34	0.004	2.50	1.54-4.07	<0.001
Automatic controlled natural ventilation				0.39	0.17-0.84	0.008
Other types of ventilation				3.69	0.91-17.48	0.03
Cooling devices used	3.53	1.18-10.57	0.02			
Heating failure	2.90	1.53-5.50	<0.001	3.67	1.61-8.46	<0.001
Use of low energy light bulbs	1.59	1.14-2.22	0.007	1.74	1.08-2.80	0.02
Flat chain/auger feeder	1.45	1.05-2.01	0.02			
Bell drinkers	2.69	0.98-7.40	0.05	3.88	1.13-15.04	0.01
Nipple drinkers without cups				1.82	1.13-2.94	0.008
Number of houses on the farm (*continuous*) Fractional polynomial: 0 -0.5	1.12 1.85 	1.07-1.18 1.46-2.35 	<0.001 <0.001 	1.17 0.29	1.10-1.25 0.19-0.47	<0.001 <0.001
Age of the eldest house	1.02	1.01-1.03	0.004	1.03	1.01-1.04	0.003
Number of feed bins on the farm (*continuous*)	1.07	1.03-1.11	<0.001	1.12	1.07-1.17	<0.001
Straw as litter				1.56	0.96-2.54	0.05
Husbandry/management						
Breed A	1.63	1.06-2.48	0.02	6.54	2.52-21.49	<0.001
Breed B				4.37	1.32-16.64	0.004
Chickens grown separated by sex	0.68	0.47-0.98	0.04	0.52	0.32-0.87	0.008
Feeding equipment failures in last flock	2.41	1.69-3.43	<0.001	3.97	2.40-6.57	<0.001
Number of people working on the farm (*continuous*) Fractional polynomial: 2 3	0.99 173.15 0.03	0.92-1.07 2.83-10591 0.001-0.65	0.83 0.014 0.025			
Number of flocks per year (*continuous*)	1.30	1.07-1.58	0.008	2.15	1.55-2.99	<0.001

Table 5. 1 (continued)

	Wet litter – all causes			Wet litter - disease		
Variable	**OR**	**95% C.I.**	**p-value**	**OR**	**95% C.I.**	**p-value**
Number of chickens placed in last flock (*continuous, per 10000*)	1.05	1.03-1.08	<0.001	1.09	1.06-1.12	<0.001
Number of parent flocks that produced the chicks for the last flock (*continuous*)	1.12	1.05-1.20	<0.001	1.14	1.05-1.23	0.001
Number of hatcheries that supplied the chicks for the last flock (*continuous*)	1.54	1.18-2.00	0.001	1.91	1.35-2.70	<0.001
Age at slaughter (days) of last flock (*continuous*)	0.97	0.94-1.00	0.04	0.96	0.92-1.00	0.04
Average weight at slaughter of last flock (*continuous, per 100 gram*)	0.97	0.94-1.00	0.04	0.95	0.91-0.98	0.006
Hygiene & Biosecurity						
House(s) cleaned by: Contract cleaner	Ref.			Ref.		
Company cleaner	1.15	0.59-2.24	0.68	1.40	0.64-3.09	0.40
Farm manager	0.62	0.42-0.92	0.02	0.27	0.14-0.55	<0.001
House(s) disinfected by: Contract cleaner	Ref.			Ref.		
Company cleaner	1.11	0.60-2.06	0.75	1.27	0.60-2.70	0.53
Farm manager	0.63	0.43-0.92	0.02	0.31	0.17-0.59	<0.001
Use of ammonia as a disinfectant				2.90	1.35-6.25	0.002
Hand wash facilities in each shed	0.47	0.25-0.88	0.01			
Hand sanitizers in each shed	1.58	1.02-2.44	0.04			
Using hand sanitizers before entering shed: Never	Ref.			Ref.		
Occasionally	1.33	0.84-2.11	0.22	1.97	0.95-4.11	0.07
Always	1.79	1.11-2.89	0.02	2.76	1.31-5.83	0.008
Separate clothing for each shed	0.54	0.37-0.78	<0.001	0.28	0.15-0.51	<0.001
Change clothes before entering shed: Never				Ref.		
Occasionally				0.48	0.27-0.86	0.014
Always				0.41	0.22-0.76	0.005
Plastic over-boots for each shed	1.44	1.02-2.02	0.04			
Litter is disposed by: Spread on field	0.67	0.47-0.94	0.02			
Deep stacked and then spread on field	0.67	0.47-0.94	0.02			
Disposed of to a power plant	2.02	1.44-2.86	<0.001	2.23	1.37-3.64	0.001
Litter is spread on fields away from the farm				1.90	0.95-3.82	0.05
Cattle on the farm				0.51	0.27-0.93	0.02
Cattle on fields adjoining the farm				0.64	0.39-1.03	0.05
Spread of manure on fields of farm	0.69	0.48-0.98	0.04	0.36	0.20-0.65	<0.001
Spread of cow manure on fields of farm	0.68	0.47-0.98	0.04	0.37	0.20-0.70	<0.001
Spread of cow manure on fields adjoining the farm				0.49	0.30-0.82	0.003
People other than the farm manager, employees or area manager entering the shed	1.67	1.20-2.32	0.002	1.76	1.10-2.84	0.01
Electricians entering the shed	1.61	1.15-2.27	0.006	1.81	1.11-2.95	0.01

Table 5. 1 (continued)

	Wet litter – all causes			Wet litter - disease		
Variable	**OR**	**95% C.I.**	**p-value**	**OR**	**95% C.I.**	**p-value**
Service engineers entering the shed	1.62	1.04-2.52	0.03			
Collection lorries entering the farm				6.68	0.98-287.14	0.04
Length of turn-around (days) before the last flock (*continuous*)				0.93	0.88-0.97	0.003
Minimum time between disinfection and restocking (days) the flock (*continuous*)	0.95	0.91-1.00	0.04	0.88	0.81-0.95	0.001
Fractional polynomial: 3				0.24	0.11-0.50	<0.001
3				5.00	1.52-16.43	0.008
Number of feed lorries entering the farm during the last flock (*continuous*)	1.03	1.02-1.04	<0.001	1.04	1.03-1.06	<0.001
Number of rodent baits used (*continuous*)	1.03	1.02-1.04	<0.001	1.04	1.02-1.05	<0.001
Fractional polynomial: 0				2.32	1.66-3.25	<0.001
Feed & Water						
Addition of whole wheat to the feed	1.88	1.13-3.13	0.01	2.30	1.00-5.43	0.03
Wheat added by blending in the mill				0.55	0.31-0.98	0.03
Source of water supply: Mains	1.78	1.23-2.59	0.002	1.91	1.07-3.42	0.02
Well				0.30	0.07-1.13	0.05
Borehole	0.50	0.34-0.73	<0.001	0.53	0.30-0.94	0.02
Water is analysed	0.59	0.42-0.84	0.004			
Water is analysed bacteriologically	0.59	0.42-0.84	0.004			
Maximum amount (%) of whole wheat added (*continuous*)	0.96	0.93-1.00	0.04			
Prevention/Treatment						
Use of preventive antibiotics in last flock				1.63	0.98-2.72	0.05
Use of curative antibiotics in last flock	2.31	1.62-3.31	<0.001	8.03	4.51-14.37	<0.001
Use of competitive exclusion (CE) products in last flock				0.35	0.12-0.92	0.02
Type of CE product Aviquard				Ref.		
Broilact				14.50	1.06-198.80	0.05
Method of CE product administration Water				0.04	0.00-0.53	0.001
Spray				25.43	2.24-1416.03	<0.001
Use of coccidiostatic agents in last flock				2.77	0.98-9.63	0.04
Flock performance						
EPEF of last flock (*continuous*)	0.98	0.97-0.99	<0.001	0.97	0.96-0.99	<0.001

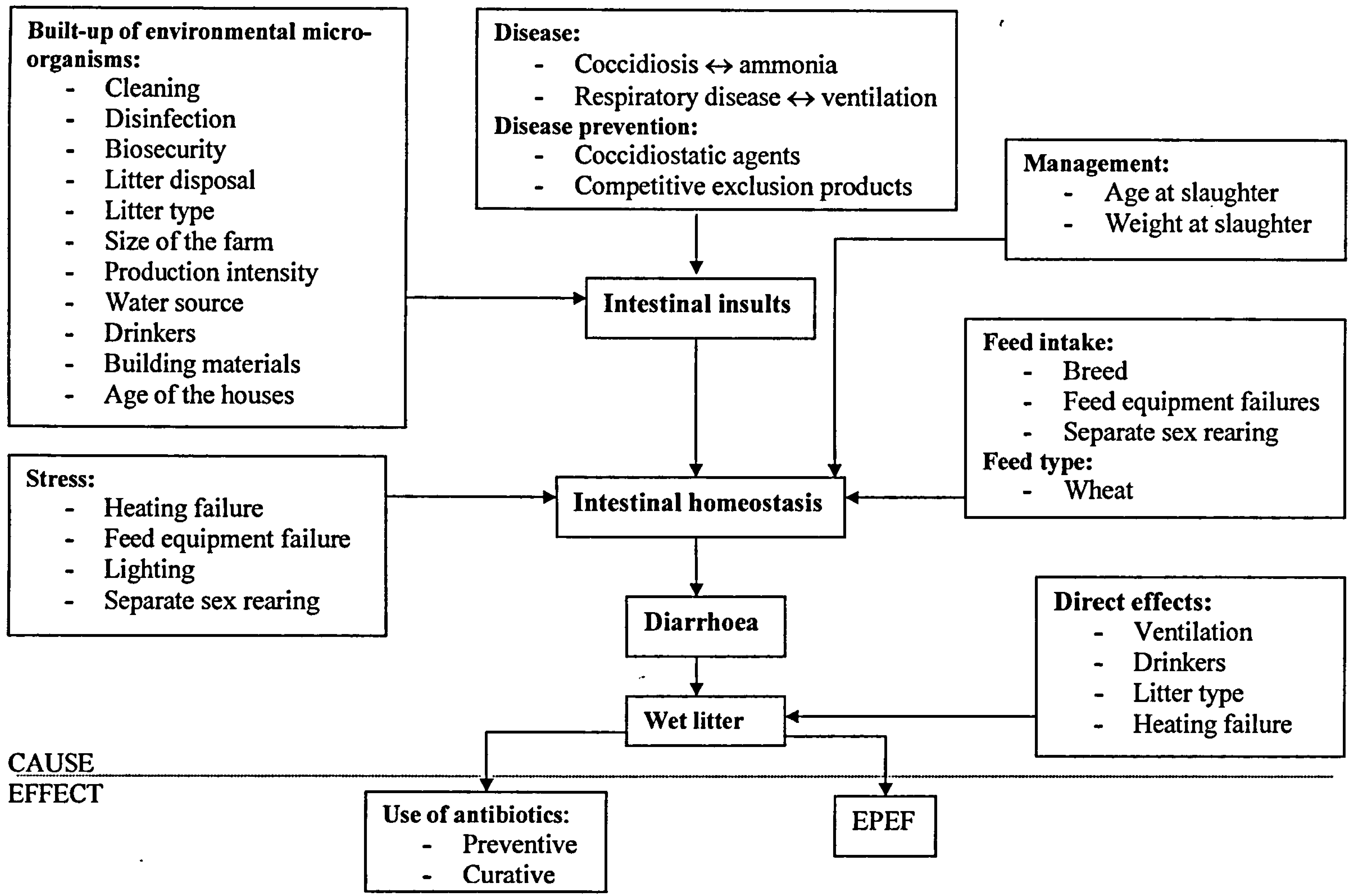

Figure 5. 3: Hypothetical causal pathway of wet litter cases with a farmer reported disease aetiology, based on univariate analysis ($p \leq 0.05$).

Multivariable analysis

The final multilevel logistic regression models for wet litter due to all causes (model 1) and wet litter because of a suspected disease aetiology (model 2) are listed in Table 5. 2 and Table 5. 3, respectively. Because observations with missing values for any of the variables in the final model were excluded, model 1 was built on 444 observations, which included 248 cases and 196 non-cases. Similarly, model 2 consisted of 313 observations, including 106 cases and 207 non-cases.

Consistent risk factors for both models were clinical coccidiosis and feed equipment breakdowns, which increased the odds for wet litter, and the availability of separate farm clothing, which decreased the odds for wet litter.

In the model where all cases of wet litter were considered (model 1), thinning the flock, using side ventilation systems, an increased number of rodent baits on the farm, using plastic overshoes and having pigs on the farm all increased the odds for wet litter. The association between the number of people working on the farm and the occurrence of wet litter was not linear but could best be represented by introducing the power terms two and three. The association showed an increased odds for wet litter for farms with an increasing number of people working on it with a maximum of six workers after which the odds decreased. A decrease in the odds for wet litter was seen with an increased slaughter age and using birds of a specific breed.

In the model where only those cases of wet litter were considered with a reported disease aetiology (model 2), the use of hand sanitizers and having houses with concrete walls increased the odds for wet litter.

In model 1, the variance estimate of the random effect was very small, indicating that the proportion of variation attributable to poultry company was negligible. In contrast in model 2, 27.3% of the residual variation resided at the company level.

Table 5. 2: Hiearchical multivariable logistic regression model of risk factors associated with the occurrence of wet litter (model 1).

Variable	Coefficient	S.E.	O.R.	95% C.I.	p-value
Coccidiosis in the last flock	1.620	0.654	5.05	1.40-18.19	0.01
Flock was thinned	1.350	0.402	3.86	1.75-8.49	0.001
Side ventilation	0.552	0.236	1.74	1.09-2.76	0.02
Separate farm clothing for each house	-1.116	0.272	0.33	0.19-0.56	<0.001
Feeding equipment failures in the last flock	0.701	0.236	2.02	1.27-3.20	0.003
Number of rodent baits *(continuous)*	0.021	0.007	1.02	1.01-1.04	0.005
Plastic overshoes for each house	0.747	0.235	2.11	1.33-3.34	0.001
Number of people working on the farm *(continuous)*					
Fractional polynomial 2	6.162	2.494	474	3.57-63012	0.01
3	-4.118	1.677	0.02	0.00-0.44	0.01
Age at slaughter *(continuous)*	-0.092	0.022	0.91	0.87-0.95	<0.001
Breed C	-0.794	0.247	0.43	0.27-0.71	0.001
Pigs on the farm	1.424	0.665	4.15	1.13-15.29	0.03
Constant	2.787	1.019	-	-	-
Variance estimate of the random effect: 8.1×10^{-7}	ICC: 2.5×10^{-7}				

Table 5. 3: Hierarchical multivariable logistic regression model of risk factors associated with the occurrence of wet litter with a reported disease aetiology (model 2).

Variable	Coefficient	S.E.	O.R.	95% C.I.	p-value
Coccidiosis in the last flock	4.145	0.917	63.13	10.47-380.48	<0.001
Separate farm clothing for each house	-1.475	0.411	0.23	0.10-0.51	<0.001
Feeding equipment failures in the last flock	1.315	0.320	3.72	1.99-6.98	<0.001
Use of hand sanitizers before entering house					
Never	0	-	1.00	-	-
Occasionally	1.352	0.501	3.87	1.45-10.33	0.007
Always	2.098	0.551	8.15	2.77-24.01	<0.001
Concrete walls	1.299	0.354	3.66	1.83-7.33	<0.001
Constant	-2.921	0.534	-	-	-
Variance estimate of the random effect: 1.238	ICC: 0.273				

Model cross-validation

The cross-validation results for the models for wet litter due to all causes, wet litter with a disease aetiology and wet litter with a disease aetiology after reweighting are shown in Table 5. 4, Table 5. 5, and Table 5. 6, respectively. They are presented as mean coefficients with corresponding standard deviations (SD) and mean sensitivities and specificities of the multivariable models.

The relative magnitudes and signs of the mean coefficients of the cross validation were similar to those obtained by the multivariable logistic regression models and the standard deviations of the coefficients were small, indicating a high reliability of these estimates. Cross validation demonstrated high estimates of sensitivities and specificities for the model of all cases of wet litter, confirming the importance of the risk factors identified by this model. Reweighting improved the sensitivity of the model of wet litter with a disease aetiology from 0.562 to 0.704 while only slightly decreasing the specificity from 0.790 to 0.736.

Table 5. 4: Cross-validation of a multivariable logistic regression model of risk factors associated with wet litter (model 1).

Variable	Mean Coefficient	SD
Coccidiosis in the last flock	0.976	0.0752
Flock was thinned	0.923	0.0752
Side ventilation	0.524	0.0619
Separate farm clothing for each house	-0.774	0.0579
Feeding equipment failures in the last flock	0.609	0.0646
Number of rodent baits *(continuous)*	0.023	0.0025
Plastic overshoes for each house	0.585	0.0631
Number of people working on the farm (*continuous*)		
Fractional polynomial 2	0.350	0.0963
3	-0.434	0.0541
Age at slaughter *(continuous)*	-0.045	0.0029
Breed C	-0.553	0.0643
Pigs on the farm	0.727	0.0727
Model Evaluation		
Sensitivity	0.770	0.0091
Specificity	0.583	0.0102

Table 5. 5: Cross-validation of a multivariable logistic regression model of risk factors associated with wet litter with a reported disease aetiology (model 2), without reweighting.

Variable	Mean Coefficient	SD
Coccidiosis in the last flock	1.759	0.0971
Separate farm clothing for each house	-1.061	0.0718
Feeding equipment failures in the last flock	0.999	0.0875
Use of hand sanitizers before entering house	0.278	0.0343
Never (0); Occasionally (1); Always(2)		
Concrete walls	0.658	0.0701
Model Evaluation		
Sensitivity	0.562	0.0135
Specificity	0.790	0.0117

Table 5. 6: Cross-validation of a multivariable logistic regression model of risk factors associated with wet litter with a reported disease aetiology (model 2), with reweighting.

Variable	Mean Coefficient	SD
Coccidiosis in the last flock	1.926	0.1080
Separate farm clothing for each house	-1.154	0.0813
Feeding equipment failures in the last flock	1.146	0.1005
Use of hand sanitizers before entering house Never (0); Occasionally (1); Always(2)	0.421	0.0418
Concrete walls	0.785	0.0734
Model Evaluation		
Sensitivity	0.704	0.0046
Specificity	0.736	0.0062

Discussion

The results of our survey indicate that wet litter is a common phenomenon in UK broiler flocks with over half (56.1%) of the farm managers who completed the questionnaire reporting this condition in their last flock. This is in contrast with studies carried out in Northern Ireland (McIlroy *et al.*, 1987; Bruce *et al.*, 1990; Menzies *et al.*, 1998) whereby in 1984/1985 15 % of flocks experienced acute outbreaks of wet litter and 35% reported poor litter conditions, in 1986/1987 2% of flocks experienced an acute outbreak of wet litter and 24% reported wet or sticky litter and in 1993/1994 no acute outbreaks of wet litter were reported and poor litter conditions only occurred sporadically. The reduction in prevalence over these three study periods was attributed to better management and improvements in house design, notably a change in the type of drinker system from bell to nipple drinkers (Menzies *et al.*, 1998). This trend away from the use of bell drinkers seems to have continued as only a minority (3.6%) of farm managers in our survey reported to use bell drinkers as opposed to 39.6% using nipple drinkers and 69.0% using nipple drinkers with cups. Several studies have found a significant reduction in water usage and improved litter friability with nipple and small cup drinker systems as compared to bell drinker systems (Bray & Lynn, 1986; Tucker & Walker, 1992). In spite of this, 23.5% of the farmers in our study who identified a cause for the wet litter ascribed it to their drinker system and both bell drinkers and nipple drinkers without cups were associated with wet litter in univariate analysis. However, after adjusting for confounding variables in multivariable analyses, they were not significant risk factors.

The only variable associated with the design of the broiler houses which remained in the multivariable model of all causes of wet litter was side ventilation. This was associated with an increased risk of wet litter and was reportedly used on 36.7% of the farms. Inadequate ventilation can lead to an increased relative humidity inside the house and to incorrect air velocity patterns. If the air speed of incoming air is too slow it will fall to the ground, rather than mixing with air that is already in the roof space, and create patches of condensation on the litter. The use of side ventilation, whereby air is drawn in from one side of the house and is extracted from the opposite side, might be more prone to in-correct air circulation patterns. Inadequate ventilation is particularly a problem in the winter when the incoming air is cold and moist and ventilation may be reduced to conserve heat (Payne, 1967; Tucker & Walker, 1992).This is consistent with the seasonal variation in the prevalence of wet litter reported here and elsewhere (McIlroy *et al.*, 1987; Bruce *et al.*, 1990). However, seasonal patterns in wet litter occurrence could also be explained by seasonal variations in feed composition (Pattison, 1987) or increased susceptibility to disease during the winter months.

The majority of farmers (61%) who identified a cause for the wet litter, attributed it to a disease rather than to a house design or management problem. This suggests that the high prevalence encountered in our survey was not so much caused by a breakdown in litter management techniques, but rather by a disease process whereby large quantities of wet faeces are shed. Indeed clinical coccidiosis, reported in 5.8% of the flocks, was a consistent risk factor in both multivariable models and has been recognised as a cause for diarrhoea in broilers (reviewed by McDougald & Reid, 1997). In recent years, a poorly defined enteritis-like condition has been blamed for the apparent increase in wet litter cases seen on UK broiler farms (Pattison, 2002) and non-specific enteritis was most often mentioned as a cause in our survey. To what extent this condition, which has also been termed 'dysbacteriosis' because of an apparent overgrowth of certain intestinal bacteria, is responsible for the wet litter occurrences in our survey is unclear because both a case definition and a validated method of diagnosis are lacking.

The studies in Northern Ireland (McIlroy *et al.*, 1987; Bruce *et al.*, 1990; Menzies *et al.*, 1998) reported that over 70% of acute outbreaks of wet litter occurred in flocks over 4 weeks of age. Older birds are heavier and will excrete larger volumes of waste products, thereby placing a larger burden on the absorptive capacity of the litter (Menzies *et al.*, 1998). In contrast, in our survey only 11% of wet litter cases were reported in flocks older than 4 weeks and multivariable analysis demonstrated a decreased odds of having wet litter with increased slaughter age . Slaughter weight was not included in the final model because it was highly correlated with slaughter age but a similar effect was seen for both variables in univariate analysis. The fact that wet litter is occurring at a younger age in our study as compared to the studies in Northern Ireland suggests different underlying aetiologies.

Two other variables were consistently present in both multivariable models, namely feed equipment failures and separate farm clothing for each house. Feed equipment failures have not been previously identified as risk factors for wet litter. However, because of the genetic predisposition of modern broilers for hyperphagia (Richards, 2003), periods of feed absence will often be followed by periods of engorgement. Overeating could perhaps lead to digestive disturbances resulting in wet droppings. Also, absence of feed has been demonstrated to lead to increased stress levels of broilers (Scott *et al.*, 1983), possibly leading to maldigestion.

Farms that had farm clothing for each separate house were at a reduced risk for developing wet litter than farms that had no separate clothing available, indicating that biosecurity has a role to play in the epidemiology of wet litter and providing supporting evidence for the possible infectious nature of the condition. It must be noted however, that although 30.2% of farm managers indicated that they had separate farm clothing available for each house, discussions with people familiar with the UK broiler industry suggest that there are very few farms who actually use separate clothing for each house. Two other biosecurity measures, the availability of plastic over-boots for each house (in model 1) and the use of hand sanitizers (in model 2) were positively associated with the occurrence of wet litter. It is possible that farm managers who make the considerable effort to have separate farm clothing for each house, are more likely to practice a high standard of biosecurity. In contrast, hand sanitizers and plastic over-boots are measures that are easy to implement and might be resorted to by farms with histories of disease and/or wet litter. Moreover, they may not be particularly effective;.although several studies have shown that the use of hand sanitizers can be useful in reducing the risk of bacterial contamination, they have primarily been tested on clean hands and are likely to be less effective when used on dirty, greasy hands (Montville *et al.*, 2002). The availability of plastic over-boots for each house does not mean they are used consistently and, depending on the type of over-boot, they are known to easily come off the boot or tear while walking in litter.

'Thinning', that is removing part of the flock for slaughter before the end of the rearing period, has to our knowledge not specifically been mentioned as a risk factor for wet litter . Greene and others (1985), investigating contact dermatitis in broilers, observed that wet litter would tend to get drier after 'thinning', but they did not associate the practice of 'thinning' with the occurrence of wet litter. 'Thinning' has been demonstrated to be a risk factor for the introduction of infectious disease agents (Hald *et al.*, 2001), and to contribute to a breakdown of coccidiosis control because withdrawal periods of the coccidiostatic agents have to be observed (Braunius, 1988). It is conceivable that 'thinning' also leads to higher stress levels among the birds and to a disruption of the house environment while bird loading is taking place. However, the majority of wet litter cases in our survey (98.9%) occurred before the average 'thinning' age (38 days) which is inconsistent with the criterion of causality, where cause should precede the outcome (Evans, 1978). This makes it unlikely that 'thinning' is a major risk factor but suggests that management factors associated with 'thinning' practices such as husbandry or intensity of production may be more likely risk factors. Flocks that are going to be 'thinned' might have higher chick density levels which places a high burden on the absorptive capacity of the litter. Unfortunately, due to too many missing values of the variable 'density', this aspect could not be explored in the present study and will need to be addressed in future studies.

As far as we are aware, no previous studies have looked at the effect of breed on the occurrence of wet litter. In our study, one specific breed had a decreased odds of developing wet litter when all cases were considered (model 1) but not so when only those cases with a disease aetiology were considered. The decreased risk of this hybrid was in agreement with anecdotal reports from the broiler industry at the time of the survey.

An increased odds of wet litter with an increase in the number of rodent baits and an increase in the number of people working on the farm (model 1), could indicate that larger farms are at a higher risk rather than any direct effect of each of these variables. Other indications for this possibility were found in univariate analysis. Increases in the number of houses on the farm, number of chicks placed, number of parent flocks and number of hatcheries that supplied the chicks, and number of feed bins on the farm all increased the odds for wet litter. However, none of these variables remained significant in the multivariable models.

The presence of pigs on the farm was significantly associated with the occurrence of wet litter in our first multivariable model. Although pigs have been implicated as a risk factor for campylobacter infection in broilers (van de Giessen *et al.*, 1996), there is insufficient information available to conclude that pigs present a biosecurity risk in relation to wet litter. They are more likely to represent a proxy variable, in that farms that keep pigs in addition to broilers might be characterised by a specific management type or geographical region.

There was a significant association between house walls made of concrete and wet litter cases with a disease aetiology. Concrete walls, reportedly used on 30.7% of the farms, may be more prone to condensation and damp problems, especially if they are poorly insulated. If this was the case, we would have expected that this variable would also have remained significant in our first model.

We have applied a standard cross-validation technique (Kohavi, 1995) to determine the predictive accuracy of our multivariable logistic regression models. This addresses one of the major criticisms of observational epidemiology; that associations identified in statistical models of cross-sectional data are rarely validated, either by testing them on other data sets or in intervention studies.

In conclusion, we have found that wet litter occurred frequently on UK broiler farms during the time period of our study and seems to have increased in prevalence compared to study periods during the 1980s and 1990s. Although total flock mortality did not seem to be affected by the occurrence of wet litter, the performances of the affected flocks were impaired as indicated by a decrease in EPEF and there was no direct benefit in using antibiotics to try to prevent the condition. Coccidiosis had a strong, consistent association with wet litter, emphasising the continued importance of coccidiosis control. Extended periods of feed absence, such as will happen when feed equipment breaks down, should be avoided and strict adherence to biosecurity measures is an important aspect of wet litter prevention.

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Chapter 6. The epidemiology of coccidiosis in broiler chickens in the UK

Summary

Using information collected for a survey designed to study necrotic enteritis in UK broiler flocks, an assessment was made of the occurrence and risk factors for clinical coccidiosis. Data were collected from 603 conventional broiler farms, owned or contracted by nine UK poultry companies, by means of a postal questionnaire directed at farm managers. One-hundred and sixteen farmers (19.2%; 95% C.I. 16.2-22.7) indicated that they had clinical coccidiosis on their farm during 2001 in at least one crop and 35 farmers (5.8%; 95% C.I. 4.1-8.1) reported clinical coccidiosis in their most recently harvested crop at the time of completing the questionnaire. The disease was reported more often during the colder months of the year (October to March) than during the months April to September. Univariate statistical analysis showed strong associations between the occurrence of coccidiosis and that of necrotic enteritis (OR 6.63, 95% C.I. 2.66-16.51, $p<0.001$) and wet litter (OR=9.14, 95% C.I. 2.81-47.25, $p<0.001$) as had previous multivariable logistic regression models reported elsewhere. Other univariate associations that increased the odds for the occurrence of coccidiosis included larger farms, visits by people into the poultry house other than employees or the area manager, and lorries coming onto the farm. In order to account for the correlated nature of the data, a random effects (cluster-specific) model and a population average model were constructed. Both models demonstrated associations between coccidiosis and visits by representatives of the feed mill and age at slaughter. In addition, the population average model found an increased odds for coccidiosis with an increased number of chicks at placement and a decreased odds for coccidiosis for those farms that had bacterial counts taken prior to the last harvested crop.

Introduction

Avian coccidiosis is caused by protozoa of the genus *Eimeria* of which seven species are known to occur in domesticated fowls (*Gallus gallus*) (Shirley, 1986). It is one the most common diseases of poultry and is almost universal in its occurrence. The disease is of economic importance because its sub-clinical form results in decreased weight gain and egg production and increased feed conversion. Costs for the poultry industry in the UK alone were estimated to be in excess of £38.5 million in 1995 of which 98% involved broilers (Williams, 1999). Clinical outbreaks are presumed rare and usually associated with breakdown in cover by anticoccidial compounds in the feed (Braunius, 1988).

As part of a questionnaire survey on necrotic enteritis (NE) in broilers, information was collected on disease occurrence and associated risk factors on broiler farms in the UK. This information was used to make an initial assessment of the prevalence of coccidiosis on broiler producing farms in this country and to determine what factors were commonly associated with its occurrence.

Materials and Methods

Study design and data collection

The design of this cross-sectional survey has been described in detail in Chapter 4 and briefly was as follows. The study was designed to investigate the occurrence of necrotic enteritis (NE) in broiler flocks in the UK. The sampling frame consisted of 942 company and contract farms, producing broilers for nine UK based poultry companies. Total sample size, obtained by stratified random sampling, was based on an expected prevalence of NE of 5% with 95% confidence limits and 1% precision and consisted of 857 farms.

Data were collected by means of a questionnaire mailed to farm managers during the first week of February 2002. The questionnaire contained a total of 206 questions addressing a wide variety of topics including occurrence, prevention and management of the most important broiler diseases on the farm, farm characteristics such as number, age, and building materials of the houses, flock husbandry, flock performance, climate control, hygiene and biosecurity and feed and water supply. Questions focused on the most recently harvested crop of chickens, encompassing the total number of chickens reared on the farm. Additionally, information was also collected on the occurrence of disease on the farm during the year 2001. The final deadline for the receipt of completed questionnaires was the 20th of May 2002. A copy of the questionnaire can be found in Appendix 2.

To validate disease occurrence, post-mortem reports were collected from company veterinarians and private veterinary practices for those farms that had returned a questionnaire, selecting those reports that covered the farm's most recently harvested crop at the time of the survey.

Statistical analysis

The questionnaire data were entered by a commercial company into a Microsoft Access database and checked and corrected. Statistical analysis was carried out using EpiInfo Version 6 (CDC, Atlanta, Georgia, USA) and Stata 7 (Stata Corporation, College Station, Texas, USA).The occurrence of clinical coccidiosis in the most recently harvested crop was used as dependent variable. Independent dichotomous variables were analysed with 2x2 contingency tables and tested for significance with the chi-squared test or Fisher's exact test. Independent categorical variables were analysed in a univariate logistic regression model, by creating dummy variables. Continuous variables were also analysed using univariate logistic regression and their best fitting form were assessed using the following fractional polynomials: -2, -1, -0.5, 0, 0.5, 1, 2, 3 (Royston *et al.*, 1999). In addition, continuous variables were categorized according to their quartile values to explore the nature of the relationship with the outcome variable (Hosmer & Lemeshow, 2000).

Variables with a statistical significance of $p<0.25$ in the univariate analysis were tested in a multilevel logistic regression model with poultry company as random effect (cluster-specific model). Prior to model building, correlation between all eligible variables was examined and pairs of variables with a correlation of 0.7 or higher were not included in the model at the same time to avoid collinearity. Variables with more than 10% missing values were not included to avoid numerical instability of the model. The model was built manually, starting with variables with the lowest p-values in the univariate analysis. Variables with a term-wise Wald test p-value of ≤ 0.05 or variables that significantly improved the model fit (likelihood ratio chi squared statistic $p \leq 0.05$) were retained in the model. A variable was considered a confounder when its entry into or removal from the model caused a considerable change (>25%) in the coefficient estimates of other variables in the model. Interaction terms were tested for all variables left in the final model and for those that seemed biologically plausible. In the output of the random effects model, Stata includes an estimate of the variance of the random effect and the proportion of the total variance that is accounted for by the random effect (rho). A likelihood ratio test can be used to test the null hypothesis that rho=0.

Diagnostic statistics to evaluate the fit of the model are not available for random effects models in Stata. Instead, it was assumed that the data were not correlated and the fit of a standard logistic regression model containing the same variables was assessed with the Pearson χ^2 statistic, Hosmer-Lemeshow χ^2 statistic, and by determining the area under the receiver operating characteristic (ROC) curve (Hosmer & Lemeshow, 2000).

The quadrature approximation, which is used by Stata to compute the log likelihood in its random effects models, was assessed by re-estimating the model using two different numbers of quadrature points. The coefficient estimates for the original model (with 12 quadrature points) and the two re-estimated models (with 8 and 16 quadrature points) were compared and a relative difference of less than 10^{-2} (1%) between the estimated coefficients was taken as evidence of the validity of the quadrature approach.[1]

Because there was some doubt about the stability of the quadrature approximation, a population average model was constructed using generalized estimating equations (GEE) (Zeger *et al.*, 1988). The modelling procedure was the same as was used for the random effects model but because the GEE approach is not based on likelihood theory, variables were included or excluded from the model on the basis of their

[1] Stata Reference Manual, Release 6, Volume 3 P-St, pp 116-121, Stata Press, College Station, Texas.

Wald test. An exchangeable correlation was assumed. The Hosmer-Lemeshow chi-squared statistic may be used to assess the fit of a population average model provided the intra-cluster correlation coefficient (ICC) is not too large and the model contains sufficient clusters and sufficient observations per cluster (Hosmer & Lemeshow, 2000).

Results

Descriptive epidemiology

One-hundred and sixteen farmers (19.2%; 95% C.I. 16.2-22.7) indicated that they had clinical coccidiosis on their farm during 2001 in a median number of two crops (I.Q.R. 1-3). The monthly frequency distribution of these coccidiosis cases is shown in Figure 6. 1. Coccidiosis occurred significantly more frequently during the months October to March than during the months April to September (z=3.11, p=0.002).

Thirty-five farmers (5.8%; 95% C.I. 4.1-8.1) reported clinical coccidiosis in their most recently harvested crop, occurring at a median age of 25 days (I.Q.R. 21-28; range 15-50). The frequency distribution by poultry company of these cases of clinical coccidiosis is shown in Figure 6. 2. *Eimeria tenella* was identified as the causal organism in 37.1% of the cases. No other coccidial species were reported.

Thirteen cases (37.1%) of clinical coccidiosis in the most recently harvested crop were treated with an anti-microbial compound. In more than half of the cases (n=21; 60.0%), the farm manager was involved in making the diagnosis of coccidiosis, either with a veterinarian (n=4), an area manager (n=4), with both (n=1), or by himself (n=12). Ten cases (28.6%) of coccidiosis were exclusively diagnosed by a veterinarian.

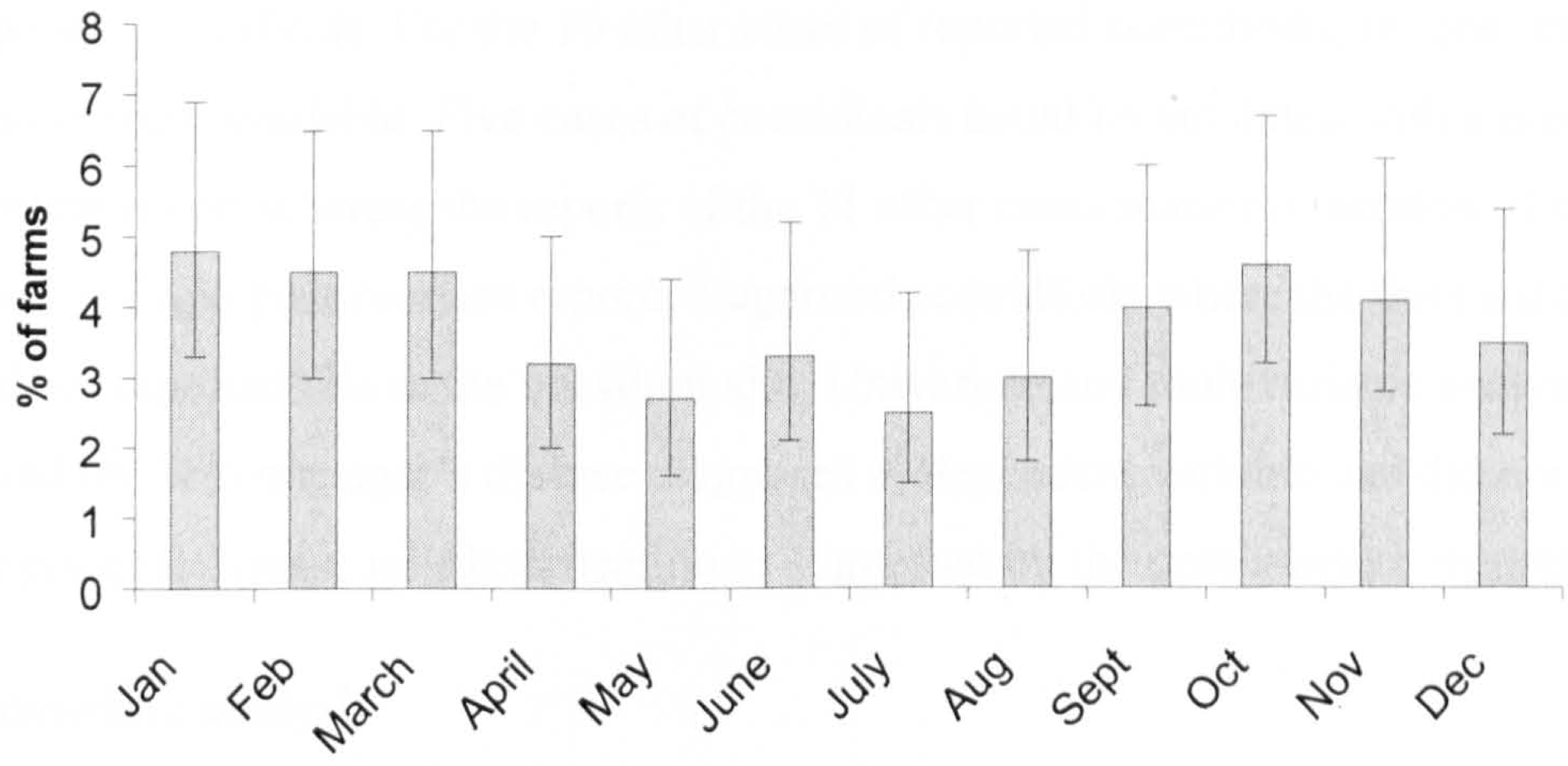

Figure 6. 1: Monthly distribution of farmer reported clinical coccidiosis cases during 2001 (error bars represent 95% confidence intervals).

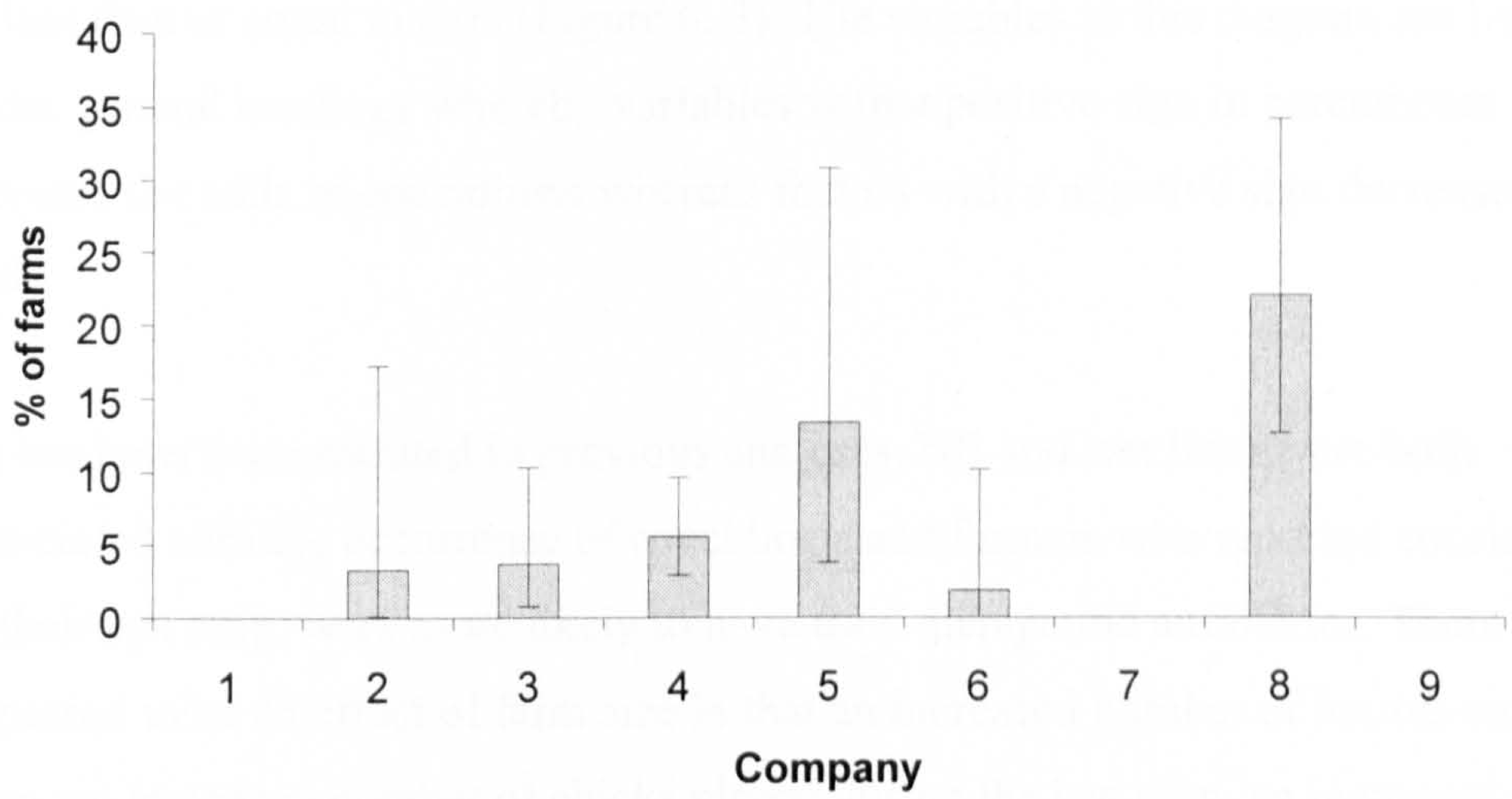

Figure 6. 2: Prevalence of farmer reported clinical coccidiosis cases among nine UK broiler companies (error bars represent 95% confidence intervals).

Disease validation

Post-mortem reports were collected for 123 flocks, 16 of which had farm manager reported coccidiosis. For the 19 other cases of reported coccidiosis, no post-mortem reports were available. Five cases of coccidiosis could be validated with a post-mortem report whereas the reports of the 11 other cases made no mention of the disease. Eight post-mortem reports diagnosed coccidiosis where the farm manager had not reported this in the questionnaire. Univariate and multivariable analyses was based on farm manager's disease diagnoses as dependent variable and did not correct for possible disease misclassification as suggested by the post-mortem reports.

Univariate analysis

Categorical and continuous variables significantly associated ($p \leq 0.05$) with the occurrence of coccidiosis in the most recently harvested crop are listed in Table 6. 1. Full univariate results can be found in Appendix 9. First or second-degree fractional polynomials of the continuous variables are shown if these significantly improved the fit of the univariate logistic regression model ($p \leq 0.05$). On the basis of univariate analysis, a hypothetical causal pathway was constructed of variables with a p-value of less than or equal to 0.05 (Figure 6. 3). The variables in this diagram are listed under general headings whereby variables with a positive sign in parentheses increase the odds of coccidiosis whereas factors with a negative sign decrease the odds.

As has been demonstrated in previous analyses, NE and wet litter were both associated with the occurrence of coccidiosis and farmers who reported coccidiosis in their last crop, were more likely to have used therapeutic antibiotics. There appeared to be an effect of farm size in that an increased number of houses on the farm, an increased number of chicks placed during the last crop, an increased number of hatcheries supplying the chicks, and an increased number of feed lorries coming on the farm all increased the odds for coccidiosis. Hygiene and biosecurity issues that were positively associated with the occurrence of coccidiosis were mostly related to visits by people into the poultry house and lorries on the farm whereas measures such as hand washing, boot dipping and farm clothing did not have a measurable effect on the occurrence of coccidiosis.

Table 6. 1: Univariate associations (p≤0.05) between the occurrence of clinical coccidiosis and explanatory variables.

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Disease					
Wet litter in the last crop					
	Yes	32/310 (10.3)	9.14	2.81-47.25	<0.001
	No	3/242 (1.2)	Ref.	-	-
Necrotic enteritis in the last crop					
	Yes	12/71 (16.9)	6.63	2.66-16.51	<0.001
	No	13/437 (3.0)	Ref.	-	-
Environment					
Number of houses on the farm					<0.001[1]
	1-2	2/143 (1.4)	Ref.	-	-
	3-4	6/147 (4.1)	3.00	0.60-15.12	0.18
	5-7	8/113 (7.1)	5.37	1.12-25.82	0.04
	8-20	19/151 (12.6)	10.15	2.32-44.41	0.002
Number of houses on the farm (*continuous*)		-	1.17	1.07-1.27	<0.001
Age of the newest house					0.04[1]
	<6 months-5 years	9/164 (5.5)	Ref.	-	-
	6-11 years	2/109 (1.8)	0.32	0.07-1.52	0.15
	12-26 years	13/137 (9.5)	1.81	0.75-4.36	0.19
	27-60 years	11/131 (8.4)	1.58	0.63-3.93	0.33
Average age of the houses					0.007[1]
	6 months-9 years	2/124 (1.6)	Ref.	-	-
	10-18 years	14/126 (11.1)	7.63	1.70-34.30	0.008
	19-30 years	9/127 (7.1)	4.65	0.98-21.98	0.05
	31-60 years	4/113 (3.5)	2.24	0.40-12.46	0.36
Roof ventilation					
	Yes	18/372 (4.8)	0.50	0.24-1.05	0.04
	No	17/183 (9.3)	Ref.	-	-
Side ventilation					
	Yes	19/204 (9.3)	2.15	1.02-4.55	0.03
	No	16/351 (4.6)	Ref.	-	-
Automatic controlled natural ventilation					
	Yes	1/96 (1.0)	0.13	0.01-0.92	0.02
	No	34/459 (7.4)	Ref.	-	-
Use of cooling devices					
	Yes	4/21 (19.0)	3.80	1.00-13.18	0.01
	No	31/532 (5.8)	Ref.	-	-
Use of low energy light bulbs					
	Yes	21/235 (8.9)	2.14	1.01-4.59	0.03
	No	14/320 (4.4)	Ref.	-	-
Wooden walls					
	Yes	29/356 (8.1)	2.82	1.12-8.46	0.02
	No	6/197 (3.0)	Ref.	-	-
Husbandry/management					
Number of chickens placed in the last crop					0.02[1]
	8300-46500	4/137 (2.9)	Ref.	-	-
	47000-90000	7/129 (5.4)	1.91	0.55-6.68	0.31
	90200-144000	7/138 (5.1)	1.78	0.51-6.21	0.37
	144200-582260	17/142 (12.0)	4.52	1.48-13.81	0.008
Number of chickens placed in the last crop (*continuous, per 10000*)		-	1.06	1.03-1.10	<0.001
Number of hatcheries that supplied the chicks for the last crop					0.04[1]
	1	16/327 (4.9)	Ref.	-	-
	2-9	19/201 (9.5)	2.03	1.02-4.04	0.04

Table 6. 1 (continued)

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Husbandry/management					
Number of hatcheries that supplied the chicks for the last crop (*continuous*)		-	1.78	1.15-2.75	0.01
Age at slaughter (days) of the last crop					0.003[1]
	37-42	2/107 (1.9)	Ref.	-	-
	43-47	18/163 (11.0)	6.52	1.48-28.70	0.01
	48-52	9/112 (8.0)	4.59	0.97-21.75	0.06
	53-72	5/159 (3.1)	1.70	0.32-8.95	0.53
Age at slaughter (days) of the last crop (*continuous*)		-	0.99	0.93-1.06	0.81
Fractional polynomial: -2		-	-	-	0.01
-2		-	-	-	0.01
Average weight at slaughter (grams) of the last crop (*continuous, per 100 gram*)		'-	0.94	0.88-1.00	0.05
Changes in management or employees during the last crop					
	Yes	5/34 (14.7)	2.83	0.88-8.48	0.04
	No	30/522 (5.7)	Ref.	-	-
Hygiene & biosecurity					
Who cleaned the houses before the last crop					0.006[1]
	Contract cleaner	30/357 (8.4)	Ref.	-	-
	Company cleaner	1/37 (2.7)	0.30	0.04-2.29	0.25
	Farm manager	2/127 (1.6)	0.17	0.04-0.74	0.02
Who disinfected the houses before the last crop					0.01[1]
	Contract cleaner	28/348 (8.0)	Ref.	-	-
	Company cleaner	1/42 (2.4)	0.28	0.04-2.10	0.22
	Farm manager	3/141 (2.1)	0.25	0.07-0.83	0.02
How were the houses disinfected					0.04[1]
	Spray disinfectant	9/235 (3.8)	Ref.	-	-
	Fumigation	0/5 (0)	-	-	-
	Both	24/294 (8.2)	2.23	1.02-4.90	0.05
Were bacterial counts taken from inside the house before the last crop					
	Yes	7/223 (3.1)	0.36	0.14-0.89	0.01
	No	25/300 (8.3)	Ref.	-	-
Is cattle kept on the farm					
	Yes	3/139 (2.2)	0.26	0.06-0.93	0.02
	No	32/414 (7.7)	Ref.	-	-
Is litter disposed of to a power plant					
	Yes	19/212 (9.0)	2.01	0.96-4.25	0.04
	No	16/343 (4.7)	Ref.	-	-
Is cow manure spread on fields of the farm					
	Yes	4/154 (2.6)	0.31	0.09-0.96	0.02
	No	30/381 (7.9)	Ref.	-	-
Is cow manure spread on fields adjoining the farm					
	Yes	8/259 (3.1)	0.31	0.13-0.76	0.004
	No	23/249 (9.2)	Ref.	-	-
Did people other than the farm manager, employees or area manager enter the house during the last crop					
	Yes	25/275 (9.1)	2.72	1.21-6.24	0.007
	No	10/282 (3.5)	Ref.	-	-

Table 6. 1 (continued)

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Did a feed representative enter the house during the last crop					
	Yes	4/15 (26.7)	5.91	1.47-21.98	0.001
	No	31/535 (5.8)	Ref.	-	-
Did an electrician enter the house during the last crop					
	Yes	22/207 (10.6)	3.03	1.41-6.57	0.001
	No	13/344 (3.8)	Ref.	-	-
Did hatchery lorries come on the farm during the last crop					
	Yes	31/537 (5.8)	0.25	0.07-1.07	0.01
	No	4/20 (20.0)	Ref.	-	-
Did oil lorries come on the farm during the last crop					
	Yes	19/211 (9.0)	2.03	0.95-4.36	0.04
	No	15/323 (4.6)	Ref.	-	-
Number of feed lorries that came coming on the farm during the last crop					0.04[1]
	1-10	4/125 (3.2)	Ref.	-	-
	11-17	9/130 (6.9)	2.25	0.67-7.50	0.19
	18-26	5/124 (4.0)	1.27	0.33-4.85	0.73
	27-96	15/133 (11.3)	3.85	1.24-11.92	0.02
Number of feed lorries that came on the farm during the last crop (*continuous*)		-	1.03	1.01-1.06	0.001
Feed and water					
Feed equipment failures during the last crop					
	Yes	20/207 (9.7)	2.54	1.18-5.48	0.008
	No	14/346 (4.0)	Ref.	-	-
Use of feeders other than flat chain/auger, pan or tube	Yes	2/4 (50.0)	15.54	1.10-153.20	<0.001
	No	33/553 (6.0)	Ref.	-	-
Number of feed bins on the farm					0.02[1]
	1-3	3/105 (2.9)	Ref.	-	-
	4-6	2/107 (1.9)	0.65	0.11-3.96	0.64
	7-9	17/193 (8.8)	3.28	0.94-11.48	0.06
	10-40	12/148 (8.1)	3.00	0.83-10.91	0.10
Number of feed bins on farm (*continuous*)		-	1.05	0.99-1.11	0.13
Fractional polynomial: -1		-	0.69	0.49-0.98	0.04
Maximum (%) amount of whole wheat added during the last crop					
	≤20	29/299 (9.7)	1.00	-	-
	>20	1/81 (1.2)	0.12	0.02-0.87	0.04
Maximum (%) amount of whole wheat added during the last crop (*continuous*)		-	0.89	0.84-0.95	<0.001
Use of bell drinkers	Yes	5/22 (22.7)	4.93	1.46-15.70	0.001
	No	30/533 (5.6)	Ref.	-	-
Has the drinking water been analysed					
	Yes	13/301 (4.3)	0.43	0.19-0.95	0.02
	No	18/188 (9.6)	Ref.	-	-
Has the drinking water been analysed bacteriologically	Yes	8/227 (3.5)	0.37	0.15-0.91	0.02-
	No	23/258 (8.9)	Ref.	-	
Prevention/treatment					
Curative antibiotics use during the last crop					
	Yes	18/208 (8.7)	2.03	0.93-4.46	0.05
	No	14/314 (4.5)	Ref.	-	-

[1] Likelihood ratio test statistic

Figure 6. 3: Hypothetical causal pathway of clinical coccidiosis based on univariate risk factors ($p \leq 0.05$). Dotted arrows represent unexplained associations.

Multivariable analysis

The final hierarchical logistic regression model with clinical coccidiosis as dependent variable and poultry company as random effect, based on 535 observations, is shown in Table 6. 2. Because associations between coccidiosis and NE and coccidiosis and wet litter have been demonstrated previously in multivariable analyses (Chapter 4 & 5 respectively), these variables were not included in the multivariable models.

Age at slaughter of the most recently harvested crop was both significant as a categorical variable and after transformation of the continuous variable with the power terms –2 –2. Inclusion of the fractional polynomials produced a model with a significantly lower deviance than the model containing the categorical variable (likelihood ratio test $\chi^2=5.56$; p=0.02). The relationship between the continuous variable 'age at slaughter' and the logodds of coccidiosis is shown in Figure 6. 4 as an univariable locally weighted smoothed scatterplot (lowess) on the logit scale (Cleveland, 1979).

The proportion of the total variance attributable to poultry company was highly significant (p<0.001). Model diagnostic tests based on a non-hierachical logistic regression model with the same variables, resulted in a Pearson $\chi^2=86.9$ (p<0.001), Hosmer-Lemeshow $\chi^2=11.58$ (p=0.12) and an area under the ROC curve of 0.70. Re-estimation of the random effects model with different quadrature points resulted in relative differences between the original coefficients and the re-estimated coefficients slightly greater than 1% (data not shown).

Table 6. 2: Hierarchical multivariable logistic regression model of risk factors associated with the occurrence of clinical coccidiosis.

Variable	Coefficient	S.E.	Odds Ratio	95% C.I.	p-value
Visit(s) of a feed representative into the poultry house	2.440	0.708	11.47	2.86 – 46.05	0.001
Average age at slaughter (days) of the last crop *(continuous)*					
Fractional polynomial: -2	-1270.144	593.398	-	-	0.032
-2	1133.118	561.365	-	-	0.044
Constant	-2.335	0.384	-	-	-
Variance estimate of the random effect: 2.103 rho=0.391 LRT of rho=0 $\chi^2=17.74$ p<0.001					

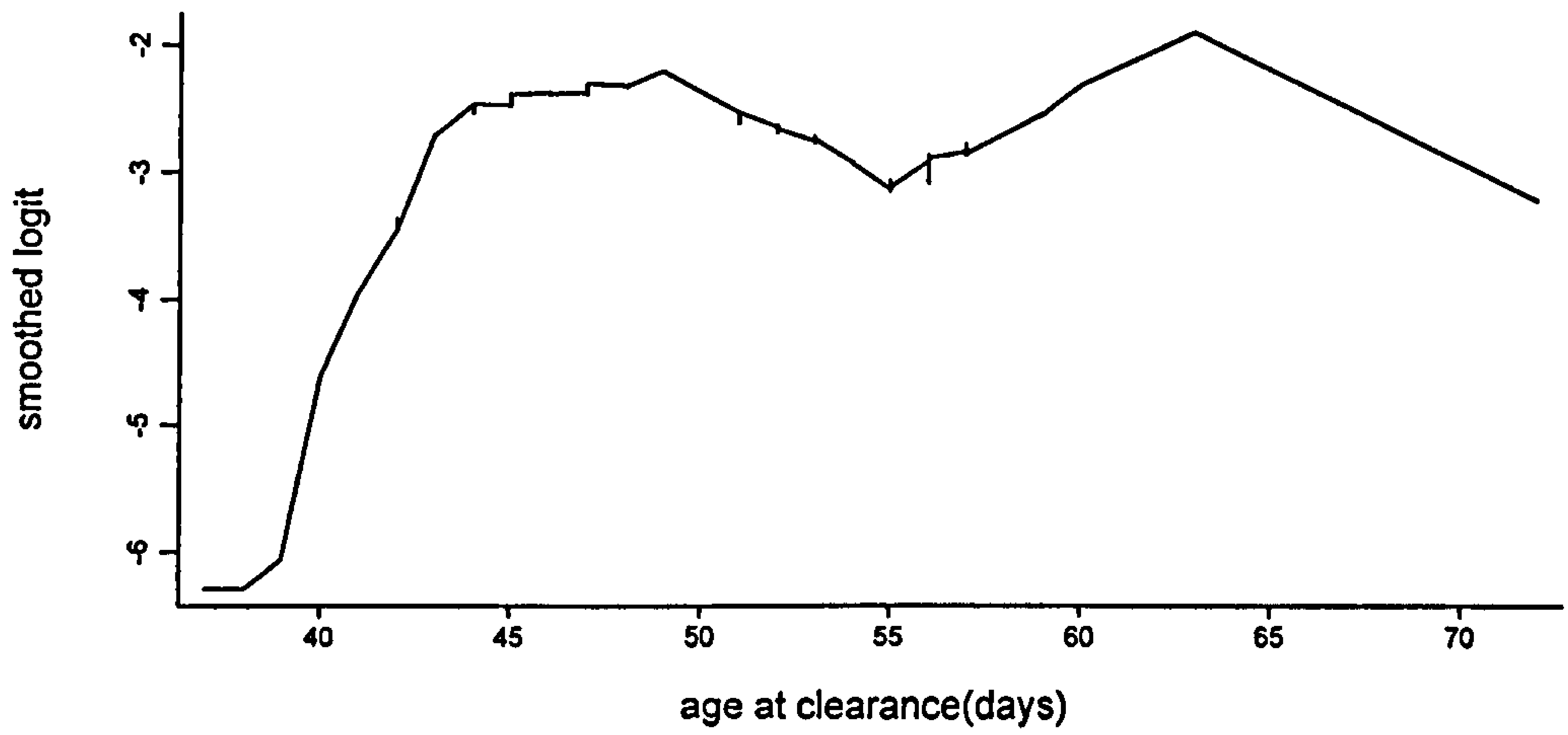

Figure 6. 4 Univariate locally weighted smoothed scatterplot of the relationship between clinical coccidiosis and age at slaughter.

The less than optimal fit of the cluster-specific model and the apparent instability of the quadrature approximation prompted us to re-model the data using a population averaged approach. The obtained model, based on 496 observations, was similar to the random effects model except that it contained two extra variables; number of chicks placed during the last crop and if bacterial counts were taken from the inside of the house prior to the last crop (Table 6. 3). Because the number of chicks placed during the last crop was correlated with the total number of houses on the farm (correlation coefficient 0.73) and the number of feed lorries coming on the farm (correlation coefficient 0.84), they were modelled separately from each other to avoid collinearity. The resulting three models contained the same variables and the regression coefficients did not differ in sign and by less than 15% in magnitude. For this reason, only the model containing the total number of chicks is presented here. Because the ICC was not excessively large (0.39) and there were nine clusters with a minimum of 18 observations per cluster, it was felt that the Hosmer-Lemeshow test could be used to assess the fit of the model. The resulting p value (0.90) provides evidence that the model adequately fits the data.

Table 6. 3: Population average model with exchangeable correlation of risk factors associated with the occurrence of clinical coccidiosis.

Variable	Coefficient	S.E.	Odds Ratio	95% C.I.	p-value
Visit(s) of a feed representative into the poultry house	2.100	0.676	8.17	2.17 – 30.69	0.002
Age at slaughter (days) of the most recently harvested crop *(continuous)*					
Fractional polynomial: -2	-1498.919	676.959	-	-	0.027
-2	1410.553	639.408	-	-	0.027
Total number of chicks *(continuous, per 10000)*	0.052	0.018	1.05	1.02 – 1.09	0.004
Bacterial counts taken	-1.065	0.471	0.34	0.14 – 0.87	0.024
Constant	-2.453	0.444	-	-	-
Hosmer-Lemeshow χ^2 =3.45, p=0.90					

Discussion

Almost six percent of farm managers responded positively when asked if clinical coccidiosis occurred in their most recently harvested crop, albeit only a relatively small percentage of these reported cases (14%) could be validated with a veterinary post-mortem report. Although 'clinical coccidiosis' was not defined in our questionnaire, it is assumed that farmers could make the distinction between cases of coccidiosis with manifest clinical signs, and sub-clinical cases that can only be detected through routine post-mortem inspections. However, it cannot be ruled out that our prevalence figure includes some of these latter cases. Most on-farm surveys of coccidiosis in poultry rely on the detection of oocysts in faecal or litter samples and/or on intestinal lesion scores (Williams *et al.*, 1996; McDougald *et al.*, 1997; Razmi & Kalideri, 2000; Al-Natour *et al.*, 2002). More often than not therefore, these surveys measure sub-clinical coccidosis and as such the obtained frequency measures are not comparable to the prevalence figure obtained in our study. Judging from these surveys, sub-clinical coccidiosis is very common, with broiler farm prevalence figures ranging from 38% in Iran (Razmi & Kalideri, 2000), 50% to 63% in the Netherlands (Braunius, 1988; Graat *et al.*, 1998), 78% in Jordan (Al-Natour *et al.*, 2002) to 88% in Argentina (McDougald *et al.*, 1997).

Fewer studies have addressed the occurrence of clinical coccidiosis, usually by monitoring consignments of birds submitted to veterinary diagnostic laboratories. Lee & Onderka (1978) reported that 406 consignments of chickens which were submitted to the regional laboratories of the Ontario Veterinary Services Branch during 1973 to 1977 were diagnosed with clinical coccidiosis, but they failed to provide information on the total number of consignments or the size of the population from which this sample was drawn. Lundén *et al* (2000) found that 11 of 57 flocks (19%) of laying hens followed between 1992 and 1996 suffered an outbreak of clinical coccidiosis during their production time.

As was the case for NE (chapter 4) and wet litter (chapter 5), farmers reported significantly more occurrences of coccidiosis during the colder months of the year (October to March). Similar seasonal observations were made in the Netherlands (Braunius, 1988; Graat *et al.*, 1998) but in Canada coccidiosis was more frequently diagnosed during the months May to October (Lee & Onderka, 1978). It is generally believed that humid conditions inside the poultry house, such as are common in the UK and the Netherlands during the winter months, favour outbreaks of coccidiosis due to an increased sporulation and survival of oocysts within the litter, thereby increasing the chances of infection. Lee & Onderka (1978) ascribed the increased coccidiosis prevalence from May to October in Ontario to warmer and more humid conditions during these months. In countries with defined seasonal rainfall, coccidiosis appears to occur more often during the rainy seasons (Maungyai *et al.*, 1990; Razmi & Kalideri, 2000). However, several controlled experiments have failed to confirm a positive relationship between litter moisture and sporulation rates. Graat *et al* (1994) found no difference between the maximum percentage of *E. acervulina* oocysts that sporulated in dry or clammy litter although onset of sporulation was earlier at higher temperatures and higher relative humidities. Waldenstedt *et al* (2001) found that sporulation of *E. maxima* oocysts was most efficient under the driest conditions studied whereas the least sporulation was observed in the samples with the highest moisture content. They and others (Williams, 2005) suggested that high moisture levels in the litter encourage bacterial growth leading to low oxygen levels which limit sporulation and high ammonia levels which exert a lethal effect on the oocysts.

Our survey found a strong association between the occurrence of wet litter and that of coccidiosis in univariate analysis (OR=9.14, 95% C.I. 2.81-47.25, $p<0.001$) which remained highly significant in a multilevel logistic regression model with the occurrence of wet litter as outcome variable (Chapter 5). The location of these variables on a possible causal pathway is unknown although the median age of occurrence of wet litter of 24 days compared to that of 25 days for coccidiosis might suggest that wet litter precedes coccidiosis. In light of the experimental results discussed above, the observed association between wet litter and coccidiosis might not be due to a favourable effect of the litter moisture on the sporulation and infectivity of the oocysts, and alternative explanations should be explored.

The occurrence of coccidiosis was also associated with another important enteric disease of broilers, namely NE (OR 6.63, 95% C.I. 2.66-16.51, $p<0.001$). Previous analysis (Chapter 4) had demonstrated this to be a significant variable in a multivariable model with NE as outcome and therefore NE was not included in the multivariable models described in this paper. As is the case for wet litter, we can only guess at the direction of the association between coccidiosis and NE although a median age of occurrence of 25 days for coccidiosis and 26 days for NE, combined with experimental evidence suggests that coccidiosis precedes NE (Al-Sheikhly & Al-Saieg, 1980; Shane *et al.*, 1985). Williams (2005) argued that on a purely patho-physiological basis NE seems unlikely to predispose chickens to coccidiosis because the destruction of enterocytes removes potential development sites for coccidia and the presence of a diphtheritic membrane obstructs the intraluminal dissemination of extracellular coccidial stages.

Apart from its association with NE, coccidiosis is often reported to occur more frequently or more severely in conjunction with other diseases, in particular those that have a suppressive effect on the host's immune status such as Marek's disease and infectious bursal disease (IBD) (Biggs *et al.*, 1968; McDougald *et al.*, 1980). No such associations were detected in this survey although it must be noted that only a very small proportion of the respondents (n=9; 1.5%) reported an immuno-suppressive disease in their most recently cleared crop.

Two risk factors were significant in both our cluster-specific model and our population average model; visits by a feed representative and age at slaughter. It is unclear whether the observed increased odds for coccidiosis because of one or more visits by a feed representative inside the poultry house is due to an increased biosecurity risk or whether these visits represent a proxy measure for some other unmeasured risk factor, possibly related to feed. Alternatively, the association could be explained in that flocks with outbreaks of clinical coccidiosis could warrant visits by representatives of the feed mill in order to review the efficacy of the anticoccidial agents which had been in use. Biosecurity issues have been identified as risk factors in a previous paper addressing the epidemiology of coccidiosis in broiler chickens (Graat *et al.*, 1998). It observed that the admittance of visitors to the farm, including an increased number of veterinary visits to the farm during the grow-out period, and employing staff who might also work on other farms increased the risk for *E. acervulina* infection in a multivariable model. The same model also included an effect of feed mill, most likely associated with the type of anticoccidial agents and associated shuttle programmes utilised by the different mills. Insufficient data on feed composition were available in the present study to investigate the role of coccidiostatic agents or indeed other feed components.

The second common variable in both our multivariable models was the age at which the last birds of the crop were sent for slaughter. In a univariable context, this variable does not present a linear relationship with the outcome variable but rather shows a sharp increase in the odds for disease up to a slaughter age of approximately 45 days after which the risk levels off (Figure 6. 4). Presumably, flocks that are slaughtered at an older age are exposed to the disease agent for a longer time and are therefore at a higher risk to develop coccidiosis. Why this relationship does not hold true for flocks that are kept beyond 45 days of age, resulting in the flattened curve as shown in Figure 6. 4, is not directly apparent but could be associated with different management practices of flocks that are kept until an older age. In particular feeding regimes and bird densities are likely to be different for these types of flocks and might contribute to a lessened risk which offsets the increased risk of a longer exposure time. Insufficient information was available on feeding regimes to explore the possible association with age at slaughter. The variable 'density' contained more than 10% missing values and was therefore not included in the multivariable model.

No significant association was found between density and coccidiosis in univariate analysis.

Observational studies of infectious diseases of intensively reared livestock often identify the size of a farm as a risk factor (Refregier-Petton *et al.*, 2001; Thomas *et al.*, 2005). Perhaps this can be explained by increased exposure to the infectious agent due to increased movements and contacts on and off the farm. Farm size has also been implicated as a risk factor in epidemiological studies of coccidiosis (Braunius, 1980; Razmi & Kalideri, 2000) although several other papers reported no association (Henken *et al.*, 1992; Graat *et al.*, 1998; Al-Natour *et al.*, 2002). In our survey, no variables associated with farm size remained significant in the cluster-specific model, but in the population average model an increase in the number of chicks placed, the number of houses on the farm and the number of feed lorries coming on the farm were all associated with an increase in the odds for coccidiosis, albeit in separate models.

A variable which was only significant in the population average model was bacterial counts taken inside the house prior to the last harvested crop. The fact that farms on which bacterial counts were taken were less likely to report coccidiosis may well be a proxy measure for farms that have superior hygiene measures in place.

Coccidiosis lends its importance in the poultry industry to the great economic losses that are associated with its occurrence. Williams (1999) estimated that 81% of these losses are due to the effects on mortality, weight gain and feed conversion. In a case-control study (Graat *et al.*, 1998), a 12% decrease in feed conversion and a 5% decrease in daily weight gain was found in *Eimeria*-positive flocks as compared to negative control flocks. In the present study, no associations were detected between coccidiosis positive flocks and the production parameters total mortality, feed conversion or EPEF. A possible explanation for this could be that our survey focused on clinical coccidiosis cases, which inherently are treated more readily with anti-coccidial agents than sub-clinical cases of coccidiosis, thereby mitigating the potential damaging effects of the infection.

In conclusion, we have provided an initial assessment of the occurrence of clinical coccidiosis in broiler flocks in the UK during 2001. It must be noted that this study was not specifically designed to investigate this particular disease but was part of a larger inquiry into enteric disorders of broilers. As such, the results must be treated with caution but may provide a starting point for future observational studies in the UK.

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Chapter 7: General discussion

The application of veterinary epidemiology towards animal disease has as ultimate aims to safe-guard animal welfare and to protect public health. Both of these aspects are highly relevant with regard to the study of NE. The systematic review of case-reports of NE demonstrated that mortality was a consistent feature of the disease (Chapter 2) and an observational study found a strong association between the occurrence of NE and total flock mortality (Chapter 4). Thus, the welfare of UK broilers is compromised by NE associated mortality.

The consistent isolation of *Clostridium perfringens* from cases of NE has potential implications for public health in that certain enterotoxin producing strains are capable of causing food poisoning in humans (Niilo, 1978). More important perhaps, is the widespread use of antimicrobial compounds in animal feed to stimulate growth and to prevent diseases such as NE. Increasing concerns over the development of multi-resistant bacterial strains because of this practice has led to the withdrawal of many of these compounds from animal feed in recent years which in turn has led to speculations of increased disease incidence and increased therapeutic drug use. This ongoing debate is not the focus of the present thesis and interested readers are referred elsewhere (Wierup, 2001; Casewell *et al.*, 2003; Phillips *et al.*, 2004; Turnidge, 2004). It must be appreciated however that discussions such as these can only be settled with adequate data on disease occurrence coupled with information on prophylactic and therapeutic drug use. In this respect, observational studies such as described in this thesis can be important measuring tools.

Another reason for the usefulness of observational studies in the investigation of NE is to aid in the formulation of intervention strategies. In order to become less reliant on anti-microbial compounds to control NE, alternative control strategies are needed and this requires knowledge of the risk factors for the disease. Although putative risk factors can be tested in controlled experimental trials, the interaction of many potential causal factors can often only be adequately assessed in observational studies.

A cross-sectional study design was used to determine the prevalence and risk factors for the major enteric disorders in broiler chickens. This type of observational study is relatively easy and inexpensive to conduct and is often an important precursor to subsequent longitudinal studies when little is known about the frequency of the disease in a specific population. Furthermore, it can generate important hypotheses about the possible causes of disease. There are also several disadvantages associated with cross-sectional studies. It is often difficult to differentiate between cause and effect because both the exposure and outcome are measured at the same time. This is especially important for exposures which are subject to change as opposed to exposures which are more time-invariant such as building materials of the poultry houses, number of houses on the farm or other poultry farms in the neighbourhood. Several examples for this so-called reverse–causation problem were found in this study and it was particularly relevant with the detected associations between NE and wet litter, NE and coccidosis, and wet litter and coccidosis.

Like all observational studies, cross-sectional studies are prone to bias and confounding and particular attention was paid during the design and analysis of the study described in this thesis to minimise these aspects. By trying to take a random sample of the UK broiler population it was hoped that selection bias would be minimal. The chosen sampling frame for this study consisted of farms growing broilers for the major integrated poultry companies in the UK. Two of the eleven companies that were approached declined to participate in this study and no information on these companies was available to determine if their absence represented a significant form of bias. In addition, farms that operated outside the integrated industry were also not included and these might well be very different with regard to disease occurrence and management factors than farms operating within integrated companies. Another form of selection bias might have been introduced by the proportion of selected farms which did not return the questionnaire. By maximising the response through follow-up with reminder cards and second mailings of the questionnaire it was hoped that this non-response bias was minimal. However, a brief analysis of non-responder characteristics indicated that these farms tended to be smaller in size than responding farms and farm size was often associated with the analysed outcomes.

Information bias is introduced into a cross-sectional study by misclassification of the outcome and/or exposures. Because we relied to a large extent on farmer reported disease occurrence, there was considerable room for error in the accuracy of diagnoses and hence scope for misclassification of the outcome. The nature (differential or non-differential) and extent of the misclassification of disease status in this study is unclear and consequently so are the effects of the misclassification on the apparent disease prevalence and the reported associations between exposure factors and the outcome.

Validation of farmer reported disease was attempted in several ways. Veterinary post-mortem reports were consulted to validate NE and coccidiosis diagnoses. However, post-mortem reports could only be obtained for relatively few crops in which disease had occurred (26% and 46% for crops with outbreaks of NE and coccidiosis respectively) and even less cases could be confirmed. It appears therefore that the majority of disease diagnoses in broilers is not accompanied by post-mortem reports and might be taking place on farms rather than in diagnostic laboratories. An attempt was also made to validate the accuracy of NE diagnoses by collecting intestinal and liver samples of birds with clinical signs of NE and examining these histologically. The protocol of this collection scheme can be found in Appendix 10. Unfortunately, the response rate for this scheme by the participating veterinary surgeons was too low to draw conclusions and the collected data was not incorporated into this thesis.

There are some indications that misclassification of exposure status occurred in this study. For instance, although 30.2% of farm managers indicated that they had separate farm clothing available for each house, a factor which decreased the odds for the occurrence of wet litter, informal feedback from poultry experts suggested that there are very few farms in the UK where this is actually the case. External validation of exposure factors was not carried out in this study although some data had been collected for this purpose. By repeating some of the questions in the questionnaire under a different topic and in a different format, we obtained a measure of the internal validity. For example, when asked in two separate sections of the questionnaire about the number of feed lorries visiting the farm during the last harvested crop, the agreement between the responses to the two questions was 67.4%.

The issue of confounding was tackled by collecting information on a wide range of variables and by using multivariable analysis techniques. Despite these precautions, confounding of the variables in the multivariable models by some unmeasured exposure factors can never be completely ruled out.

Conducting a study in a highly integrated industry such as the poultry industry presented its own set of unique challenges. Farms producing broilers for a specific company are often operated according to company protocol, potentially leaving little scope for variation beyond company level. Indeed, using multi-level modelling techniques it was found that statistically significant proportions of the variation was attributed to company for all analysed outcomes except that of wet litter due to all causes. Alternative approaches to dealing with clustered data are available, including estimating robust standard errors and using generalised estimating equations (GEE) (Zeger *et al.*, 1988) and in recent years Bayesian statistics has gained in popularity, in particular by the application of Markov chain Monte Carlo (MCMC) estimation (Dohoo *et al.*, 2001). The analysis described in Chapter 6 of this thesis threw some doubt on the validity of the random effects model as computed by Stata and a population-averaged model using GEEs was adopted instead.

Confidentiality issues are another aspect which perhaps directly result from the integrated nature of the UK broiler industry. Although confidentiality of the information which was collected was assured at all levels, some data was extremely difficult to obtain. In particular this was true for information on feed rations. It seems reasonable to assume that feed has an important role to play in the pathogenesis of enteric disorders but because of our inability to obtain detailed feed data, this could not be confirmed in this study. Future studies will need to devise strategies to circumvent this confidentiality hurdle in order to investigate in detail the effects of feed components on the occurrence of NE and wet litter in particular.

Conclusions

It stands to reason that the control of disease in food animals should increasingly be based on preventive management strategies such that the use of antimicrobial compounds can be reduced. To achieve this goal, an in-depth knowledge of the prevalence and risk factors for disease is necessary. Based on this premise, an epidemiological study of NE was initiated which subsequently also encompassed wet litter and coccidiosis. The study consisted of two components: first, the construction of an evidence-based case definition for NE as an aid to review putative risk factors from experimental studies, and secondly the collection of field observations.

Traditional literature reviews are increasingly being critiqued for their lack of transparency in identifying, selecting and validating included information and for their vulnerability to bias (Mulrow, 1994). Reviews based on the methodology developed at the Cochrane Collaboration address these shortcomings. The systematic reviews described in this thesis not only resulted in an evidence-based case definition of NE and a detailed examination of the merits of experimental risk factors for the disease, they also demonstrated the importance of consistent scientific standards while studying this disease. It is hoped that as future literature reviews will increasingly adopt the 'systematic' approach, the need for studies adhering to rigorous scientific standards will become more apparent.

Our cross-sectional study resulted in the first population-based prevalence estimates of NE and wet litter in UK broiler flocks. These estimates and the demonstrated risk factors for these conditions are in obvious need of validation but may provide important information for future epidemiological study design.

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Appendix 1: Cover letter accompanying postal questionnaire.

Department of Veterinary Clinical Science
and Animal Husbandry

Faculty of Veterinary Science

Leahurst
Chester High Road
Neston
CH64 7TE

Telephone: 0151 794 7582
Facsimile: 0151 794 6028

Leahurst, 28 January 2002.

RE: STUDY ON WET LITTER AND ENTERITIS IN BROILERS.

Dear Sir/Madam,

The broiler industry in the UK faces many new challenges. One of these is the emergence of new or re-appearing diseases. Lately, wet litter and enteritis seem to be occurring more frequently. It is important that we investigate these diseases in order to be able to prevent them.

In the epidemiology group at Liverpool Veterinary School we are conducting a study with this purpose in mind. ---------- has recognized the importance of this study and has provided us with a list of their broiler growers. Your farm has been selected from this list and accompanying this letter you will find a questionnaire which we would like you to fill in. It is important that you complete this questionnaire even if you have not experienced wet litter or enteritis on your farm.

The information collected in this study will be strictly confidential. In any scientific publication or report from this work, it will not be possible to identify individual farms, individual farmers or individual companies. We have enclosed a self-addressed freepost envelope in which you can send the questionnaire directly back to us. We would like to receive the questionnaire no later than the 1st of March, 2002.

We hope you will able to participate as the information you provide us with will enable us to come up with strategies to prevent wet litter and enteritis in broilers. If you require any further information please contact Patrick Hermans at telephone number 0151-794-6079 or email address hermans@liv.ac.uk.

Many thanks for your co-operation.

Yours faithfully,

Patrick Hermans

Professor Kenton Morgan

iversity switchboard
ephone: 0151 794 2000
simile: 0151 708 6502

Appendix 2: Postal questionnaire.

THE UNIVERSITY
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STUDY ON WET LITTER AND ENTERITIS IN BROILERS.

QUESTIONNAIRE.

Epidemiology Group
Department of Veterinary Clinical Science
& Animal Husbandry
Faculty of Veterinary Science

Farm ID No.

Section A: Disease occurrence on the farm.

I. Wet litter.

1. Has wet litter occurred on your farm since January 1, 2001? **(please tick ☑ in correct box)** — ☐Yes ☐ No ☐ Don't know

IF YES CONTINUE
IF NO OR DON'T KNOW GO TO QUESTION 9.

2. In how many crops has wet litter occurred since January 1, 2001? **(please tick ☑ in correct box)** — ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8

3. In which months did wet litter occur? **(please tick ☑ in more than one box if appropriate)** — ☐ Jan ☐ May ☐ Sept ☐ Feb ☐ June ☐ Oct ☐ Mar ☐ July ☐ Nov ☐ Apr ☐ Aug ☐ Dec ☐ Don't know

4. Did wet litter occur in your last harvested crop? **(please tick ☑ in correct box)** — ☐Yes ☐ No ☐ Don't know

IF YES CONTINUE
IF NO OR DON'T KNOW GO TO QUESTION 9.

5. In your last crop, how old were the birds when wet litter occurred? — ....days

6. Was the cause of wet litter found in your last crop? **(please tick ☑ in correct box)** — ☐Yes ☐ No ☐ Don't know

   IF YES, what was it? **(please tick ☑ in more than one box if appropriate)** — ☐Ventilation problem ☐ Drinker problem ☐ Coccidiosis ☐ Enteritis ☐ Viral infection ☐ Don't know ☐ Other **(please specify)** ....

7. If a disease was the cause, by whom was it diagnosed? **(please tick ☑ in correct box)** — ☐ Veterinarian ☐ Area manager ☐ Yourself ☐ Other **(please specify)** ....

8. Was wet litter treated in your last crop? **(please tick ☑ in correct box)** — ☐Yes ☐ No ☐ Don't know

   IF YES, with what? — .... ☐ Don't know

II. Necrotic enteritis.

9. Has necrotic enteritis been diagnosed on your farm since January 1, 2001? **(please tick ☑ in correct box)**

☐Yes ☐ No
☐ Don't know

IF YES CONTINUE
IF NO OR DON'T KNOW GO TO QUESTION 16.

10. In how many crops was necrotic enteritis diagnosed since January 1, 2001? **(please tick ☑ in correct box)**

☐1 ☐2 ☐3 ☐4
☐5 ☐6 ☐7 ☐8

11. In which months was necrotic enteritis diagnosed? **(please tick ☑ in more than one box if appropriate)**

☐ Jan ☐ May ☐ Sept
☐ Feb ☐ June ☐ Oct
☐ Mar ☐ July ☐ Nov
☐ Apr ☐ Aug ☐ Dec
☐ Don't know

12. Did necrotic enteritis occur in your last harvested crop? **(please tick ☑ in correct box)**

☐Yes ☐ No
☐ Don't know

IF YES CONTINUE
IF NO OR DON'T KNOW GO TO QUESTION 16.

13. In your last crop, how old were the birds when necrotic enteritis occurred? ....days

14. Who diagnosed necrotic enteritis in your last crop? **(please tick ☑ in correct box)**

☐ Veterinarian
☐ Area manager
☐ Yourself
☐ Other **(please specify)**
....

15. Was necrotic enteritis treated in your last crop? **(please tick ☑ in correct box)**

☐Yes ☐ No
☐ Don't know

IF YES, with what? ....
☐ Don't know

III. Coccidiosis.

16. Has clinical coccidiosis been diagnosed on your farm since January 1, 2001? **(please tick ☑ in correct box)**

☐Yes ☐ No
☐ Don't know

IF YES CONTINUE
IF NO OR DON'T KNOW GO TO QUESTION 24.

17. In how many crops was coccidiosis diagnosed on your farm since January 1, 2001? **(please tick ☑ in correct box)**

☐1 ☐2 ☐3 ☐4
☐5 ☐6 ☐7 ☐8

18. In which months was coccidiosis diagnosed?
(please tick ☑ in more than one box if appropriate)

☐ Jan ☐ May ☐ Sept
☐ Feb ☐ June ☐ Oct
☐ Mar ☐ July ☐ Nov
☐ Apr ☐ Aug ☐ Dec
☐ Don't know

19. Did coccidiosis occur in your last harvested crop?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

IF YES CONTINUE
IF NO OR DON'T KNOW GO TO <u>QUESTION 24</u>.

20. In your last crop, how old were the birds when coccidiosis occurred?

......................days

21. Who diagnosed coccidiosis in your last crop?
(please tick ☑ in correct box)

☐ Veterinarian
☐ Area manager
☐ Yourself
☐ Other **(please specify)**
....

22. What type of coccidiosis was diagnosed in your last crop?
(please tick ☑ in correct box)

☐ *E. acervulina*
☐ *E. maxima*
☐ *E. tenella*
☐ Don't know
☐ Other **(please specify)**
....

23. Was coccidiosis treated in your last crop?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

IF YES, with what?

....
☐ Don't know

24. Were anticoccidials used in the feed during the last crop?
(please tick ☑ in correct box)
IF YES, FILL IN THE FOLLOWING TABLE,
IF NO OR DON'T KNOW GO TO <u>QUESTION 25</u>.

☐Yes ☐ No
☐ Don't know

Product name	Inclusion rates	Period of usage (days)
1............................	1...................☐ Don't know	1. From......To....days ☐ Don't know
2............................	2. ....☐ Don't know	2. From......To....days ☐ Don't know
3............................	3. ....☐ Don't know	3. From......To....days ☐ Don't know
4............................	4. ....☐ Don't know	4. From......To....days ☐ Don't know

IV. Respiratory disease.

25. Were any of the following respiratory diseases diagnosed on your farm since January 1, 2001?
(please tick ☑ in more than one box if appropriate)

- ☐ Infectious Bronchitis
- ☐ Turkey Rhino Tracheitis (TRT)
- ☐ Ornithobacterium Rhino Tracheale (ORT)
- ☐ None of these
- ☐ Don't know

IF NONE OR DON'T KNOW GO TO QUESTION 33.

26. In how many crops were respiratory diseases diagnosed on your farm since January 1, 2001?
(please tick ☑ in correct box)

☐1 ☐2 ☐3 ☐4
☐5 ☐6 ☐7 ☐8

27. In which months of the year were they diagnosed?
(please tick ☑ in more than one box if appropriate)

☐ Jan	☐ May	☐ Sept
☐ Feb	☐ June	☐ Oct
☐ Mar	☐ July	☐ Nov
☐ Apr	☐ Aug	☐ Dec

☐ Don't know

28. Were any respiratory diseases diagnosed in your last harvested crop? **(please tick ☑ in correct box)**

☐Yes ☐ No
☐ Don't know

IF YES CONTINUE
IF NO OR DON'T KNOW GO TO QUESTION 33.

29. In your last crop, how old were the birds when the respiratory disease occurred?

..........................days

30. Who diagnosed the respiratory disease in your last crop?
(please tick ☑ in correct box)

- ☐ Veterinarian
- ☐ Area manager
- ☐ Yourself
- ☐ Other **(please specify)**

....

31. Which respiratory disease was diagnosed in your last crop?
(please tick ☑ in more than one box if appropriate)

- ☐ Infectious Bronchitis
- ☐ Turkey Rhino Tracheitis (TRT)
- ☐ Ornithobacterium Rhino Tracheale (ORT)
- ☐ None of these
- ☐ Don't know

32. Was the respiratory disease treated in your last crop?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

IF YES, with what?

....

☐ Don't know

V. Other diseases.

33. Did any of the following diseases occur on your farm since January 1, 2001?
(please tick ☑ in more than one box if appropriate)

☐ Gumboro disease (IBD)
☐ Chicken Anaemia Virus (CAV)
☐ Marek's disease
☐ None of these
☐ Don't know

IF NONE OR **DON'T KNOW** GO TO QUESTION 40.

34. In how many crops were any of these diseases diagnosed on your farm since January 1, 2001?
(please tick ☑ in correct box)

☐1 ☐2 ☐3 ☐4
☐5 ☐6 ☐7 ☐8

35. In which months of the year were they diagnosed?
(please tick ☑ in more than one box if appropriate)

☐ Jan	☐ May	☐ Sept
☐ Feb	☐ June	☐ Oct
☐ Mar	☐ July	☐ Nov
☐ Apr	☐ Aug	☐ Dec

☐ Don't know

36. Did any of these diseases occur in your last harvested crop?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

IF YES CONTINUE
IF NO OR **DON'T KNOW** GO TO QUESTION 40.

37. In your last crop, how old were the birds when any of these diseases occurred?

.........................days

38. Who diagnosed them in your last crop?
(please tick ☑ in correct box)

☐ Veterinarian
☐ Area manager
☐ Yourself
☐ Other **(please specify)**

....

39. Were any of these diseases treated in your last crop?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

IF YES, with what?

....

☐ Don't know

Section B: General farm questions

40.	What is the total number of houses on the farm?	....
41.	What is the age in years of the newest house?	......................................years
42.	What is the age in years of the oldest house?	......................................years
43.	What is the average age in years of the houses?	......................................years
44.	What building materials were used for the walls of the houses? **(please tick ☑ in more than one box if appropriate)**	☐ Wood ☐ Concrete ☐ Brick ☐ Breeze block ☐ Asbestos ☐ Plaster board ☐ Metal lining and wood ☐ Metal lining and plastic ☐ Other **(please specify)** ....
45.	What building materials were used for the roofs of the houses? **(please tick ☑ in more than one box if appropriate)**	☐ Wood ☐ Asbestos ☐ Metal lining and wood ☐ Metal lining and plastic ☐ Other **(please specify)** ....
46.	What floor type is used in the houses? **(please tick ☑ in more than one box if appropriate)**	☐ Concrete ☐ Earth ☐ Other **(please specify)** ....
47.	What litter type was used inside the houses during the last crop? **(please tick ☑ in more than one box if appropriate)**	☐ Straw ☐ Wood shavings ☐ Saw dust ☐ Wood bark ☐ Other **(please specify)** ....
48.	What is the approximate minimum distance between adjacent houses?	......................................feet
49.	What is the approximate maximum distance between adjacent houses?	......................................feet
50.	How many people were working on the farm during the last crop (that is from day-old to clearance not including cleaning)?	....
51.	How many cycles (that is crops) did you have last year? **(please tick ☑ in correct box)**	☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8
52.	Are you a member of a quality assurance scheme? **(please tick ☑ in correct box)**	☐Yes ☐ No
	IF YES, which:	☐ Assured Chicken ☐ Freedom food ☐ Retailer Scheme ☐ Other **(please specify)** ....
53.	For how many years have you been a broiler farmer?	......................................years
54.	For how many years have you been growing broilers for your present poultry company?	......................................years

Section C: Information of the last crop.

55. What was the date of placement of your last harvested crop (day/month/year)? ....../....../......

56. What was the total number of chicks at placement in your last harvested crop? ....

57. What was the breed of the chickens of your last crop? **(please tick ☑ in more than one box if appropriate)**
☐ Ross ☐ Cobb
☐ Hybro ☐ Hubbard
☐ Don't know
☐ Other **(please specify)**
....

58. From how many different parent flocks did the chickens originate in your last crop ? ....
☐ Don't know

59. How many different hatcheries supplied the chicks for your last crop? ....
☐ Don't know

60. Were the chickens grown separated by sex during your last crop? **(please tick ☑ in correct box)**
☐Yes ☐ No
☐ Only in some houses

IF YES, were they in separate houses or in the same house? **(please tick ☑ in correct box)**
☐ Separate houses
☐ Same house

61. Was the flock thinned or was there total depopulation? **(please tick ☑ in correct box)**
☐ Thinned
☐ Total depopulation

IF THINNED at what age(s) did this occur? ....days

IF THINNED at what weight(s) did this occur? ....grams

62. What was the maximum density (kg/m^2) during the last crop? ....kg/m^2

63. At what age were the last birds cleared from your site? ....days

64. What was the average weight of the last birds at slaughter? ....grams

65. What was the feed conversion of the last crop? ....

66. What was the total mortality (%) in the last crop? ....%

67. What were the weekly number of dead and culled birds in percentages in the last crop? **(please fill in the table)**

Week no.	Dead (%)	Culled (%)
1		
2		
3		
4		
5		
6		
7		
8		

68. What was the EPEF of the last crop? ....

☐ Don't know

Section D: Climate control.

69. What ventilation type(s) is (are) used on the farm?
(please tick ☑ in correct box)

☐ Roof extract
☐ Side extract
☐ Automatically controlled natural ventilation (ACNV)
☐ Other **(please specify)**
....

70. Did any ventilation failures occur during your last crop?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

71. Were cooling devices other than fans (i.e. misting, evaporative cooling) used in the house during the last crop?
(please tick ☑ in correct box)

☐Yes
☐ No

72. What heating type was used in the houses during the last crop?
(please tick ☑ in more than one box if appropriate)

☐ Canopy brooding
☐ Whole house heating
☐ Other **(please specify)**
....

73. Did any heating failures occur during your last crop?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

74. What type of lighting was used in the houses during the last crop?
(please tick ☑ in more than one box if appropriate)

☐ Normal light bulbs
☐ Fluorescent lamps
☐ Low energy bulbs
☐ Other **(please specify)**
....

75. Do you use a lighting programme?
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES, please fill in the following table.

Age (weeks)	Daily hours of light	Daily hours of dark	No. of light periods
1			
2			
3			
4			
5			
6			
7			
8			

76. Did you need to make any changes in lighting programme or light intensity during the last crop?
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES, why did you need to make these changes? ....

77. Did any lighting failures occur during your last crop?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

Section E: Hygiene and biosecurity.

78. Is the site run on 'all in/ all out' basis (i.e. all birds moved unto the site or cleared from the site at approximately the same time? **(please tick ☑ in correct box)**

☐ Yes
☐ No

79. Were the houses cleaned before the last crop?
(please tick ☑ in correct box)

☐ Yes
☐ No

IF YES CONTINUE
IF NO GO TO <u>QUESTION 82</u>

80. Who cleaned the houses before the last crop?
(please tick ☑ in correct box)

☐ Contract cleaner
☐ Company cleaner
☐ Yourself

81. How were the houses cleaned before the last crop?
(please tick ☑ in more than one box if appropriate)

☐ Dry brush cleaned
☐ Compressed air cleaned
☐ With water
☐ With water and detergent
☐ Other **(please specify)**
....

If a detergent was used, what was the name of the product? ....

☐ Don't know

What concentration of the detergent was used? ....

☐ Don't know

82. Were the houses disinfected before the last crop?
(please tick ☑ in correct box)

☐ Yes
☐ No

IF YES, CONTINUE
IF NO GO TO <u>QUESTION 86</u>.

83. Who disinfected the houses before the last crop?
(please tick ☑ in correct box)

☐ Contract cleaner
☐ Company cleaner
☐ Yourself

84. How were the houses disinfected before the last crop? **(please tick ☑ in correct box)**

☐ Spray disinfectant alone
☐ Fumigation alone
☐ Spray disinfectant and fumigation

If spray disinfectant was used, what was the name of the product? ....

☐ Don't know

What concentration of spray disinfectant was used? ....

☐ Don't know

If fumigation was used, what was the name of the product? ....

☐ Don't know

What concentration of fumigation product was used? ....

☐ Don't know

85. Was ammonia used as a disinfectant before the last crop

☐Yes
☐ No

86. Were bacterial counts taken of the inside of the houses? **(please tick ☑ in correct box)**

☐Yes
☐ No

87. Was the <u>inside</u> of the water system cleaned before the last crop? **(please tick ☑ in correct box)**

☐Yes
☐ No

IF YES, with which product? ....

☐ Don't know

88. What was the length of turn-around before your last harvested crop (time between the last chickens off the farm and the new chicks on the farm)? ....days

89. What was the minimum time between disinfection and restocking your last crop? .... days

90. Are there hand washing facilities? **(please tick ☑ in correct box)**

☐Yes
☐ No

IF YES, do you have hand washing facilities for each house? **(please tick ☑ in correct box)**

☐Yes
☐ No

91. Do you wash your hands before entering a house? **(please tick ☑ in correct box)**

☐ Always ☐ Occasionally
☐ Never

92. Are hand sanitizers available? **(please tick ☑ in correct box)**

☐Yes
☐ No

IF YES, are there hand sanitizers available for each house? **(please tick ☑ in correct box)**

☐Yes
☐ No

93. Do you sanitize your hands before entering a house? **(please tick ☑ in correct box)**

☐ Always ☐ Occasionally
☐ Never

94. Are there shower facilities?
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES, are there shower facilities for each house?

☐Yes
☐ No

95. Do you shower before entering a house?
(please tick ☑ in correct box)

☐ Always ☐ Occasionally
☐ Never

96. Is farm clothing available (for example overalls)?
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES, is separate clothing available for each house?
(please tick ☑ in correct box)

☐Yes
☐ No

97. Do you change clothing before entering a house?
(please tick ☑ in correct box)

☐ Always ☐ Occasionally
☐ Never

98. Are there barrier systems (that is a step-over partition) before entrance to a house?
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES, are there barrier systems for each house?
(please tick ☑ in correct box)

☐Yes
☐ No

99. Are there boot dip facilities?
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES, are there boot dip facilities for each house?
(please tick ☑ in correct box)

☐Yes
☐ No

100. Do you dip your boots before entering a house?
(please tick ☑ in correct box)

☐ Always ☐ Occasionally
☐ Never

101. Are there farm boots available?
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES, are there farm boots available for each house
(please tick ☑ in correct box)

☐Yes
☐ No

102. Do you change boots before entering a house?
(please tick ☑ in correct box)

☐ Always ☐ Occasionally
☐ Never

103. Are there plastic over boots available?
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES, are there plastic over boots available for each house?
(please tick ☑ in correct box)

☐Yes
☐ No

104. Do you use plastic over boots before entering a house?
(please tick ☑ in correct box)

☐ Always ☐ Occasionally
☐ Never

105. How often do you see rodents on your farm?
(please tick ☑ in correct box)

☐ Never
☐ Every day
☐ Every few days
☐ Every week
☐ Every month
☐ Every crop
☐ Every 6 months

106. Where do you see rodents?
(please tick ☑ in more than one box if appropriate)

☐ Outside the houses
☐ Inside the houses
☐ Other **(please specify)**
....

107. Are there rodent control measures?
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES, what kind?
(please tick ☑ in more than one box if appropriate)

☐ Poison ☐ Cats
☐ Other **(please specify)**
....

108. What is the number of rodent baiting points? ....

109. How frequent are the baiting points checked?

☐ Daily
☐ Every other day
☐ Weekly
☐ Fortnightly
☐ Monthly
☐ Every crop

110. Do you see litter beetles in the house?
(please tick ☑ in correct box)

☐Yes
☐ No

111. Are there any litter beetle control measures?
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES:
What kind of litter beetle control measures do you use? ....

When do you use these litter beetle control measures? ....

How often do you use these litter beetle control measures? ....

112. Are there other domestic animals on the farm?
(including any not farmed by yourself)
(please tick ☑ in correct box)

☐Yes
☐ No

IF YES which and how many?
(please tick ☑ in more than one box if appropriate)

☐ Cattle No..........
☐ Sheep No..........
☐ Pigs No.........
☐ Horses No..........
☐ Dogs No..........
☐ Cats No..........
☐ Other **(please specify)**
....

113.	Are there other poultry farms near you (within a radius of 2 miles)? **(please tick ☑ in correct box)**	☐Yes ☐ No ☐ Don't know
	IF YES, what type and what approximate distance (miles) as the crows flies? **(please tick ☑ in more than one box if appropriate)**	☐ Broiler Distance.........miles ☐ Broiler breeder Distance.........miles ☐ Breeder rearing Distance.........miles ☐ Layer Distance.........miles ☐ Layer breeder Distance.........miles ☐ Layer rearing Distance.........miles ☐ Turkey Distance.........miles ☐ Turkey breeder Distance.........miles ☐ Turkey breeder rearing Distance.........miles ☐ Free range broilers Distance.........miles ☐ Free range layers Distance.........miles ☐ Other **(please specify)** ....
114.	Are there other domestic animals on adjoining fields or premises? **(please tick ☑ in correct box)**	☐Yes ☐ No
	IF YES, which? **(please tick ☑ in more than one box if appropriate)**	☐ Cattle ☐ Sheep ☐ Pigs ☐ Horses ☐ Dogs ☐ Cats ☐ Other **(please specify)** ....
115.	Have you seen other animals, domestic or wild, in the poultry house? **(please tick ☑ in correct box)**	☐Yes ☐ No
	IF YES, when: **(please tick ☑ in correct box)**	☐ During occupation ☐ While empty
116.	Have you seen wild birds in the poultry houses? **(please tick ☑ in correct box)**	☐Yes ☐ No
	IF YES, when: **(please tick ☑ in correct box)**	☐ During occupation ☐ While empty

117. How is the poultry litter disposed of?
(please tick ☑ in more than one box if appropriate)

- ☐ Spread on field
- ☐ Deep stacked and then spread on field
- ☐ Power plant
- ☐ Mushroom composting
- ☐ Don't know
- ☐ Other **(please specify)**

....

If the litter is spread on the field, where is it spread?
(please tick ☑ in more than one box if appropriate)

- ☐ On the farm
- ☐ On adjacent premises to the farm
- ☐ Elsewhere

118. Prior to the last crop, was all litter removed from the farm before disinfection of the house? **(please tick ☑ in correct box)**

- ☐Yes
- ☐ No

119. Prior to the last crop, was all litter removed from the farm before the new crop arrived? **(please tick ☑ in correct box)**

- ☐Yes
- ☐ No

120. Is other manure or slurry spread on fields on your farm?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

IF YES, what kind?
(please tick ☑ in more than one box if appropriate)

- ☐ Cow manure/slurry
- ☐ Pig manure/slurry
- ☐ Human sludge
- ☐ Other **(please specify)**

....

121. Is manure or slurry spread on fields adjoining your farm?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

IF YES, which kind?
(please tick ☑ in more than one box if appropriate)

- ☐ Cow manure/slurry
- ☐ Pig manure/slurry
- ☐ Poultry litter/slurry
- ☐ Human sludge
- ☐ Don't know
- ☐ Other **(please specify)**

....

122. How many times a day do you (or your employee(s)) walk through the houses to examine the birds?

..........................time(s)

123. On average, how often does the area manager visit the farm?
(please tick ☑ in correct box)

- ☐..................time(s) daily
- ☐................time(s) weekly
- ☐..............time(s) monthly
- ☐..................time(s) crop
- ☐ Never

124. Were there any changes in management or employees on the farm during your last crop?
(please tick ☑ in correct box)

- ☐Yes
- ☐ No

125. Did people other than yourself, employees or the area manager enter into the houses during the last crop?
(please tick ☑ in correct box)

☐ Yes
☐ No

IF YES, who and how often (total number of visits per crop)?
(please tick ☑ in more than one box if appropriate)

☐ Veterinarian
visits/crop.............
☐ Feed representative
visits/crop.............
☐ Electrician
visits/crop.............
☐ Service engineer
visits/crop.............
☐ Other **(please specify)**
....
visits/crop.............

126. Are there any commercial vehicles coming onto the farm?
(please tick ☑ in correct box)

☐ Yes
☐ No

IF YES, which and how often
(total number of visits per crop)
(please tick ☑ in more than one box if appropriate)

☐ Feed lorry
visits/crop.............
☐ Hatchery lorry
visits/crop.............
☐ Collection vehicle
(transport to slaughter house)
visits/crop.............
☐ Gas lorry
visits/crop.............
☐ Oil lorry
visits/crop.............
☐ Straw/shavings lorry
visits/crop.............
☐ Other (please specify)
....
visits/crop.............

Section F: Feeders and Feeding.

127. What is the total number of feed bins on the farm? ....

128. Are single or double bins used per house

☐ Single
☐ Double

129. How frequently are the insides of feed bins cleaned?
(please tick ☑ in correct box)

☐ Never
☐ Once per month
☐ Once per crop
☐ Once per 6 months
☐ Once per year
☐ Other **(please specify)**
....

130. How frequently are the insides of feed bins disinfected?
(please tick ☑ in correct box)

☐ Never
☐ Once per month
☐ Once per crop
☐ Once per 6 months
☐ Once per year
☐ Other **(please specify)**
....

131. What type(s) of feeders are used in the houses?
(please tick ☑ in more than one box if appropriate)

☐ Flat chain/auger
☐ Pan feeders
☐ Tube feeders
☐ Other **(please specify)**
....

132. What is the approximate number of broilers per feeder?

.............birds/metre of track
................birds/pan feeder
.........................birds/tube
☐ Don't know
☐ Other **(please specify)**
....

133. What feeding regimen was used during the last crop?
(please tick ☑ in correct box)

☐ Ad libitum
☐ Controlled

134. Did any feeder failures occur in the last crop?

☐Yes ☐ No
☐ Don't know

IF YES, how many times? ....

135. How many different types of feed did you have during the last crop? **(please tick ☑ in correct box)**

☐2 ☐3 ☐4 ☐5 ☐6

136. Which company(ies) supplied the feed? ....

137. What were the delivery dates of the feed delivered during the last crop (day/month/year)?

1…./…./…..16…./…./….
2…./…./…..17…./…./….
3…./…./…..18…./…./….
4…./…./…..19…./…./….
5…./…./…..20…./…./….
6…./…./…..21…./…./….
7…./…./…..22…./…./….
8…./…./…..23…./…./….
9…./…./…..24…./…./….
10…/…./….25…./…./….
11…/…./….26…./…./….
12…/…./….27…./…./….
13…/…./….28…./…./….
14…/…./….29…./…./….
15…/…./….30…./…./….

138. Was whole wheat added to the feed during the last crop? ☐Yes ☐ No ☐ Don't know
(please tick ☑ in correct box)

IF YES CONTINUE
IF NO OR DON'T KNOW GO TO QUESTION 142.

IF YES, what minimum percentage? ………………………….%
And what maximum percentage? ………………………….%

139. Who supplied the wheat?
(please tick ☑ in correct box)

☐ Own farm
☐ Other farm
☐ Feed mill

140. Where was the wheat added?
(please tick ☑ in correct box)

☐ Blended on the farm
☐ Blended in the mill
☐ Blended in the wagon
☐ Dumped on the wagon

141. Was the wheat treated? ☐Yes ☐ No ☐ Don't know
(please tick ☑ in correct box)

IF YES, with what?
(please tick ☑ in more than one box if appropriate)

☐ Organic acids
☐ Virkon
☐ SalCurb
☐ Don't know
☐ Other **(please specify)**
………………………………

142. How many feed lorries entered the farm during the last crop? ………………………………

Section G: Water and drinkers.

143. What type of drinkers are used in the houses?
(please tick ☑ in more than one box if appropriate)

☐ Bell drinkers
☐ Nipples without cups
☐ Nipples with cups
☐ Other **(please specify)**
....

144. What is the approximate number of broilers per drinker?

.................birds/belldrinker
.......................birds/nipple
☐ Don't know
☐ Other **(please specify)**
....

145. What water regimen was used during the last crop?
(please tick ☑ in correct box)

☐ Ad libitum
☐ Controlled

146. What was the source of your water supply during the last crop?
(please tick ☑ in more than one box if appropriate)

☐ Mains
☐ Well
☐ Borehole
☐ Stream
☐ Other **(please specify)**
....

147. Has your water been analysed?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

IF YES, what kind of analysis?
(please tick ☑ in more than one box if appropriate)

☐ Bacteriological
☐ Chemical
☐ Don't know

148. Did any failure in water supply occur during your last crop?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

149. Was a sanitizer used in the water during the last crop?
(please tick ☑ in correct box)

☐Yes ☐ No
☐ Don't know

IF YES:
What was the product name of the sanitizer?

....
☐ Don't know

What concentration of the sanitizer was used?

....
☐ Don't know

How often was the sanitizer used?

☐.................time(s) daily
☐...............time(s) weekly
☐............time(s) monthly
☐..................time(s) crop

Section H: Disease management.

150. Which vaccines were used during your last crop (**please fill in the table**)?

Vaccine	Age(s) of application (days)	Method of application	Product name
Newcastle Disease ☐Yes ☐ No ☐ Don't know (please tick ☑ in correct box)	............days	☐ Drinking water ☐ Spray ☐ Other (please specify)	.... ☐ Don't know
Gumboro Disease ☐Yes ☐ No ☐ Don't know (please tick ☑ in correct box)	............days	☐ Drinking water ☐ Other (please specify)	.... ☐ Don't know
Infectious Bronchitis ☐Yes ☐ No ☐ Don't know (please tick ☑ in correct box)	............days	☐ Drinking water ☐ Spray ☐ Other (please specify)	.... ☐ Don't know
Turkey Rhino Tracheitis ☐Yes ☐ No ☐ Don't know (please tick ☑ in correct box)	............days	☐ Drinking water ☐ Spray ☐ Other (please specify)	.... ☐ Don't know
Other (please specify)	............days	☐ Drinking water ☐ Spray ☐ Other (please specify)	.... ☐ Don't know

151. In your last crop were preventive antibiotics used? ☐Yes ☐ No
(that is antibiotics given before disease appeared) ☐ Don't know
(please tick ☑ in correct box)

IF YES FILL IN THE FOLLOWING TABLE,
IF NO OR DON'T KNOW GO TO QUESTION 152.

Name of antibiotic	Age of the birds when administered	Route of administration	Reason for medication
....	From ....days To ....days ☐ Don't know	☐ Water ☐ Feed ☐ Other (please specify)	.... ☐ Don't know
....	From ....days To ....days ☐ Don't know	☐ Water ☐ Feed ☐ Other (please specify)	.... ☐ Don't know
....	From ....days To .... days ☐ Don't know	☐ Water ☐ Feed ☐ Other (please specify)	.... ☐ Don't know
....	From ....days To ....days ☐ Don't know	☐ Water ☐ Feed ☐ Other (please specify)	.... ☐ Don't know

152.	Did your last crop require treatment? **(please tick ☑ in correct box)**	☐Yes ☐ No ☐ Don't know

IF YES, FILL IN THE FOLLOWING TABLE
IF NO OR DON'T KNOW GO TO <u>QUESTION 153</u>.

Name of product	Age of the birds when administered	Route of administration	Reason for medication
....	From ....days To .... days ☐ Don't know	☐ Water ☐ Feed ☐ Other (please specify)	.... ☐ Don't know
....	From ....days To .... days ☐ Don't know	☐ Water ☐ Feed ☐ Other (please specify)	.... ☐ Don't know
....	From ....days To .... days ☐ Don't know	☐ Water ☐ Feed ☐ Other (please specify)	.... ☐ Don't know
....	From ....days To .... days ☐ Don't know	☐ Water ☐ Feed ☐ Other (please specify)	.... ☐ Don't know

153.	Were competitive exclusion products used during your last crop (for example Aviguard or Broilact)? **(please tick ☑ in correct box)**	☐Yes ☐ No ☐ Don't know

IF YES CONTINUE
IF NO OR DON'T KNOW GO TO QUESTION 154

Which product(s)?
(please tick ☑ in more than one box if appropriate)

☐ Aviguard
☐ Broilact
☐ Other (please specify)
....

How was it administered?
(please tick ☑ in correct box)

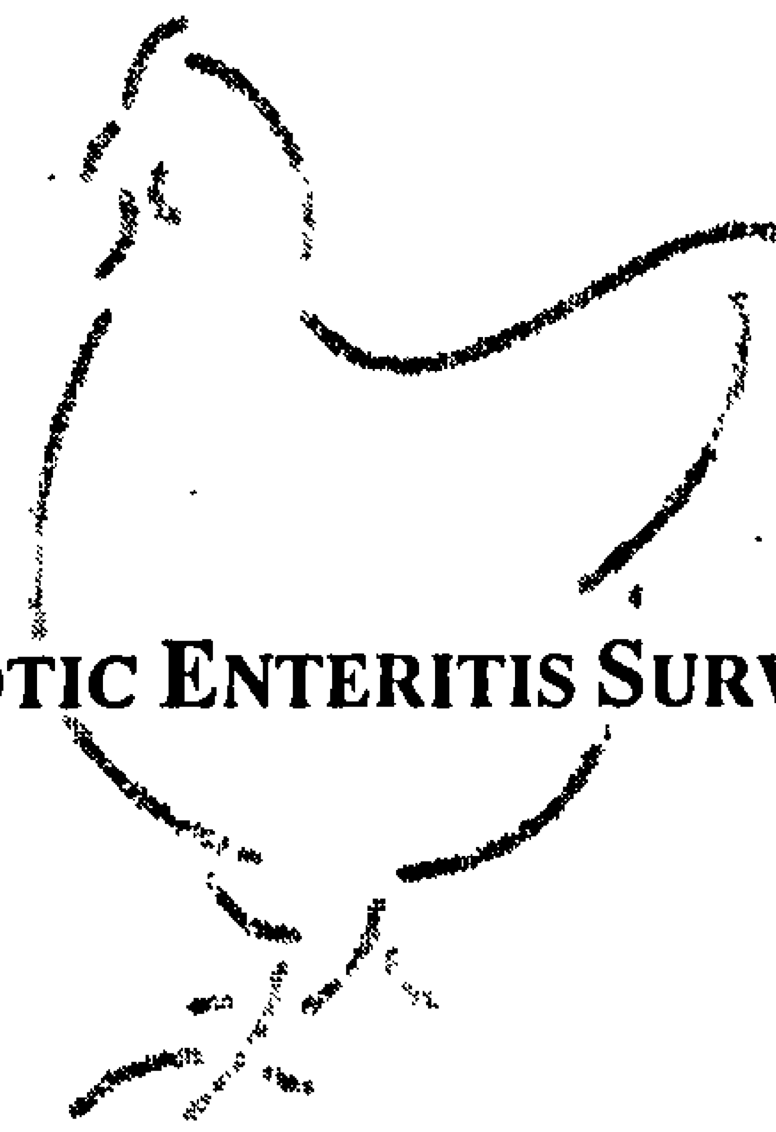
☐ Water
☐ Feed
☐ Other (please specify)
....

Thank you for completing this questionnaire. Please return the questionnaire in the enclosed pre-addressed envelope. If you would like to receive a copy of the results obtained from this study, please indicate so below.

154.	Would you like to receive a copy of results obtained from this study?	☐Yes ☐ No

Appendix 3: Reminder card.

THE UNIVERSITY
of LIVERPOOL



NECROTIC ENTERITIS SURVEY

This is to remind you that we have not, as yet, received your questionnaire for <u>the Study on Wet Litter and Enteritis in Broilers.</u> We would be most grateful if you would fill in your survey form as soon as possible and send it back to us in the pre-addressed pre-paid envelope provided. The information from the questionnaire will further our knowledge of wet litter and enteritis and therefore it is of great importance that you reply even if you have not had any instances of wet litter or enteritis in your flock. If you have returned your questionnaire during the last few days, please ignore this card.

MANY THANKS FOR YOUR COOPERATION.

Patrick Hermans
Dept. Veterinary Clinical Sciences & Animal Husbandry.
Leahurst, Neston.
CH64 0TT.

THE UNIVERSITY
of LIVERPOOL

Department of Veterinary Clinical Science and Animal Husbandry

Faculty of Veterinary Science

Leahurst
Chester High Road
Neston
CH64 7TE

Telephone: 0151 794 7582
Facsimile: 0151 794 6028

Appendix 4: Reminder letter.

2 April, 2002.

STUDY ON WET LITTER AND ENTERITIS IN BROILERS.

Dear Sir/Madam,

The response rate to our wet litter and enteritis survey has been good to date and we hope that we can still include your farm in this study. Understandably many questionnaires may have gone astray. So we are sending a second copy to anyone we have not heard from.

The information coming from your farm is important to us, even if you haven't had any instances of wet litter or enteritis in your flock. Please, fill in this survey form as soon as possible and return it to us in the pre-paid envelope. Your help will be much appreciated.

If, upon receipt of this letter, you have already returned your questionnaire, we kindly ask you to ignore this mailing and we thank you for your support.

Yours faithfully,

Patrick Hermans

Professor Kenton Morgan

University switchboard
Telephone: 0151 794 2000
Facsimile: 0151 708 6502

Appendix 5: Telephone questionnaire.

Farm ID No.: ....
Farm Name: ....
Contact person: ....
Telephone: ....
Called on: ....
Call back on: ....
Date completed: ....

Hello, my name is .... And I am calling from the University of Liverpool, School of Veterinary Medicine. In February of this year we sent you a questionnaire dealing with wet litter and enteritis in broilers. Did you receive this questionnaire?
? yes
? no
(If yes continue, if no apologize that the questionnaire wasn't delivered and say that in that case you have no further questions)

Can I take a minute of your time to ask you three simple questions.

1. **We didn't receive a completed questionnaire from you and we were wondering what the reason was that you didn't return it?**

☐ too busy
☐ not interested
☐ no wet litter/enteritis problems on this farm
☐ lost/misplaced the questionnaire
☐ did not keep broilers at this time
☐ other (please specify)...

2. **What was the total number of chicks delivered to your farm for your first crop of this year?**

   ....…....... no of chicks

3. **How many houses were there on your farm at this time?**

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10 ☐11 ☐12 ☐10 ☐11 ☐12 ☐13 ☐14 ☐15 ☐16 ☐17 ☐18 ☐19 ☐20 ☐21 ☐22 ☐23 ☐24 ☐25 ☐ Other (please specify)..........

Appendix 6: Complete results of the univariate analysis of the associations between the occurrence of necrotic enteritis and explanatory variables.

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Disease					
Wet litter in the last crop					
	Yes	54/277 (19.5)	2.82	1.56–5.13	<0.001
	No	19/240 (7.9)	Ref.	-	-
Coccidiosis in the last crop					
	Yes	12/25 (48.0)	6.63	2.66-16.51	<0.001
	No	59/483 (12.2)	Ref.	-	-
Respiratory diseases in the last crop					
	Yes	4/16 (25.0)	2.19	0.57-7.69	0.18
	No	63/476 (13.2)	Ref.	-	-
Immunosuppressive diseases in the last crop					
	Yes	3/9 (33.3)	3.20	0.61-15.04	0.09
	No	66/498 (13.3)	Ref.	-	-
Environment					
Number of houses on the farm					0.39[1]
	1-2	14/140 (10.0)	Ref.	-	-
	3-4	23/140 (16.4)	1.77	0.87-3.60	0.12
	5-7	16/101 (15.8)	1.69	0.79-3.65	0.18
	8-20	20/137 (14.6)	1.54	0.74-3.19	0.25
Number of houses on the farm *(continuous)*		-	1.03	0.96-1.10	0.43
Fractional polynomial: -2		-	-	-	0.02
Age of the newest house (years)					0.13[1]
	<6 months-5 years	26/150 (17.3)	Ref.	-	-
	6-11 years	10/106 (9.4)	0.50	0.23-1.08	0.08
	12-26 years	14/125 (11.2)	0.60	0.30-1.21	0.15
	27-60 years	22/123 (17.9)	1.04	0.56-1.94	0.91
Age of the newest house *(continuous)*		-	1.01	0.99-1.03	0.51
Fractional polynomials: 0.5		-	-	-	0.008
0.5		-	-	-	0.008
Age of the eldest house (years)					0.16[1]
	6 months-12 years	17/126 (13.5)	Ref.	-	-
	13-28 years	18/124 (14.5)	1.09	0.53-2.23	0.82
	29-37 years	13/132 (9.8)	0.70	0.33-1.51	0.36
	38-60 years	22/109 (20.2)	1.62	0.81-3.24	0.17
Age of the eldest house *(continuous)*		-	1.01	0.99-1.03	0.46
Average age of the houses (years)					0.11[1]
	6 months-9 years	20/119 (16.8)	Ref.	-	-
	10-18 years	16/114 (14.0)	0.81	0.40-1.65	0.56
	19-30 years	10/115 (8.7)	0.47	0.21-1.06	0.07
	31-60 years	21/108 (19.4)	1.19	0.61-2.35	0.61
Average age of the houses *(continuous)*		-	1.00	0.98-1.03	0.69
Minimum distance between houses (feet)					0.28[1]
	1-16 feet	19/116 (16.4)	Ref.	-	-
	18-22 feet	12/106 (11.3)	0.65	0.30-1.42	0.28
	23-30 feet	26/134 (19.4)	1.23	0.64-2.36	0.54
	32-10560 feet	13/104 (12.5)	0.73	0.34-1.56	0.42
Minimum distance between houses *(continuous, per 10 feet increase)*		-	0.92	0.78-1.08	0.32

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Environment (continued)					
Maximum distance between houses					0.64[1]
	8-20 feet	19/121 (15.7)	Ref.	-	-
	21-30 feet	24/141 (17.0)	1.10	0.57-2.13	0.77
	33-40 feet	12/71 (16.9)	1.09	0.50-2.41	0.83
	41-10560 feet	14/119 (11.8)	0.72	0.34-1.50	0.38
Maximum distance between houses *(continuous, per 10 feet increase)*		-	0.96	0.89-1.03	0.25
Wooden house walls					
	Yes	45/327 (13.8)	0.89	0.52-1.52	0.64
	No	29/190 (15.3)	Ref.	-	-
Concrete house walls					
	Yes	29/162 (17.9)	1.50	0.87-2.59	0.12
	No	45/355 (12.7)	Ref.	-	-
Brick house walls					
	Yes	10/101 (9.9)	0.60	0.28-1.28	0.16
	No	64/416 (15.4)	Ref.	-	-
Breeze block house walls					
	Yes	33/242 (13.6)	0.90	0.53-1.53	0.68
	No	41/275 (14.9)	Ref.	-	-
Asbestos house walls					
	Yes	27/181 (14.9)	1.08	0.62-1.86	0.77
	No	47/336 (14.0)	Ref.	-	-
Plaster board house walls					
	Yes	8/32 (25.0)	2.12	0.83-5.25	0.07
	No	66/485 (13.6)	Ref.	-	-
Metal lining & wooden house walls					
	Yes	17/96 (17.7)	1.37	0.72-2.59	0.29
	No	57/421 (13.5)	Ref.	-	-
Metal lining & plastic house walls					
	Yes	12/53 (22.6)	1.90	0.88-4.01	0.07
	No	62/464 (13.4)	Ref.	-	-
Other type of house walls					
	Yes	10/72 (13.9)	0.96	0.44-2.08	0.92
	No	64/446 (14.3)	Ref.	-	-
Wooden roof					
	Yes	19/147 (12.9)	0.83	0.45-1.52	0.53
	No	55/364 (15.1)	Ref.	-	-
Asbestos roof					
	Yes	39/273 (14.3)	0.97	0.57-1.64	0.89
	No	35/238 (14.7)	Ref.	-	-
Metal lining & wood roof					
	Yes	33/206 (16.0)	1.23	0.72-2.09	0.42
	No	41/305 (13.4)	Ref.	-	-
Metal lining & plastic roof					
	Yes	11/65 (16.9)	1.24	0.57-2.62	0.55
	No	63/446 (14.1)	Ref.	-	-
Other type of roof					
	Yes	16/109 (14.7)	1.04	0.54-1.97	0.89
	No	58/409 (14.2)	Ref.	-	-
Concrete floor					
	Yes	74/517 (14.3)	-	-	-
	No	0/5 (0)	Ref.	-	-
Earth floor					
	Yes	1/4 (25.0)	2.03	0.04-25.65	0.53
	No	73/518 (14.1)	Ref.	-	-
Other type of floor					
	Yes	1/12 (8.3)	0.54	0.03-4.22	0.56
	No	73/510 (14.3)	Ref.	-	-

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Environment (continued)					
Type of litter used during the last crop					
Straw as litter					
	Yes	25/166 (15.1)	1.10	0.63-1.92	0.71
	No	49/354 (13.8)	Ref.	-	-
Wood shavings as litter					
	Yes	53/371 (14.3)	1.02	0.57-1.83	0.95
	No	21/149 (14.1)	Ref.	-	-
Saw dust as litter					
	Yes	14/68 (20.6)	1.69	0.84-3.39	0.11
	No	60/452 (13.3)	Ref.	-	-
Wood bark as litter					
	Yes	1/1 (100)	-	-	-
	No	73/519 (14.1)	Ref.	-	-
Other type of litter					
	Yes	2/11 (18.2)	1.36	0.00-7.01	0.70
	No	71/505 (14.1)	Ref.	-	-
Roof ventilation					
	Yes	46/353 (13.0)	0.74	0.43-1.28	0.24
	No	28/166 (16.9)	Ref.	-	-
Side ventilation					
	Yes	34/187 (18.2)	1.62	0.95-2.76	0.06
	No	40/332 (12.0)	Ref.	-	-
Automatic controlled natural ventilation					
	Yes	9/90 (10.0)	0.62	0.27-1.37	0.20
	No	65/429 (15.2)	Ref.	-	-
Other types of ventilation					
	Yes	2/13 (15.4)	1.21	0.00-6.11	0.81
	No	72/506 (14.2)	Ref.	-	-
Did ventilation failures occur during the last crop					
	Yes	2/13 (15.4)	1.10	0.00-5.46	0.91
	No	72/506 (14.2)	Ref.	-	-
Were cooling devices used during the last crop					
	Yes	3/18 (16.7)	1.21	0.27-4.65	0.77
	No	71/500 (14.2)	Ref.	-	-
Canopy brood heating					
	Yes	19/155 (12.3)	0.78	0.43-1.42	0.40
	No	55/364 (15.1)	Ref.	-	-
Whole house heating					
	Yes	66/423 (15.6)	2.03	0.90-4.80	0.07
	No	8/96 (8.3)	Ref.	-	-
Did heating failures occur during the last crop					
	Yes	10/49 (20.4)	1.66	0.73-3.69	0.18
	No	63/471 (13.4)	Ref.	-	-
Use of light bulbs					
	Yes	13/144 (9.0)	0.52	0.26-1.02	0.04
	No	60/375 (16.0)	Ref.	-	-
Use of fluorescent lights					
	Yes	49/301 (16.3)	1.57	0.90-2.76	0.09
	No	24/218 (11.0)	Ref.	-	-
Use of low energy light bulbs					
	Yes	33/215 (15.3)	1.20	0.70-2.04	0.48
	No	40/304 (13.2)	Ref.	-	-
Other types of light					
	Yes	3/6 (50.0)	6.34	0.83-48.03	0.01
	No	70/514 (13.6)	Ref.	-	-

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Environment (continued)					
Use of a light regime during the last crop					
	Yes	58/415 (14.0)	1.07	0.54-2.18	0.83
	No	13/99 (13.1)	Ref.	-	-
Change in light regime/intensity during the last crop					
	Yes	11/59 (18.6)	1.45	0.67-3.10	0.30
	No	62/455 (13.6)	Ref.	-	-
Did lighting failures occur during the last crop					
	Yes	1/10 (10.0)	0.68	0.03-5.42	0.71
	No	71/505 (14.1)	Ref.	-	-
Husbandry/Management					
Number of people working on the farm					
	≤2	55/418 (13.2)	Ref.	-	-
	>2	17/97 (17.5)	1.40	0.77-2.54	0.27
Number of people working on the farm *(continuous)*		-	0.99	0.89-1.11	0.90
Number of crops per year					0.17[1]
	2-5	15/123 (12.2)	Ref.	-	-
	6	46/272 (16.9)	1.47	0.78-2.74	0.23
	7-8	13/125 (10.4)	0.84	0.38-1.84	0.66
Number of crops per year *(continuous)*		-	0.95	0.71-1.26	0.72
Month of placement of the last crop					0.68[1]
	January – March	17/127 (13.4)	Ref.	-	-
	April – June	0/2 (0)	-	-	-
	July – September	0/3 (0)	-	-	-
	October - December	55/370 (14.9)	1.13	0.63-2.03	0.68
Number of chickens placed in the last crop					0.11[1]
	8300-46500	11/131 (8.4)	Ref.	-	-
	47000-90000	19/124 (15.3)	1.97	0.90-4.34	0.09
	90200-144000	21/124 (16.9)	2.22	1.02-4.83	0.04
	144200-582260	23/131 (17.6)	2.32	1.08-4.99	0.03
Number of chickens placed in the last crop *(continuous, per 10000 chickens increase)*		-	1.03	1.00-1.05	0.05
Breed of chickens used during the last crop					
	Breed A				
	Yes	69/426 (16.2)	3.29	1.28-10.74	0.009
	No	5/90 (5.6)	Ref.	-	-
	Breed B				
	Yes	3/18 (16.7)	1.20	0.27-4.63	0.77
	No	71/498 (14.3)	Ref.	-	-
	Breed C				
	Yes	32/232 (13.8)	0.92	0.54-1.57	0.75
	No	42/284 (14.8)	Ref.	-	-
Number of parent flocks that supplied the chicks for the last crop					0.29[1]
	1-2	9/70 (12.9)	Ref.	-	-
	3-4	27/120 (22.5)	1.97	0.87-4.47	0.11
	5-6	14/97 (14.4)	1.14	0.46-2.81	0.77
	7-24	19/107 (17.8)	1.46	0.62-3.45	0.38
Number of parent flocks that supplied the chicks for the last crop *(continuous)*		-	1.02	0.96-1.10	0.50
Number of hatcheries that supplied the chicks for the last crop					
	1	39/305 (12.8)	Ref.	-	-
	2-9	33/188 (17.6)	1.45	0.88-2.40	0.15
Number of hatcheries that supplied the chicks for the last crop *(continuous)*		-	1.46	1.05-2.05	0.03

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Husbandry/Management (continued)					
Maximum density (kg/m^2) during the last crop					0.63[1]
	16.86-35.00	15/83 (18.1)	Ref.	-	-
	35.10-37.60	15/80 (18.8)	1.05	0.47-2.31	0.91
	37.63-38.12	10/83 (12.0)	0.62	0.26-1.58	0.28
	38.20-51.30	13/81 (16.0)	0.87	0.38-1.96	0.73
Maximum density during the last crop *(continuous, per 1 kg/m² increase)*		-	0.97	0.93-1.02	0.24
Age at slaughter (days) of the last crop					0.26[1]
	37-42	14/104 (13.5)	Ref.	-	-
	43-47	16/148 (10.8)	0.78	0.36-1.68	0.52
	48-52	13/99 (13.1)	0.97	0.43-2.19	0.95
	53-72	29/155 (18.7)	1.48	0.74-2.96	0.27
Age at slaughter of the last crop *(continuous)*		-	1.04	1.00-1.09	0.05
Weight at slaughter (grams) of the last crop					0.16[1]
	1680-2195	14/107 (13.1)	Ref.	-	-
	2200-2520	16/118 (13.6)	1.04	0.48-2.25	0.92
	2525-3390	13/107 (12.1)	0.92	0.41-2.06	0.84
	3400-5100	27/124 (21.8)	1.85	0.91-3.74	0.09
Weight at slaughter of the last crop *(continuous, per 100 grams increase)*		-	1.03	0.99-1.07	0.14
Flock was thinned					
	Yes	64/464 (13.8)	0.88	0.37-2.14	0.75
	No	8/52 (15.4)	Ref.	-	-
Chickens were grown separated by sex					
	Yes	53/329 (16.1)	1.84	0.97-3.53	0.05
	No	15/158 (9.5)	Ref.	-	-
Number of times per day chickens are examined					
	≤ 3	46/354 (13.0)	Ref.	-	-
	> 3	27/162 (16.7)	1.34	0.80-2.24	0.27
Number of times per day chickens are examined *(continuous)*		-	0.95	0.74-1.21	0.67
Number of years having been a broiler farmer					0.83[1]
	1-8 years	20/133 (15.0)	Ref.	-	-
	9-15 years	19/158 (12.0)	0.77	0.39-1.52	0.45
	16-25 years	15/102 (14.7)	0.97	0.47-2.01	0.94
	26-52 years	19/124 (15.3)	1.02	0.52-2.02	0.95
Number of years having been a broiler farmer *(continuous)*		-	1.01	0.99-1.03	0.52
Number of years having farmed for the company					0.50[1]
	<6 months-4 years	18/142 (12.7)	1.00	-	-
	5-9 years	22/120 (18.3)	1.55	0.79-3.04	0.21
	10-15 years	19/129 (14.7)	1.19	0.59-2.38	0.62
	16-45 years	15/125 (12.0)	0.94	0.45-1.95	0.87
Number of years having farmed for the company *(continuous)*		-	1.00	0.97-1.02	0.79
Are you a member of quality assurance scheme					
	Yes	72/495 (14.5)	-	-	-
	No	0/19 (0)	Ref.	-	-

Variable	Risk of NE (%)	OR	95% C.I.	p-value
Hygiene & biosecurity				
Use of all in/all out				
Yes	66/466 (14.2)	1.06	0.45-2.91	0.89
No	7/52 (13.5)	Ref.	-	-
Length of turn-around (days) before the last crop				0.02[1]
1-6	22/129 (17.1)	Ref.	-	-
7-9	19/145 (13.1)	0.73	0.38-1.43	0.36
10-12	23/118 (19.5)	1.18	0.62-2.25	0.62
14-62	9/125 (7.2)	0.38	0.17-0.86	0.02
Length of turn-around before the last crop *(continuous)*	-	0.97	0.93-1.02	0.25
Second degree fractional polynomial: 3.	-	-	-	0.008
3	-	-	-	0.008
Minimum time between disinfection and restocking the last crop (days)				0.60[1]
0.5-2	16/103 (15.5)	Ref.	-	-
3-4	27/160 (16.9)	1.10	0.56-2.17	0.77
4.5-6	14/107 (13.1)	0.82	0.38-1.78	0.61
7-48	17/144 (11.8)	0.73	0.35-1.52	0.40
Minimum time between disinfection and restocking the last crop *(continuous)*	-	0.94	0.87-1.02	0.12
Were the houses cleaned before the last crop				
Yes	74/517 (14.3)	-	-	-
No	0/1 (0)	Ref.	-	-
Who cleaned the houses				0.07[1]
Contract cleaner	57/329 (17.3)	Ref.	-	-
Company cleaner	4/36 (11.1)	0.60	0.20-1.75	0.35
Farm manager	11/120 (9.2)	0.48	0.24-0.95	0.04
How were the houses cleaned				0.12[1]
With water and detergent	63/431 (14.6)	Ref.	-	-
With water	6/45 (13.3)	0.90	0.37-2.21	0.82
Dry brush and/or compressed air	3/6 (50.0)	5.84	1.15-29.59	0.03
Were the houses disinfected before the last crop				
Yes	74/515 (14.4)	-	-	-
No	0/0	Ref.	-	-
Who disinfected the houses				0.04[1]
Contract cleaner	57/325 (17.5)	Ref.	-	-
Company cleaner	4/42 (9.5)	0.49	0.17-1.44	0.20
Farm manager	12/129 (9.3)	0.48	0.25-0.93	0.03
How were the houses disinfected				0.82[1]
Spray disinfectant	33/217 (15.2)	Ref.	-	-
Fumigation	0/5 (0)	-	-	-
Both	40/276 (14.5)	0.95	0.57-1.56	0.83
Use of ammonia as a disinfectant before the last crop				
Yes	14/40 (35.0)	3.60	1.66-7.74	<0.001
No	56/430 (13.0)	Ref.	-	-
Were bacterial counts taken from inside the house				
Yes	18/211 (8.5)	0.41	0.22-0.76	0.002
No	52/283 (18.4)	Ref.	-	-
Was the inside of the water system cleaned				
Yes	63/467 (13.5)	0.59	0.27-1.35	0.16
No	10/48 (20.8)	Ref.	-	-
Are hand wash facilities available				
Yes	73/508 (14.4)	1.84	0.26-80.53	0.55
No	1/12 (8.3)	Ref.	-	-

Variable	Risk of NE (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)				
Are hand wash facilities available in each house				
Yes	17/182 (9.3)	0.47	0.25-0.88	0.01
No	55/308 (17.9)	Ref.	-	-
Do you wash your hands before entering the house				0.98[1]
Never	13/92 (14.1)	Ref.	-	-
Occasionally	37/269 (13.8)	0.97	0.49-1.92	0.93
Always	22/152 (14.5)	1.03	0.48-2.23	0.94
Are hand sanitizers available				
Yes	67/457 (14.7)	1.30	0.56-3.53	0.53
No	7/60 (11.7)	Ref.	-	-
Are hand sanitizers available in each house				
Yes	56/374 (15.0)	0.97	0.49-1.94	0.92
No	14/91 (15.4)	Ref.	-	-
Do you sanitise your hands before entering the house				0.61[1]
Never	10/90 (11.1)	Ref.	-	-
Occasionally	35/235 (14.9)	1.40	0.66-2.96	0.38
Always	29/191 (15.2)	1.43	0.67-3.08	0.36
Are shower facilities available				
Yes	17/73 (23.3)	2.09	1.08-4.02	0.02
No	57/449 (12.7)	Ref.	-	-
Do you shower before entering the house				0.54[1]
Never	50/376 (13.3)	Ref.	-	-
Occasionally	6/36 (16.7)	1.35	0.53-3.41	0.53
Always	0/1 (0)	-	-	-
Is farm clothing available				
Yes	71/509 (14.0)	0.49	0.12-2.86	0.28
No	3/12 (25.0)	Ref.	-	-
Is separate clothing available for each house				
Yes	10/151 (6.6)	0.32	0.15-0.67	<0.001
No	60/327 (18.3)			
Do you change clothes before entering the house				0.14[1]
Never	42/254 (16.5)	Ref.	-	-
Occasionally	16/128 (12.5)	0.72	0.39-1.34	0.30
Always	11/118 (9.3)	0.52	0.26-1.05	0.07
Are there barriers before the entrance to the house				
Yes	50/385 (13.0)	0.70	0.39-1.23	0.18
No	24/136 (17.6)	Ref.	-	-
Are there barriers before the entrance to each house				
Yes	46/357 (12.9)	0.66	0.26-1.87	0.34
No	7/38 (18.4)	Ref.	-	-
Are there boot dip facilities available				
Yes	73/517 (14.1)	-	-	-
No	0/5 (0)	Ref.	-	-
Are there boot dip facilities for each house				
Yes	71/478 (14.9)	-	-	-
No	0/4 (0)	Ref.	-	-
Do you dip your boots before entering the house				0.32[1]
Always	69/495 (13.9)	Ref.	-	-
Occasionally	5/23 (21.7)	1.71	0.61-4.77	0.30
Never	0/5 (0)	-	-	-

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Are there farm boots available					
	Yes	67/486 (13.8)	0.80	0.31-2.44	0.63
	No	6/36 (16.7)	Ref.	-	-
Are there farm boots available for each house					
	Yes	24/240 (10.0)	0.49	0.28-0.88	0.01
	No	40/218 (18.3)	Ref.	-	-
Do you change boots before entering the house					0.31[1]
	Never	32/192 (16.7)	Ref.	-	-
	Occasionally	10/63 (15.9)	0.94	0.43-2.05	0.88
	Always	30/255 (11.8)	0.67	0.39-1.14	0.14
Are plastic over boots available					
	Yes	58/415 (14.0)	0.91	0.48-1.75	0.77
	No	16/106 (15.1)	Ref.	-	-
Are plastic over boots available for each house					
	Yes	40/319 (12.5)	0.59	0.32-1.11	0.08
	No	21/108 (19.4)	Ref.	-	-
Do you use plastic over boots before entering the house					0.54[1]
	Never	44/286 (15.4)	Ref.	-	-
	Occasionally	16/120 (13.3)	0.85	0.46-1.58	0.60
	Always	11/88 (12.5)	0.69	0.34-1.39	0.30
Are rodents seen on the farm					0.52[1]
	Never	21/128 (16.4)	Ref.	-	-
	Every six months	25/204 (12.3)	0.71	0.38-1.33	0.29
	Every crop	10/78 (12.8)	0.75	0.33-1.69	0.49
	Every month	9/35 (25.7)	1.31	0.55-3.12	0.54
	Every week	5/19 (26.3)	1.34	0.45-3.99	0.60
	Every few days	0/15 (0)	-	-	-
	Daily	0/9 (0)	-	-	-
Are rodents seen in- or outside the shed					0.84[1]
	Outside	39/269 (14.5)	Ref.	-	-
	Inside	10/80 (12.5)	0.84	0.40-1.77	0.65
	Both	5/42 (11.9)	0.80	0.30-2.15	0.65
Are there rodent control measures in place					
	Yes	74/520 (14.2)	-	-	-
	No	0/1 (0)	Ref.	-	-
What type of rodent control measures					0.71[1]
	Poison	53/396 (13.4)	Ref.	-	-
	Other (i.e. cats, traps, dogs)	1/4 (25.0)	2.16	0.22-21.12	0.51
	Poison and other	16/102 (15.7)	1.20	0.66-2.21	0.55
Number of rodent baits used					0.31[1]
	1-9	14/137	Ref.	-	-
	10-16	15/117	1.29	0.60-2.80	0.52
	17-30	15/122	1.23	0.57-2.67	0.60
	31-150	21/115	1.96	0.95-4.06	0.07
Number of rodent baits used *(continuous)*		-	1.01	1.00-1.02	0.19
Presence of litter beetles in the house					
	Yes	32/201 (15.9)	1.28	0.75-2.18	0.33
	No	41/318 (12.9)	Ref.	-	-
Litter beetle control measures					
	Yes	33/210 (15.7)	1.28	0.75-2.18	0.33
	No	36/274 (13.1)	Ref.	-	-
Are there other domestic animals on the farm					
	Yes	49/346 (14.2)	1.06	0.60-1.88	0.83
	No	23/171 (13.5)	Ref.	-	-

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Are there cattle on the farm					
	Yes	11/128 (8.6)	0.51	0.24-1.04	0.04
	No	61/389 (15.7)	Ref.	-	-
Are there sheep on the farm					
	Yes	11/87 (12.6)	0.88	0.41-1.83	0.70
	No	61/430 (14.2)	Ref.	-	-
Are there pigs on the farm					
	Yes	3/15 (20.0)	1.57	0.34-6.25	0.49
	No	69/502 (13.7)	Ref.	-	-
Are there horses on the farm					
	Yes	7/68 (10.3)	0.68	0.27-1.63	0.35
	No	65/448 (14.5)	Ref.	-	-
Are there dogs on the farm					
	Yes	37/268 (13.8)	0.98	0.58-1.67	0.93
	No	35/249 (14.1)	Ref.	-	-
Are there cats on the farm					
	Yes	30/209 (14.4)	1.06	0.62-1.82	0.82
	No	42/308 (13.6)	Ref.	-	-
Are there other types of animals on the farm					
	Yes	3/12 (25.0)	2.07	0.43-8.73	0.27
	No	68/491 (13.8)	Ref.	-	-
Are (is) there (a) poultry farm(s) within a 2 mile radius					
	Yes	44/328 (13.4)	0.81	0.47-1.39	0.41
	No	30/187 (16.0)	Ref.	-	-
Are (is) there (a) broiler farm(s) within a 2 mile radius					
	Yes	33/265 (12.5)	0.73	0.43-1.23	0.20
	No	41/250 (16.4)	Ref.	-	-
Are (is) there (a) broiler breeder farm(s) within a 2 mile radius					
	Yes	9/45 (20.0)	1.56	0.66-3.59	0.26
	No	65/470 (13.8)	Ref.	-	-
Are (is) there (a) breeder rearing farm(s) within a 2 mile radius					
	Yes	3/21 (14.3)	0.99	0.22-3.73	0.99
	No	71/494 (14.4)	Ref.	-	-
Are (is) there (a) layer farm(s) within a 2 mile radius					
	Yes	5/43 (11.6)	0.77	0.25-2.15	0.59
	No	69/471 (14.6)	Ref.	-	-
Are (is) there (a) layer breeder farm(s) within a 2 mile radius					
	Yes	1/11 (9.0)	0.59	0.03-4.62	0.61
	No	73/503 (14.5)	Ref.	-	-
Are (is) there (a) layer rearing farm(s) within a 2 mile radius					
	Yes	0/11 (0)	-	-	-
	No	74/429 (17.2)	Ref.	-	-
Are (is) there (a) turkey farm(s) within a 2 mile radius					
	Yes	6/36 (16.7)	1.21	0.43-3.21	0.69
	No	68/478 (14.2)	Ref.	-	-
Are (is) there (a) turkey breeder farm(s) within a 2 mile radius					
	Yes	1/10 (10.0)	0.66	0.03-5.24	0.69
	No	73/504 (14.5)	Ref.	-	-

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Are (is) there (a) turkey breeder rearing farm(s) within a 2 mile radius					
	Yes	0/2 (0)	-	-	-
	No	74/512 (14.5)	Ref.	-	-
Are (is) there (a) free range broiler farm(s) within a 2 mile radius					
	Yes	1/13 (7.7)	0.49	0.02-3.74	0.49
	No	73/501 (14.6)	Ref.	-	-
Are (is) there (a) free range layer farm(s) within a 2 mile radius					
	Yes	5/35 (14.3)	0.99	0.32-2.82	0.98
	No	69/479 (14.4)	Ref.	-	-
Are (is) there (an) other poultry farm(s) within a 2 mile radius					
	Yes	4/16 (25.0)	2.04	0.53-7.14	0.22
	No	70/498 (14.1)	Ref.	-	-
Are there domestic animals on adjoining fields					
	Yes	48/352 (13.6)	0.92	0.52-1.63	0.76
	No	24/164 (14.6)	Ref.	-	-
Are there cattle on adjoining fields					
	Yes	28/239 (11.7)	0.70	0.40-1.20	0.16
	No	44/275 (16.0)	Ref.	-	-
Are there sheep on adjoining fields					
	Yes	27/205 (13.2)	0.89	0.51-1.54	0.66
	No	45/309 (14.6)	Ref.	-	-
Are there pigs on adjoining fields					
	Yes	1/12 (8.3)	0.55	0.03-4.28	0.57
	No	71/502 (14.1)	Ref.	-	-
Are there horses on adjoining fields					
	Yes	20/104 (19.2)	1.64	0.89-3.01	0.09
	No	52/410 (12.7)	Ref.	-	-
Are there dogs on adjoining fields					
	Yes	14/110 (12.7)	0.87	0.44-1.70	0.66
	No	58/404 (14.4)	Ref.	-	-
Are there cats on adjoining fields					
	Yes	12/96 (12.5)	0.85	0.41-1.73	0.64
	No	60/418 (14.4)	Ref.	-	-
Are there other domestic animals on adjoining fields					
	Yes	1/5 (20.0)	1.54	0.03-15.86	0.70
	No	71/509 (13.9)	Ref.	-	-
Are other animals, domestic or wild, seen in the poultry house					
	Yes	5/44 (11.4)	0.75	0.29-1.98	0.57
	No	69/475 (14.5)	Ref.	-	-
When are other animals seen in the house					
During occupation					
	Yes	1/9 (11.1)	0.97	0.02-11.81	0.98
	No	4/35 (11.4)	Ref.	-	-
When empty					
	Yes	4/39 (10.3)	0.47	0.03-28.08	0.52
	No	1/5 (20.0)	Ref.	-	-

Variable	Risk of NE (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)				
Are wild birds seen in the poultry house				
Yes	23/139 (16.5)	1.30	0.76-2.22	0.34
No	50/377 (13.3)	Ref.	-	-
When are wild birds seen in the house				
During occupation				
Yes	6/28 (21.4)	1.48	0.45-4.65	0.46
No	17/109 (15.6)	Ref.	-	-
When empty				
Yes	20/116 (17.2)	1.25	0.32-7.23	0.74
No	3/21 (14.3)	Ref.	-	-
Litter is disposed by spreading on the field				
Yes	25/174 (14.4)	1.04	0.59-1.81	0.89
No	48/345 (13.9)	Ref.	-	-
Litter is deep stacked and then spread on the field				
Yes	25/174 (14.4)	1.04	0.59-1.81	0.89
No	48/345 (13.9)	Ref.	-	-
Litter is disposed to a power plant				
Yes	34/203 (16.7)	1.43	0.84-2.43	0.16
No	39/316 (12.3)	Ref.	-	-
Litter is used for mushroom composting				
Yes	9/89 (10.1)	0.64	0.28-1.42	0.24
No	64/430 (14.9)	Ref.	-	-
Other types of litter disposal				
Yes	8/27 (29.6)	2.73	1.15-6.49	0.02
No	66/494 (13.4)	Ref.	-	-
If the litter is spread on a field, the field is located:				
On the farm				
Yes	18/136 (13.2)	0.90	0.44-1.82	0.74
No	25/172 (14.5)	Ref.	-	-
On adjacent premises				
Yes	10/55 (18.2)	1.48	0.63-3.43	0.32
No	33/253 (13.0)	Ref.	-	-
Elsewhere				
Yes	31/200 (15.5)	1.47	0.68-3.21	0.29
No	12/108 (11.1)	Ref.	-	-
Was the used litter removed from the farm prior to disinfection of the house				
Yes	69/485 (14.2)	1.00	0.36-3.40	0.99
No	5/35 (14.3)	Ref.	-	-
Was the used litter removed from the farm prior to arrival of the new chicks				
Yes	72/493 (14.6)	1.62	0.38-14.67	0.52
No	2/21 (9.5)	Ref.	-	-
Is manure spread on fields of the farm				
Yes	16/167 (9.6)	0.54	0.29-1.01	0.04
No	56/342 (16.4)	Ref.	-	-
Is cow manure spread on fields of the farm				
Yes	12/142 (8.5)	0.47	0.23-0.95	0.02
No	59/361 (16.3)	Ref.	-	-
Is pig manure spread on fields of the farm				
Yes	3/17 (17.6)	1.32	0.29-5.12	0.67
No	68/486 (14.0)	Ref.	-	-
Is human sludge spread on fields of the farm				
Yes	2/4 (50.0)	6.19	0.44-86.76	0.04
No	69/499 (13.8)	Ref.	-	-

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Is another type of manure spread on fields of the farm					
	Yes	2/8 (25.0)	2.06	0.00-11.72	0.37
	No	69/495 (13.9)	Ref.	-	-
Is manure spread on fields adjoining the farm					
	Yes	38/304 (12.5)	0.72	0.41-1.25	0.21
	No	30/181 (16.6)	Ref.	-	-
Is cow manure spread on fields adjoining the farm					
	Yes	26/244 (10.7)	0.55	0.31-0.97	0.03
	No	42/237 (17.7)	Ref.	-	-
Is pig manure spread on fields adjoining the farm					
	Yes	8/49 (16.3)	1.21	0.49-2.87	0.64
	No	60/432 (13.9)	Ref.	-	-
Is poultry manure spread on fields adjoining the farm					
	Yes	12/68 (17.6)	1.37	0.64-2.85	0.37
	No	56/413 (13.6)	Ref.	-	-
Is human sludge spread on fields adjoining the farm					
	Yes	3/13 (23.1)	1.86	0.39-7.67	0.35
	No	65/468 (13.9)	Ref.	-	-
Is other type of manure spread on fields adjoining the farm					
	Yes	2/6 (33.3)	3.09	0.27-22.04	0.17
	No	66/475 (13.9)	Ref.	-	-
Were there changes in management or employees during the last crop					
	Yes	8/31 (25.8)	2.24	0.87-5.58	0.06
	No	66/491 (13.4)	Ref.	-	-
Did people other than the farm manager, employees or area manager enter the house during the last crop					
	Yes	41/258 (15.9)	1.36	0.80-2.32	0.22
	No	32/263 (12.2)	Ref.	-	-
Did a veterinarian enter the house during the last crop					
	Yes	11/63 (17.5)	1.36	0.63-2.90	0.39
	No	61/454 (13.4)	Ref.	-	-
Did a feed representative enter the house during the last crop					
	Yes	3/15 (20.0)	1.56	0.34-6.22	0.50
	No	69/500 (13.8)	Ref.	-	-
Did an electrician enter the house during the last crop					
	Yes	33/193 (17.1)	1.50	0.88-2.57	0.11
	No	39/323 (12.1)	Ref.	-	-
Did a service engineer enter the house during the last crop					
	Yes	15/91 (16.5)	1.26	0.64-2.45	0.46
	No	57/422 (13.5)	Ref.	-	-
Did an other person enter the house during the last crop					
	Yes	7/66 (10.6)	0.70	0.28-1.68	0.39
	No	65/447 (14.5)	Ref.	-	-

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Did commercial vehicles come onto the farm during the last crop					
	Yes	74/521 (14.2)	-	-	-
	No	0/0 (-)	Ref.	-	-
Did feed lorries come onto the farm during the last crop					
	Yes	73/517 (14.1)	0.33	0.02-19.66	0.34
	No	1/3 (33.3)	Ref.	-	-
Did hatchery lorries come onto the farm during the last crop					
	Yes	73/504 (14.5)	2.71	0.41-115.18	0.32
	No	1/17 (5.9)	Ref.	-	-
Did collection lorries come onto the farm during the last crop					
	Yes	74/494 (15.0)	-	-	-
	No	0/27 (0)	Ref.	-	-
Did gas lorries come onto the farm during the last crop					
	Yes	65/447 (14.5)	1.13	0.51-2.60	0.74
	No	9/69 (13.0)	Ref.	-	-
Did oil lorries come onto the farm during the last crop					
	Yes	26/194 (13.4)	0.85	0.49-1.48	0.55
	No	47/306 (15.4)	Ref.	-	-
Did straw/shavings lorries come onto the farm during the last crop					
	Yes	69/485 (14.2)	0.90	0.32-3.08	0.83
	No	5/32 (15.6)	Ref.	-	-
Did other types of lorries come onto the farm during the last crop					
	Yes	23/111 (20.7)	1.84	1.01-3.31	0.03
	No	47/377 (12.5)	Ref.	-	-
Feed					
Are single or double feed bins used					0.91[1]
	Single	25/188 (13.3)	Ref.	-	-
	Double	39/265 (14.7)	1.13	0.65-1.93	0.67
	Both	9/63 (14.3)	1.09	0.48-2.47	0.84
Number of feed bins on the farm					0.11[1]
	1-3	11/105 (10.5)	Ref.	-	-
	4-6	18/164 (11.0)	1.05	0.48-2.33	0.90
	7-9	21/108 (19.4)	2.06	0.94-4.52	0.07
	10-40	24/140 (17.1)	1.77	0.82-3.79	0.14
Number of feed bins on the farm *(continuous)*		-	1.04	1.00-1.09	0.08
How often are feed bins cleaned inside					0.63[1]
	Never	29/254 (11.4)	Ref.	-	-
	Once per year	12/85 (14.1)	1.28	0.62-2.63	0.51
	Once per 6 months	8/47 (17.4)	1.59	0.68-3.74	0.29
	Once per crop	18/105 (17.1)	1.61	0.85-3.04	0.15
	More often than once per crop	1/7 (14.3))	1.29	0.15-11.12	0.82
	Other	0/3 (0)	-	-	-
How often are feed bins disinfected inside					0.94[1]
	Never	51/377 (13.5)	Ref.	-	-
	Once per year	8/62 (12.9)	0.95	0.43-2.11	0.89
	Once per 6 months	7/39 (17.9)	1.40	0.59-3.34	0.45
	Once per crop	4/25 (16.0)	1.22	0.40-3.69	0.73
	More often than once per crop	0/0	-	-	-
	Other	1/5 (20.0)	1.60	0.18-14.58	0.68

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Feed (continued)					
Type of feeders used					
Flat chain/auger feeder					
	Yes	43/284 (15.1)	1.23	0.72-2.11	0.42
	No	30/237 (12.7)	Ref.	-	-
Pan feeder					
	Yes	42/309 (13.6)	0.92	0.54-1.57	0.74
	No	31/212 (14.6)	Ref.	-	-
Tube feeder					
	Yes	2/21 (9.5)	0.64	0.10-2.96	0.55
	No	71/500 (14.2)	Ref.	-	-
Other type of feeder					
	Yes	0/2 (0)	-	-	-
	No	73/519 (14.1)	Ref.	-	-
What feeding regimen was used during the last crop					
	Ad libitum	51/369 (13.8)	Ref.	-	-
	Controlled	23/139 (16.5)	1.24	0.72-2.11	0.44
Were there any feeding equipment failures during the last crop					
	Yes	35/191 (18.3)	1.70	1.00-2.90	0.04
	No	38/326 (11.7)	Ref.	-	-
Number of feed equipment break-downs during the last crop					
	≤3	18/93 (19.4)	Ref.	-	-
	>3	12/68 (17.6)	0.89	0.40-2.00	0.78
Number of feed equipment break-downs during the last crop *(continuous)*		-	1.00	0.92-1.08	0.92
First degree fractional polynomial: -2		-	-	-	0.05
Number of feed lorries entering the farm during the last crop					0.02[1]
	1-10	8/119 (6.7)	Ref.	-	-
	11-17	19/126 (15.1)	2.46	1.03-5.87	0.04
	18-26	21/114 (18.4)	3.13	1.33-7.40	0.009
	27-96	23/127 (18.1)	3.07	1.31-7.16	0.01
Number of feed lorries entering the farm during the last crop *(continuous)*		-	1.02	1.00-1.03	0.03
First degree fractional polynomial: -1		-	-	-	0.002
Was whole wheat added to the feed during the last crop					
	Yes	67/407 (16.5)	1.63	0.70-4.42	0.24
	No	7/65 (10.8)	Ref.	-	-
Minimum (%) amount of whole wheat added					
	≤5	33/221 (14.9)	Ref.	-	-
	>5	20/116 (17.2)	1.19	0.65-2.18	0.58
Minimum amount of whole wheat added *(continuous)*		-	1.03	0.96-1.10	0.39
Maximum (%) amount of whole wheat added					
	≤20	42/280 (15.0)	Ref.	-	-
	>20	17/76 (22.4)	1.63	0.87-3.07	0.13
Maximum amount of whole wheat added *(continuous)*		-	1.00	0.96-1.05	0.89

Variable	Risk of NE (%)	OR	95% C.I.	p-value
Feed (continued)				
Who supplied the wheat				
Own farm				
Yes	13/54 (24.1)	1.69	0.80-3.54	0.13
No	56/355 (15.8	Ref.	-	-
Other farm				
Yes	7/30 (23.3)	1.56	0.57-4.06	0.33
No	62/379 (16.4	Ref.	-	-
Feed mill				
Yes	52/337 (15.4)	0.59	0.30-1.16	0.09
No	17/72 (23.6)	Ref.	-	-
Where was the whole wheat added				
Blended on the farm				
Yes	20/84 (23.8)	1.74	0.92-3.27	0.06
No	49/322 (15.2)	Ref.	-	-
Blended in the mill				
Yes	17/150 (11.3)	0.50	0.26-0.94	0.02
No	52/256 (20.3)	Ref.	-	-
Blended in the wagon				
Yes	15/61 (24.6)	1.76	0.86-3.54	0.09
No	54/345 (15.7)	Ref.	-	-
Dumped on the wagon				
Yes	25/127 (19.7)	1.31	0.73-2.34	0.33
No	44/279 (15.8)	Ref.	-	-
Was the wheat treated				
Yes	43/232 (18.5)	0.86	0.38-2.03	0.71
No	10/48 (20.8)	Ref.	-	-
With what was the wheat treated				
Organic acids				
Yes	18/77 (23.4)	1.59	0.75-3.35	0.18
No	24/149 (16.1)	Ref.	-	-
Virkon				
Yes	0/1 (0)	-	-	-
No	42/225 (18.7)	Ref.	-	-
Salcurb				
Yes	12/70 (17.1)	0.87	0.39-1.93	0.71
No	30/156 (19.2)	Ref.	-	-
Other				
Yes	3/15 (20.0)	1.10	0.23-4.54	0.88
No	39/211 (18.5)	Ref.	-	-
Water				
Type of drinkers used				
Bell drinkers				
Yes	5/19 (26.3)	2.24	0.67-6.99	0.12
No	69/501 (13.8)	Ref.	-	-
Drink nipples				
Yes	29/219 (13.2)	0.87	0.51-1.48	0.58
No	45/301 (15.0)	Ref.	-	-
Drink nipples with cups				
Yes	53/350 (15.1)	1.27	0.71-2.27	0.39
No	21/170 (12.4)	Ref.	-	-
Other type of drinkers				
Yes	1/9 (11.1)	0.75	0.00-6.11	0.79
No	73/511 (14.3)	Ref.	-	-
What water regimen was used during the last crop				
Ad libitum	70/496 (14.1)	Ref.	-	-
Controlled	2/16 (12.5)	1.15	0.26-5.17	0.86

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Water (continued)					
What was the source of the water supply during the last crop					
Mains					
	Yes	57/379 (15.0)	1.34	0.71-2.55	0.33
	No	16/137 (11.7)	Ref.	-	-
Well					
	Yes	3/35 (8.6)	0.55	0.13-1.97	0.33
	No	70/481 (14.6)	Ref.	-	-
Borehole					
	Yes	18/131 (13.7)	0.96	0.51-1.76	0.88
	No	55/385 (14.3)	Ref.	-	-
Stream					
	Yes	0/1 (0)	-	-	-
	No	73/515 (14.2)	Ref.	-	-
Other source of water supply					
	Yes	2/7 (28.6)	2.46	0.23-15.39	0.27
	No	71/509 (13.9)	Ref.	-	-
Has the water been analysed					
	Yes	38/285 (13.3)	0.87	0.49-1.54	0.60
	No	27/179 (15.1)	Ref.	-	-
What kind of water analysis					
Bacteriological					
	Yes	27/214 (12.6)	0.79	0.45-1.40	0.38
	No	38/246 (15.4)	Ref.	-	-
Chemical					
	Yes	6/55 (10.9)	0.72	0.26-1.86	0.46
	No	59/405 (14.6)	Ref.	-	-
Were there any water supply failures during the last crop					
	Yes	1/6 (16.7)	1.20	0.02-10.92	0.87
	No	73/510 (14.3)	Ref.	-	-
Was a water sanitizer used during the last crop					
	Yes	51/312 (16.3)	1.54	0.87-2.74	0.11
	No	22/195 (11.3)	Ref.	-	-
Prevention/treatment					
Which vaccines were used during the last crop					
NCD					
	Yes	17/114 (14.9)	0.92	0.45-1.88	0.81
	No	27/169 (16.0)	Ref.	-	-
IBD					
	Yes	67/485 (13.8)	0.96	0.21-9.04	0.96
	No	2/14 (14.3)	Ref.	-	-
IB					
	Yes	58/344 (16.9)	1.62	0.60-5.49	0.32
	No	5/45 (11.1)	Ref.	-	-
TRT					
	Yes	3/15 (20.0)	1.29	0.27-5.40	0.70
	No	31/191 (16.2)	Ref.	-	-
Other types of vaccination					
	Yes	1/3 (33.3)	3.46	0.03-352.33	0.37
	No	1/9 (11.1)	Ref.	-	-
Were preventive antibiotics used					
	Yes	45/268 (16.8)	1.81	1.00-3.28	0.03
	No	21/209 (10.0)	Ref.	-	-

Variable		Risk of NE (%)	OR	95% C.I.	p-value
Prevention/treatment (continued)					
Number of times preventive antibiotics were given during the last crop					
	once	37/211 (17.5)	Ref.	-	-
	2-4 times	7/58 (12.1)	0.65	0.27-1.53	0.32
Were curative antibiotics used					
	Yes	61/193 (31.6)	22.27	9.34-64.66	<0.0001
	No	6/197 (2.0)	Ref.	-	-
Number of times curative antibiotics were given during the last crop					
	once	38/132 (28.8)	Ref.	-	-
	2-4 times	23/61 (37.7)	1.50	0.79-2.84	0.22
Were coccidiostatic agents used					
	Yes	58/373 (15.5)	1.79	0.61-7.17	0.28
	No	4/43 (9.3)	Ref.	-	-
Were competitive exclusion products (CE) used					
	Yes	2/56 (3.6)	0.19	0.03-0.83	0.01
	No	65/398 (16.3)	Ref.	-	-
Which type of CE product was used					
	Aviquard	1/46 (2.2)	Ref.	-	-
	Broilact	1/5 (20.0)	11.25	0.59-215.9	1.11
Flock performance					
Feed conversion of the last crop					0.25[1]
	1.60-1.80	12/115 (10.4)	Ref.	-	-
	1.81-1.87	21/119 (17.6)	1.84	0.86-3.94	0.12
	1.88-1.92	10/86 (11.6)	1.13	0.46-2.75	0.79
	1.93-2.26	19/106 (17.9)	1.87	0.86-4.08	0.11
Feed conversion of the last crop *(continuous, per 0.1 point increase)*		-	1.26	0.92-1.72	0.15
Total mortality (%) of the last crop					0.009[1]
	0.20-2.63	8/120 (6.7)	Ref.	-	-
	2.65-3.44	21/122 (17.2)	2.91	1.23-6.86	0.02
	3.45-4.24	18/121 (14.9)	2.45	1.02-5.87	0.05
	4.25-11.80	24/113 (21.2)	3.78	1.62-8.81	0.002
Total mortality of the last crop *(continuous)*		-	1.26	1.07-1.48	0.005
EPEF of the last crop					0.33[1]
	180-270	16/79 (20.3)	Ref.	-	-
	271-285	15/85 (17.6)	0.84	0.39-1.84	0.67
	286-300	10/88 (11.4)	0.50	0.21-1.19	0.12
	301-379	17/83 (20.5)	1.01	0.47-2.18	0.97
EPEF of the last crop *(continuous, per 10 points increase)*		-	0.95	0.86-1.06	0.38

[1]. Likelihood ratio test statistic

Appendix 7: Complete results of the univariate analysis of the associations between the occurrence of wet litter and explanatory variables.

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Disease					
Necrotic enteritis in the last crop					
	Yes	54/73 (74.0)	2.82	1.56–5.13	<0.001
	No	223/444 (50.2)	Ref.	-	-
Coccidiosis in the last crop					
	Yes	32/35 (91.4)	9.17	2.77-30.33	<0.001
	No	278/517 (53.8)	Ref.	-	-
Respiratory diseases in the last crop					
	Yes	13/21 (61.9)	1.31	0.54-3.22	0.55
	No	292/528 (55.3)	Ref.	-	-
Immunosuppressive diseases in the last crop					
	Yes	9/9 (100.0)	-	-	0.007
	No	303/549 (55.2)	Ref.	-	-
Environment					
Number of houses on the farm					<0.001[1]
	1-2	68/156 (43.6)	Ref.	-	-
	3-4	78/158 (49.4)	1.26	0.81-1.97	0.31
	5-7	80/118 (67.8)	2.72	1.65-4.49	<0.001
	8-20	108/157 (68.8)	2.85	1.80-4.53	<0.001
Number of houses on the farm *(continuous)*		-	1.12	1.07-1.18	<0.001
First degree fractional polynomial: 0		-	-	-	<0.001
Age of the newest house					0.13[1]
	<6 months-5 years	86/165 (52.1)	Ref.	-	-
	6-11 years	62/120 (51.7)	0.98	0.61-1.57	0.94
	12-26 years	85/142 (59.9)	1.37	0.87-2.16	0.17
	27-60 years	89/142 (62.7)	1.57	0.99-2.49	0.05
Age of the newest house *(continuous)*		-	1.01	1.00-1.02	0.10
Age of the eldest house					0.006[1]
	6 months-12 years	69/141 (48.9)	Ref.	-	-
	13-28 years	74/138 (53.6)	1.21	0.75-1.93	0.43
	29-37 years	84/144 (58.3)	1.46	0.92-2.33	0.11
	38-60 years	90/130 (69.2)	2.35	1.43-3.86	0.001
Age of the eldest house *(continuous)*		-	1.02	1.01-1.03	0.004
Average age of the houses					0.18[1]
	6 months-9 years	65/130 (50.0)	Ref.	-	-
	10-18 years	76/128 (59.4)	1.46	0.89-2.39	0.13
	19-30 years	78/134 (58.2)	1.39	0.86-2.26	0.18
	31-60 years	76/120 (63.3)	1.73	1.04-2.86	0.03
Average age of the houses *(continuous)*		-	1.01	1.00-1.03	0.06
Minimum distance between houses					0.21[1]
	1-16 feet	86/132 (65.2)	Ref	-	-
	18-22 feet	80/131 (61.1)	0.84	0.51-1.39	0.49
	23-30 feet	81/145 (55.9)	0.68	0.42-1.10	0.12
	32-10560 feet	60/113 (53.1)	0.61	0.36-1.01	0.06
Minimum distance between houses *(continuous, per 10 feet)*		-	1.00	0.99-1.01	0.59
Maximum distance between houses					0.03[1]
	8-20 feet	93/146 (63.7)	Ref.	-	-
	21-30 feet	77/155 (49.7)	0.56	0.35-0.89	0.02
	33-40 feet	56/85 (65.9)	1.10	0.63-1.93	0.74
	41-10560 feet	78/124 (62.9)	0.97	0.59-1.59	0.89

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Environment (continued)					
Maximum distance between houses *(continuous, per 10 feet)*		-	1.00	0.99-1.02	0.52
Wooden house walls					
	Yes	231/378 (61.1)	1.60	1.14-2.25	0.007
	No	104/210 (49.5)	Ref.	-	-
Concrete house walls					
	Yes	105/182 (57.7)	1.04	0.73-1.49	0.81
	No	230/406 (56.7)	Ref.	-	-
Brick house walls					
	Yes	65/113 (57.5)	1.03	0.68-1.56	0.90
	No	270/475 (56.8)	Ref.	-	-
Breeze block house walls					
	Yes	170/278 (61.2)	1.38	1.00-1.92	0.05
	No	165/310 (53.2)	Ref.	-	-
Asbestos house walls					
	Yes	128/208 (61.5)	1.34	0.95-1.89	0.10
	No	207/380 (54.5)	Ref.	-	-
Plaster board house walls					
	Yes	18/36 (50.0)	0.74	0.38-1.46	0.38
	No	317/552 (57.4)	Ref.	-	-
Metal lining & wood house walls					
	Yes	57/104 (54.8)	0.90	0.59-1.38	0.62
	No	278/484 (57.4)	Ref.	-	-
Metal lining & plastic house walls					
	Yes	31/59 (52.5)	0.82	0.48-1.41	0.47
	No	304/529 (57.5)	Ref.	-	-
Other type of house walls					
	Yes	47/85 (55.3)	0.93	0.58-1.47	0.75
	No	288/504 (57.1)	Ref.	-	-
Wooden roof					
	Yes	89/161 (55.3)	0.92	0.64-1.33	0.67
	No	241/421 (57.2)	Ref.	-	-
Asbestos roof					
	Yes	178/308 (57.8)	1.10	0.79-1.53	0.57
	No	152/274 (55.5)	Ref.	-	-
Metal lining & wood roof					
	Yes	133/233 (57.1)	1.03	0.73-1.43	0.88
	No	197/349 (56.4)	Ref.	-	-
Metal lining & plastic roof					
	Yes	37/72 (51.4)	0.78	0.48-1.28	0.33
	No	293/510 (57.5)	Ref.	-	-
Other type of roof					
	Yes	73/127 (57.5)	1.02	0.69-1.52	0.91
	No	263/462 (56.9)	Ref.	-	-
Concrete floor					
	Yes	335/588 (57.0)	1.99	0.33-11.98	0.45
	No	2/5 (40.0)	Ref.	-	-
Earth floor					
	Yes	1/4 (25.0)	0.25	0.03-2.43	0.20
	No	336/589 (57.0)	Ref.	-	-
Other type of floor					
	Yes	11/14 (78.6)	2.85	0.79-10.31	0.10
	No	326/579 (56.3)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Environment (continued)					
Type of litter used during the last crop					
Straw as litter					
	Yes	109/188 (58.0)	1.08	0.76-1.53	0.66
	No	226/403 (56.1)	Ref.	-	-
Wood shavings as litter					
	Yes	238/420 (56.7)	1.00	0.70-1.43	0.99
	No	97/171 (56.7)	Ref.	-	-
Saw dust as litter					
	Yes	41/76 (53.9)	0.88	0.54-1.43	0.61
	No	294/515 (57.1)	Ref.	-	-
Wood bark as litter					
	Yes	3/3 (100.0)	-	-	0.13
	No	332/588 (56.5)	Ref.	-	-
Other type of litter					
	Yes	6/12 (50.0)	0.77	0.24-2.40	0.65
	No	325/574 (56.6)	Ref.	-	-
Roof ventilation					
	Yes	219/389 (56.3)	0.93	0.66-1.32	0.69
	No	116/200 (58.0)	Ref.	-	-
Side ventilation					
	Yes	142/220 (64.5)	1.66	1.18-2.34	0.004
	No	193/369 (52.3)	Ref.	-	-
Automatic controlled natural ventilation					
	Yes	54/106 (50.9)	0.75	0.49-1.14	0.17
	No	281/483 (58.2)	Ref.	-	-
Other types of ventilation					
	Yes	8/12 (66.7)	1.53	0.46-5.14	0.49
	No	327/577 (56.7)	Ref.	-	-
Ventilation failure					
	Yes	9/15 (60.0)	1.14	0.40-3.24	0.81
	No	326/573 (56.9)	Ref.	-	-
Cooling devices used					
	Yes	18/22 (81.8)	3.53	1.18-10.57	0.02
	No	316/564 (56.0)	Ref.	-	-
Canopy brood heating					
	Yes	99/180 (55.0)	0.90	0.63-1.28	0.54
	No	236/409 (57.7)	Ref.	-	-
Whole house heating					
	Yes	281/483 (58.2)	1.34	0.88-2.04	0.17
	No	54/106 (50.9)	Ref.	-	-
Other type of heating					
	Yes	3/4 (75.0)	2.29	0.24-22.11	0.46
	No	332/585 (56.8)	Ref.	-	-
Heating failure during the last crop					
	Yes	45/58 (77.6)	2.90	1.53-5.50	<0.001
	No	289/531 (54.4)	Ref.	-	-
Use of light bulbs					
	Yes	97/178 (54.5)	0.87	0.61-1.24	0.44
	No	238/411 (57.9)	Ref.	-	-
Use of fluorescent lights					
	Yes	183/339 (54.0)	0.76	0.54-1.05	0.10
	No	152/250 (60.8)	Ref.	-	-
Use of low energy light bulbs					
	Yes	156/246 (63.4)	1.59	1.14-2.22	0.007
	No	179/343 (52.2)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Environment (continued)					
Other types of light					
	Yes	5/6 (83.3)	3.85	0.45-33.15	0.19
	No	330/584 (56.5)	Ref.	-	-
Use of a light regime during the last crop					
	Yes	262/461 (56.8)	1.03	0.69-1.54	0.90
	No	68/121 (56.2)	Ref.	-	-
Change in light regime/intensity during the last crop					
	Yes	36/65 (55.4)	0.95	0.56-1.59	0.84
	No	293/517 (56.7)	Ref.	-	-
Light failure during the last crop					
	Yes	12/16 (75.0)	2.31	0.74-7.24	0.14
	No	321/568 (56.5)	Ref.	-	-
Husbandry/Management					
Number of people working on the farm					0.15[1]
	1	92/181 (50.8)	Ref.	-	-
	2	168/287 (58.5)	1.37	0.94-1.99	0.10
	>2	70/115 (60.9)	1.50	0.93-2.43	0.09
Number of people working on the farm *(continuous)*		-	0.99	0.92-1.07	0.83
Fractional polynomials: 2		-	-	-	0.014
3		-	-	-	0.025
Number of crops per year					0.004[1]
	2-5	74/142 (52.1)	Ref.	-	-
	6	167/312 (53.5)	1.06	0.71-1.57	0.78
	7-8	93/135 (68.9)	2.03	1.25-3.32	0.005
Number of crops per year *(continuous)*		-	1.30	1.07-1.58	0.008
Month of placement of the last crop					0.94[1]
	January – March	82/144 (56.9)	Ref.	-	-
	April – June	0/2 (0)	-	-	-
	July – September	2/3 (66.7)	1.51	0.13-17.06	0.74
	October - December	240/418 (57.4)	1.02	0.70-1.49	0.92
Number of chickens placed in last crop					0.001[1]
	8300-46500	65/146 (44.5)	Ref.	-	-
	47000-90000	80/144 (55.6)	1.56	0.98-2.48	0.06
	90200-144000	85/145 (58.6)	1.77	1.11-2.81	0.02
	144200-582260	96/143 (67.1)	2.55	1.58-4.10	<0.001
Number of chickens placed in the last crop *(continuous, per 10000)*		-	1.05	1.03-1.08	<0.001
Number of parent flocks that supplied the chicks for the last crop					0.004[1]
	1-2	37/79 (46.8)	Ref.	-	-
	3-4	67/127 (52.8)	1.27	0.72-2.23	0.41
	5-6	66/112 (58.9)	1.63	0.91-2.91	0.10
	7-24	83/118 (70.3)	2.69	1.49-4.87	0.001
Number of parent flocks that supplied the chicks for the last crop *(continuous)*		-	1.12	1.05-1.20	<0.001
Number of hatcheries that supplied the chicks for the last crop					
	1	186/350 (53.1)	Ref.	-	-
	2-9	133/207 (64.3)	1.58	1.11-2.26	0.01
Number of hatcheries that supplied the chicks for the last crop *(continuous)*		-	1.54	1.18-2.00	0.001

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Husbandry/Management (continued)					
Maximum density (kg/m^2) during last crop					0.45[1]
	16.86-35.00	52/94 (55.3)	Ref.	-	-
	35.10-37.60	49/87 (56.3)	1.04	0.58-1.87	0.89
	37.63-38.12	48/89 (53.9)	0.95	0.53-1.69	0.85
	38.20-51.30	57/88 (64.8)	1.49	0.82-2.70	0.19
Maximum density (kg/m^2) during last crop *(continuous)*		-	1.00	0.97-1.04	0.86
Age at slaughter (days) of the last crop					0.004[1]
	37-42	65/116 (56.0)	Ref.	-	-
	43-47	113/171 (66.1)	1.53	0.94-2.48	0.09
	48-52	54/119 (45.4)	0.65	0.39-1.09	0.10
	53-72	89/167 (53.3)	0.90	0.55-1.44	0.65
Age at slaughter of the last crop *(continuous)*		-	0.97	0.94-1.00	0.04
Weight at slaughter (grams) of the last crop					0.07[1]
	1680-2195	79/125 (63.2)	Ref	-	-
	2200-2520	81/132 (61.4)	0.92	0.56-1.53	0.76
	2525-3390	63/127 (49.6)	0.57	0.34-0.95	0.03
	3400-5100	67/129 (51.9)	0.63	0.38-1.04	0.07
Weight at slaughter of the last crop *(continuous, per 100 gram increase)*		-	0.97	0.94-1.00	0.03
Number of times per day chickens are examined					
	≤ 3	226/399 (56.6)	Ref.	-	-
	> 3	103/184 (56.0)	0.97	0.68-1.38	0.88
Number of times per day chickens are examines *(continuous)*		-	0.93	0.80-1.08	0.35
Changes in management or employees during the last crop					
	Yes	27/41 (65.9)	1.50	0.77-2.93	0.23
	No	309/550 (56.2)	Ref.	-	-
Breed of chickens used during the last crop	Breed A				
	Yes	283/482 (58.7)	1.63	1.06-2.48	0.02
	No	49/105 (46.7)	Ref.	-	-
	Breed B				
	Yes	15/21 (71.4)	1.96	0.75-5.13	0.16
	No	317/566 (56.0)	Ref.	-	-
	Breed C				
	Yes	138/260 (53.1)	0.78	0.56-1.08	0.13
	No	194/327 (59.3)	Ref.	-	-
	Breed D				
	Yes	3/7 (42.9)	0.57	0.13-2.60	0.46
	No	329/580 (56.7)	Ref.	-	-
Flock was thinned					
	Yes	308/530 (58.1)	2.36	1.32-4.21	0.003
	No	20/54(37.0)	Ref.	-	-
Chickens were grown separated by sex					
	Yes	219/407 (53.8)	0.68	0.47-0.98	0.04
	No	113/179 (63.1)	Ref.	-	-
Member of quality assurance scheme					
	Yes	319/560 (57.0)	1.21	0.53-2.80	0.65
	No	12/23 (52.2)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Husbandry/Management (continued)					
Number of years having been a broiler farmer					0.55[1]
	1-8 years	87/157 (55.4)	Ref.	-	-
	9-15 years	101/173 (58.4)	1.13	0.73-1.75	0.59
	16-25 years	72/117 (61.5)	1.29	0.79-2.10	0.31
	26-52 years	74/139 (53.2)	0.92	0.58-1.45	0.71
Number of years having been a broiler farmer *(continuous)*		-	1.00	0.98-1.01	0.50
Number of years having farmed for the company					0.24[1]
	<6 months-4 years	101/164 (61.6)	Ref.	-	-
	5-9 years	77/134 (57.5)	0.84	0.53-1.34	0.47
	10-15 years	84/143 (58.7)	0.89	0.56-1.40	0.61
	16-45 years	72/143 (50.3)	0.63	0.40-1.00	0.05
Number of years having farmed for the company *(continuous)*		-	0.99	0.97-1.00	0.13
Hygiene & biosecurity					
Use of all in/all out					
	Yes	296/529 (56.0)	0.74	0.42-1.28	0.27
	No	38/60 (63.3)	Ref.	-	-
Length of turn-around (days) before the last crop					0.002
	1-6	97/140 (69.3)	1.00	-	-
	7-9	93/165 (56.4)	0.57	0.36-0.92	0.02
	10-12	67/133 (50.4)	0.45	0.27-0.74	0.002
	14-62	73/148 (49.3)	0.43	0.27-0.70	0.001
Length of turn-around (days) before the last crop *(continuous)*		-	0.98	0.96-1.01	0.13
Minimum time between disinfection and restocking the last crop (days)					0.01[1]
	0.5-2	66/112 (58.9)	Ref.	-	-
	3-4	120/187 (64.2)	1.25	0.77-2.02	0.37
	4.5-6	69/124 (55.6)	0.87	0.52-1.47	0.61
	7-48	74/159 (46.5)	0.61	0.37-0.99	0.05
Minimum time between disinfection and restocking the last crop *(continuous)*		-	0.95	0.91-1.00	0.04
Were the houses cleaned before the last crop					
	Yes	334/588 (56.8)	-	-	-
	No	1/1 (100.0)	Ref.	-	-
Who cleaned the houses					0.04[1]
	Contract cleaner	227/372 (61.0)	Ref.	-	-
	Company cleaner	27/42 (64.3)	1.15	0.59-2.24	0.68
	Farm manager	69/140 (49.3)	0.62	0.42-0.92	0.02
How were the houses cleaned					0.92[1]
	With water and detergent	287/492 (58.3)	Ref.	-	-
	With water	31/53 (58.5)	1.01	0.57-1.79	0.98
	Dry brush and/or compressed air	4/6 (66.7)	1.43	0.26-7.87	0.68
Were the houses disinfected before the last crop					
	Yes	334/585 (57.1)	-	-	-
	No	0/0	Ref.	-	-
Who disinfected the houses					0.04[1]
	Contract cleaner	218/363 (60.1)	Ref.	-	-
	Company cleaner	30/48 (62.5)	1.11	0.60-2.06	0.75
	Farm manager	75/154 (48.7)	0.63	0.43-0.92	0.02

Variable	Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)				
How were the houses disinfected				0.12[1]
Spray disinfectant	135/250 (54.0)	Ref.	-	-
Fumigation	1/5 (20.0)	0.21	0.02-1.93	0.17
Both	185/313 (59.1)	1.23	0.88-1.72	0.23
Use of ammonia as a disinfectant before the last crop				
Yes	34/50 (68.0)	1.68	0.90-3.13	0.10
No	268/480 (55.8)	Ref.	-	-
Were bacterial counts taken from inside the shed				
Yes	138/237 (58.2)	1.11	0.79-1.56	0.55
No	177/318 (55.7)	Ref.	-	-
Was the inside water system cleaned				
Yes	297/525 (56.6)	1.02	0.59-1.77	0.95
No	32/57 (56.1)	Ref.	-	-
Are hand wash facilities available				
Yes	328/578 (56.7)	1.12	0.37-3.39	0.83
No	7/13 (53.8)	Ref.	-	-
Are hand wash facilities available in each house				
Yes	115/211 (54.5)	0.47	0.25-0.88	0.01
No	201/347 (57.9)	Ref.	-	-
Do you wash your hands before entering the house				0.62[1]
Never	57/104 (54.8)	Ref.	-	-
Occasionally	172/306 (56.2)	1.06	0.68-1.66	0.80
Always	87/144 (60.4)	1.26	0.76-2.10	0.38
Are hand sanitizers available				
Yes	297/517 (57.4)	1.51	0.92-2.50	0.10
No	33/70 (47.1)	Ref.	-	-
Are hand sanitizers available in each house				
Yes	257/426 (60.3)	1.58	1.02-2.44	0.04
No	50/102 (49.0)	Ref.	-	-
Do you sanitise your hands before entering the house				0.04[1]
Never	49.101 (48.5)	Ref.	-	-
Occasionally	147/264 (55.7)	1.33	0.84-2.11	0.22
Always	137/218 (62.8)	1.79	1.11-2.89	0.02
Are shower facilities available				
Yes	44/82 (53.7)	0.86	0.54-1.38	0.53
No	293/511 (57.3)	Ref.	-	-
Are shower facilities available in each house				
Yes	0/1 (0.0)	-	-	-
No	336/591 (56.9)	Ref.	-	-
Do you shower before entering the house				0.47[1]
Never	188/434 (43.3)	Ref.	-	-
Occasionally	22/35 (62.9)	1.29	0.63-2.63	0.48
Always	1/1 (100.0)	-	-	-
Is farm clothing available				
Yes	324/570 (56.8)	0.99	0.41-2.38	0.98
No	12/21 (57.1)	Ref.	-	-
Is separate clothing available for each house				
Yes	79/164 (48.2)	0.54	0.37-0.78	<0.001
No	239/377 (63.4)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Do you change clothes before entering the house					0.12[1]
	Never	182/301 (60.5)	Ref.	-	-
	Occasionally	69/136 (50.7)	0.67	0.45-1.01	0.06
	Always	71/133 (53.4)	0.75	0.50-1.13	0.17
Are there barriers before entrance to the house					
	Yes	248/431 (57.5)	1.08	0.75-1.56	0.68
	No	89/160 (55.6)	Ref.	-	-
Are there barriers before entrance to each house					
	Yes	231/398 (58.0)	0.74	0.39-1.40	0.35
	No	30/46 (65.2)	Ref.	-	-
Are there boot dip facilities available					
	Yes	336/588 (57.1)	5.33	0.59-48.01	0.09
	No	1/5 (20.0)	Ref.	-	-
Are there boot dip facilities available for each house					
	Yes	318/545 (58.3)	0.93	0.15-5.63	0.94
	No	3/5 (60.0)	Ref.	-	-
Do you dip your boots before entering the house					0.19[1]
	Always	312/557 (56.0)	Ref.	-	-
	Occasionally	22/31 (71.0)	1.92	0.87-4.24	0.11
	Never	2/5 (40.0)	0.52	0.09-3.16	0.48
Are farm boots available					
	Yes	313/549 (57.0)	1.16	0.63-2.13	0.63
	No	24/45 (53.3)	Ref.	-	-
Are farm boots available for each house					
	Yes	156/272 (57.4)	1.01	0.72-1.42	0.95
	No	157/275 (57.1)	Ref.	-	-
Do you change boots before entering the house					0.92[1]
	Never	128/225 (56.9)	Ref.	-	-
	Occasionally	43/74 (58.1)	1.05	0.62-1.79	0.85
	Always	156/280 (55.7)	0.95	0.67-1.36	0.79
Are plastic over boots available					
	Yes	276/472 (58.5)	1.46	0.97-2.18	0.07
	No	59/120 (49.2)	Ref.	-	-
Are plastic over boots available for each house					
	Yes	215/362 (59.4)	1.44	1.02-2.02	0.04
	No	104/206 (50.5)	Ref.	-	-
Do you use plastic over boots before entering the house					0.53[1]
	Never	181/326 (55.5)	Ref.	-	-
	Occasionally	75/136 (55.1)	0.98	0.66-1.47	0.94
	Always	68/111 (61.3)	1.27	0.82-1.97	0.29
Are rodents seen on the farm					0.75[1]
	Never	74/146 (50.7)	Ref.	-	-
	Every six months	132/226 (58.4)	1.37	0.90-2.08	0.14
	Every crop	53/89 (59.6)	1.43	0.84-2.44	0.19
	Every month	29/54 (53.7)	1.13	0.60-2.11	0.71
	Every week	16/27 (59.3)	1.42	0.62-3.26	0.41
	Every few days	9/16 (56.3)	1.25	0.44-3.54	0.67
	Daily	8/12 (66.7)	1.95	0.56-6.75	0.29

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Are rodents seen in- or outside the shed					0.12[1]
	Outside	173/304 (56.9)	Ref.	-	-
	Inside	50/85 (58.8)	1.08	0.66-1.76	0.75
	Both	38/53 (71.7)	1.92	1.01-3.64	0.05
Are there rodent control measures in place					
	Yes	335/590 (56.8)	-	-	-
	No	0/1 (0)	Ref.	-	-
What type of rodent control measures					0.99[1]
	Poison	257/453 (56.7)	Ref.	-	-
	Other (i.e. cats, traps, dogs)	3/5 (60.0)	1.14	0.19-6.91	0.88
	Poison and other	64/113 (56.6)	1.00	0.66-1.51	0.99
Number of rodents baits used					<0.001[1]
	1-9	60/146 (41.1)	Ref.	-	-
	10-16	68/130 (52.3)	1.57	0.98-2.53	0.06
	17-30	89/146 (61.0)	2.24	1.40-3.57	0.001
	31-150	94/128 (73.4)	3.96	2.37-6.61	<0.001
Number of rodents baits used *(continuous)*		-	1.03	1.02-1.04	<0.001
Presence of litter beetles in the house					
	Yes	130/230 (56.5)	0.99	0.71-1.39	0.97
	No	203/358 (56.7)	Ref.	-	-
Are there litter beetle control measures					
	Yes	134/240 (55.8)	0.89	0.63-1.25	0.49
	No	181/308 (58.8)	Ref.	-	-
Are there domestic animals on the farm					
	Yes	221/393 (56.2)	0.97	0.69-1.38	0.87
	No	111/195 (56.9)	Ref.	-	-
Are there cattle on the farm					
	Yes	72/145 (49.7)	0.69	0.48-1.01	0.06
	No	260/443 (58.7)	Ref.	-	-
Are there sheep on the farm					
	Yes	54/108 (50.0)	0.73	0.48-1.10	0.13
	No	278/480 (57.9)	Ref.	-	-
Are there pigs on the farm					
	Yes	14/18 (77.8)	2.77	0.90-8.53	0.06
	No	318/570 (55.8)	Ref.	-	-
Are there horses on the farm					
	Yes	42/76 (55.3)	0.94	0.58-1.53	0.81
	No	290/511 (56.8)	Ref.	-	-
Are there dogs on the farm					
	Yes	170/309 (55.0)	0.88	0.64-1.22	0.46
	No	162/279 (58.1)	Ref.	-	-
Are there cats on the farm					
	Yes	134/238 (56.3)	0.99	0.71-1.38	0.95
	No	198/350 (56.6)	Ref.	-	-
Are there other types of animals on the farm					
	Yes	6/12 (50.0)	0.77	0.24-2.41	0.65
	No	317/560 (56.6)	Ref.	-	-
Are (is) there (a) poultry farm(s) within a 2 mile radius					
	Yes	212/366 (57.9)	1.12	0.80-1.58	0.50
	No	120/218 (55.0)	Ref.	-	-
Are (is) there (a) broiler farm(s) within a 2 mile radius					
	Yes	163/287 (56.8)	0.99	0.72-1.38	0.98
	No	169/297 (56.9)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Are (is) there (a) broiler breeder farm(s) within a 2 mile radius					
	Yes	27/46 (58.7)	1.09	0.59-2.00	0.79
	No	305/538 (56.7)	Ref.	-	-
Are (is) there (a) breeder rearing farm(s) within a 2 mile radius					
	Yes	16/25 (64.0)	1.37	0.59-3.15	0.46
	No	316/559 (56.5)	Ref.	-	-
Are (is) there (a) layer farm(s) within a 2 mile radius					
	Yes	27/47 (57.4)	1.02	0.56-1.87	0.94
	No	305/536 (56.9)	Ref.	-	-
Are (is) there (a) layer breeder farm(s) within a 2 mile radius					
	Yes	8/11 (72.7)	2.04	0.54-7.77	0.29
	No	324/572 (56.6)	Ref.	-	-
Are (is) there (a) layer rearing farm(s) within a 2 mile radius					
	Yes	7/14 (50.0)	0.75	0.26-2.17	0.60
	No	325/569 (57.1)	Ref.	-	-
Are (is) there (a) turkey farm(s) within a 2 mile radius					
	Yes	28/43 (65.1)	1.45	0.76-2.78	0.26
	No	304/540 (56.3)	Ref.	-	-
Are (is) there (a) turkey breeder farm(s) within a 2 mile radius					
	Yes	5/10 (50.0)	0.75	0.22-2.63	0.65
	No	327/573 (57.1)	Ref.	-	-
Are (is) there (a) turkey breeder rearing farm(s) within a 2 mile radius					
	Yes	2/2 (100.0)	-	-	-
	No	330/581 (56.8)	Ref.	-	-
Are (is) there (a) free-range broiler farm(s) within a 2 mile radius					
	Yes	10/16 (62.5)	1.27	0.45-3.54	0.65
	No	322/567 (56.8)	Ref.	-	-
Are (is) there (a) free-range layer farm(s) within a 2mile radius					
	Yes	25/40 (62.5)	1.28	0.66-2.48	0.46
	No	307/543 (56.5)	Ref.	-	-
Are (is) there (an) other poultry farm(s) within a 2 mile radius					
	Yes	13/20 (65.0)	1.42	0.56-3.61	0.46
	No	319/563 (56.7)	Ref.	-	-
Are there domestic animals on adjoining fields					
	Yes	227/404 (56.2)	0.97	0.68-1.39	0.88
	No	104/183 (56.8)	Ref.	-	-
Are there cattle on adjoining fields					
	Yes	143/267 (53.6)	0.81	0.58-1.12	0.20
	No	187/318 (58.8)	Ref.	-	-
Are there sheep on adjoining fields					
	Yes	129/238 (54.2)	0.86	0.62-1.20	0.37
	No	201/347 (57.9)	Ref.	-	-
Are there pigs on adjoining fields					
	Yes	8/15 (53.3)	0.88	0.31-2.46	0.81
	No	322/570 (56.5)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Are there horses on adjoining fields					
	Yes	74/128 (57.8)	1.08	0.72-1.60	0.72
	No	256/457 (56.0)	Ref.	-	-
Are there dogs on adjoining fields					
	Yes	76/128 (59.4)	1.17	0.78-1.74	0.44
	No	254/457 (55.6)	Ref.	-	-
Are there cats on adjoining fields					
	Yes	64/111 (57.7)	1.06	0.70-1.62	0.77
	No	266/474 (56.1)	Ref.	-	-
Are there other domestic animals on adjoining fields					
	Yes	5/7 (71.4)	1.95	0.37-10.11	0.42
	No	325/578 (56.2)	Ref.	-	-
Are other animals, domestic or wild, seen in the poultry house					
	Yes	23/49 (46.9)	0.65	0.36-1.17	0.15
	No	312/541 (57.7)	Ref.	-	-
When are other animals seen in the house					
During occupation					
	Yes	4/11 (36.4)	0.57	0.14-2.26	0.42
	No	20/40 (50.0)	Ref.	-	-
When empty					
	Yes	22/44 (50.0)	2.50	0.44-14.29	0.29
	No	2/7 (28.6)	Ref.	-	-
Are wild birds seen in the poultry house					
	Yes	92/160 (57.5)	1.04	0.72-1.50	0.85
	No	240/424 (56.6)	Ref.	-	-
When are wild birds seen in the house					
During occupation					
	Yes	18/37 (48.6)	0.61	0.29-1.28	0.19
	No	76/125 (60.8)	Ref.	-	-
When empty					
	Yes	81/137 (59.1)	1.34	0.57-3.14	0.51
	No	13/25 (52.0)	Ref.	-	-
Disposal of litter					
Spread on field					
	Yes	96/193 (49.7)	0.67	0.47-0.94	0.02
	No	237/397 (59.7)	Ref.	-	-
Deep stacked and then spread on field					
	Yes	98/197 (49.7)	0.67	0.47-0.94	0.02
	No	235/393 (59.8)	Ref.	-	-
To a power plant					
	Yes	153/229 (66.8)	2.02	1.44-2.86	<0.001
	No	180/361 (49.9)	Ref.	-	-
Mushroom composting					
	Yes	56/101 (55.4)	0.95	0.62-1.47	0.82
	No	277/489 (56.6)	Ref.	-	-
Other types of disposal					
	Yes	19/29 (65.5)	1.49	0.68-3.25	0.32
	No	316/563 (56.1)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
If litter is spread on a field, the field is located:					
On the farm					
	Yes	74/156 (47.4)	0.73	0.48-1.12	0.15
	No	104/188 (55.3)	Ref.	-	-
On adjacent premises					
	Yes	29/60 (48.3)	0.85	0.49-1.48	0.56
	No	149/284 (52.5)	Ref.	-	-
Elsewhere					
	Yes	122/220 (55.5)	1.51	0.97-2.35	0.07
	No	56/124 (45.2)	Ref.	-	-
Was the used litter removed from the farm prior to disinfection of the house					
	Yes	311/551 (56.4)	0.90	0.47-1.74	0.76
	No	23/39 (59.0)	Ref.	-	-
Was the used litter removed from the farm prior to the arrival of the new chicks					
	Yes	317/560 (56.6)	0.87	0.38-1.97	0.74
	No	15/25 (60.0)	Ref.	-	-
Is manure spread on fields of the farm					
	Yes	95/190 (50.0)	0.69	0.48-0.98	0.04
	No	227/383 (59.3)	Ref.	-	-
Is cow manure spread on fields of the farm					
	Yes	80/161 (49.7)	0.68	0.47-0.98	0.04
	No	239/404 (59.2)	Ref.	-	-
Is pig manure spread on fields of the farm					
	Yes	12/19 (63.2)	1.33	0.52-3.44	0.55
	No	307/546 (56.2)	Ref.	-	-
Is human sludge spread on fields of the farm					
	Yes	2/4 (50.0)	0.77	0.11-5.50	0.79
	No	317/561 (56.5)	Ref.	-	-
Is other type of manure spread on fields of the farm					
	Yes	6/10 (60.0)	1.16	0.32-4.16	0.82
	No	313/555 (56.4)	Ref.	-	-
Is manure spread on fields adjoining the farm					
	Yes	184/341 (54.0)	0.82	0.58-1.17	0.28
	No	118/201 (58.7)	Ref.	-	-
Is cow manure spread on fields adjoining the farm					
	Yes	139/269 (51.7)	0.72	0.51-1.02	0.06
	No	160/268 (59.7)	Ref.	-	-
Is pig manure spread on fields adjoining the farm					
	Yes	34/51 (66.7)	1.67	0.91-3.07	0.10
	No	265/486 (54.5)	Ref.	-	-
Is poultry manure spread on fields adjoining the farm					
	Yes	47/74 (63.5)	1.46	0.88-2.42	0.14
	No	252/463 (54.4)	Ref.	-	-
Is human sludge spread on fields adjoining the farm					
	Yes	10/16 (62.5)	1.34	0.48-3.74	0.58
	No	289/521 (55.5)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Is other type of manure spread on fields adjoining the farm					
	Yes	3/6 (50.0)	0.79	0.16-3.97	0.78
	No	296/531 (55.7)	Ref.	-	-
Did people other than the farm manager, employees or area manager enter the house during the last crop					
	Yes	186/296 (62.8)	1.67	1.20-2.32	0.002
	No	149/296 (50.3)	Ref.	-	-
Did a veterinarian enter the house during the last crop					
	Yes	44/70 (62.9)	1.36	0.81-2.27	0.24
	No	287/517 (55.5)	Ref.	-	-
Did a feed representative enter the house during the last crop					
	Yes	10/18 (55.6)	0.96	0.37-2.46	0.93
	No	321/567 (56.6)	Ref.	-	-
Did an electrician enter the house during the last crop					
	Yes	142/223 (63.7)	1.61	1.15-2.27	0.006
	No	189/363 (52.1)	Ref.	-	-
Did a service engineer enter the house during the last crop					
	Yes	68/103 (66.0)	1.62	1.04-2.52	0.03
	No	262/480 (54.6)	Ref.	-	-
Did an other person enter the house during the last crop					
	Yes	44/74 (59.5)	1.14	0.70-1.88	0.60
	No	286/509 (56.2)	Ref.	-	-
Did commercial vehicles coming onto the farm during the last crop					
	Yes	336/592 (56.8)	-	-	-
	No	0/0 (-)	Ref.	-	-
Did feed lorries come unto the farm during the last crop					
	Yes	333/587 (56.7)	1.31	0.18-9.37	0.79
	No	2/4 (50.0)	Ref.	-	-
Did hatchery lorries come onto the farm during the last crop					
	Yes	322/567 (56.8)	1.03	0.46-2.31	0.94
	No	14/25 (56.0)	Ref.	-	-
Did collection lorries come onto the farm during the last crop					
	Yes	314/556 (56.5)	0.87	0.43-1.74	0.68
	No	21/35 (60.0)	Ref.	-	-
Did gas lorries come onto the farm during the last crop	Yes	287/509 (56.4)	0.85	0.52-1.39	0.52
	No	47/78 (60.3)	Ref.	-	-
Did oil lorries come onto the farm during the last crop	Yes	132/217 (60.8)	1.31	0.93-1.85	0.12
	No	191/352 (54.3)	Ref.	-	-
Did straw/shavings lorries come onto the farm during the last crop	Yes	309/547 (56.5)	0.67	0.35-1.31	0.24
	No	27/41 (65.9)	Ref.	-	-
Did other types of lorries come onto the farm during the last crop	Yes	70/123 (56.9)	1.01	0.68-1.52	0.95
	No	244/431 (56.6)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Feed					
Number of feed bins on the farm					0.02[1]
	1-3	56/116 (48.3)	Ref.	-	-
	4-6	102/193 (52.8)	1.20	0.76-1.90	0.44
	7-9	77/125 (61.6)	1.72	1.03-2.87	0.04
	10-40	99/152 (65.1)	2.00	1.22-3.28	0.006
Number of feed bins on the farm *(continuous)*		-	1.07	1.03-1.11	<0.001
Are single or double feed bins used					0.35[1]
	Single	128/219 (58.4)	Ref.		
	Double	156/293 (53.2)	0.81	0.57-1.15	0.24
	Both	46/72 (63.9)	1.26	0.73-2.18	0.41
	Treble	1/2 (50.0)	0.71	0.04-11.51	0.81
How often are feed bins cleaned inside					0.15[1]
	Never	171/290 (59.0)	Ref.	-	
	Once per year	42/92 (45.7)	0.58	0.36-0.94	0.03
	Once per 6 months	28/57 (49.1)	0.67	0.38-1.19	0.17
	Once per crop	64/114 (56.1)	0.89	0.57-1.38	0.60
	More often than once per crop	5/7 (71.4)	1.74	0.33-9.12	0.51
	Other	4/5 (80.0)	2.78	0.31-25.22	0.36
How often are feed bins disinfected inside					0.32[1]
	Never	252/434 (58.1)	Ref.		
	Once per year	34/68 (50.0)	0.72	0.43-1.21	0.21
	Once per 6 months	23/44 (52.3)	0.79	0.42-1.47	0.46
	Once per crop	12/27 (44.4)	0.58	0.26-1.26	0.17
	More often than once per crop	0/0	-	-	-
	Other	4/5 (80.0)	2.89	0.32-26.06	0.35
Type of feeders used					
	Flat chain/auger feeder				
	Yes	194/319 (60.8)	1.45	1.05-2.01	0.02
	No	141/273 (51.6)	Ref.	-	-
	Pan feeder				
	Yes	192/348 (55.2)	0.87	0.62-1.21	0.41
	No	143/244 (58.6)	Ref.	-	-
	Tube feeder				
	Yes	12/23 (52.2)	0.83	0.36-1.91	0.66
	No	323/569 (56.8)	Ref.	-	-
	Other type of feeder				
	Yes	3/4 (75.0)	2.31	0.24-22.37	0.46
	No	332/588 (56.5)	Ref.	-	-
What feeding regimen was used during the last crop					
	Ad libitum	242/421 (57.5)	Ref.	-	-
	Controlled	86/157 (54.8)	0.90	0.62-1.30	0.56
Were there any feeding equipment break-downs during the last crop					
	Yes	152/219 (69.4)	2.41	1.69-3.43	<0.001
	No	177/365 (48.5)	Ref.	-	-
Number of feed equipment break-downs during the last crop					
	≤3	68/106 (64.2)	Ref.	-	-
	>3	56/77 (72.7)	1.49	0.79-2.83	0.22
Number of feed equipment break-downs during the last crop *(continuous)*		-	0.97	0.92-1.03	0.34

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Feed (continued)					
Number of feed lorries entering the farm during the last crop					<0.001[1]
	1-10	53/132 (40.2)	Ref.	-	-
	11-17	82/137 (59.9)	2.22	1.36-3.62	0.001
	18-26	73/134 (54.5)	1.78	1.10-2.90	0.02
	27-96	95/136 (69.9)	3.45	2.08-5.72	<0.001
Number of feed lorries entering the farm during the last crop *(continuous)*		-	1.03	1.02-1.04	<0.001
Addition of whole wheat to the feed					
	Yes	274/457 (60.0)	1.88	1.13-3.13	0.01
	No	31/70 (44.3)	Ref.	-	-
Minimum (%) amount of whole wheat added					0.008[1]
	0-5	139/249 (55.8)	Ref.	-	-
	5.1-6	30/37 (81.1)	3.39	1.41-8.13	0.005
	6.1-40	57/90 (63.3)	1.37	0.83-2.25	0.22
Minimum (%) amount of whole wheat added *(continuous)*		-	1.03	0.97-1.10	0.29
Maximum (%) amount of whole wheat added					0.41[1]
	2-15	102/158 (64.6)	Ref.	-	-
	16-20	92/156 (59.0)	0.79	0.50-1.25	0.31
	20.5-43	47/83 (56.6)	0.72	0.42-1.24	0.23
Maximum (%) amount of whole wheat added *(continuous)*		-	0.96	0.93-1.00	0.04
Who supplied the wheat					
	Own farm				
	Yes	33/60 (55.0)	0.82	0.47-1.41	0.47
	No	239/399 (59.9)	Ref.	-	-
	Other farm				
	Yes	18/30 (60.0)	1.03	0.49-2.20	0.93
	No	254/429 (59.2)	Ref.	-	-
	Feed mill				
	Yes	229/381 (60.1)	1.23	0.75-2.00	0.42
	No	43/78 (55.1)	Ref.	-	-
Where was the whole wheat added:					
	Blended on the farm				
	Yes	57/92 (62.0)	1.14	0.71-1.82	0.59
	No	213/362 (58.8)	Ref.	-	-
	Blended in the mill				
	Yes	102/174 (58.6)	0.94	0.64-1.39	0.77
	No	168/280 (60.0)	Ref.	-	-
	Blended in the wagon				
	Yes	37/68 (54.4)	0.78	0.47-1.32	0.36
	No	233/386 (60.4)	Ref.	-	-
	Dumped on the wagon				
	Yes	87/136 (64.0)	1.31	0.87-1.98	0.20
	No	183/318 (57.5)	Ref.	-	-
Was the wheat treated					
	Yes	143/257 (55.6)	0.94	0.51-1.74	0.85
	No	28/49 (57.1)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Feed (continued)					
With what was the wheat treated					
	Organic acids				
	Yes	46/86 (53.5)	0.88	0.52-1.48	0.62
	No	92/162 (56.8)	Ref.	-	-
	Virkon				
	Yes	0/1 (0)	-	-	-
	No	138/247 (55.9)	Ref.	-	-
	Salcurb				
	Yes	44/75 (58.7)	1.19	0.69-2.06	0.53
	No	94/173 (54.3)	Ref.	-	-
	Other				
	Yes	12/18 (66.7)	1.65	0.60-4.55	0.33
	No	126/230 (54.8)	Ref.	-	-
Water					
Type of drinkers used					
	Bell drinkers				
	Yes	17/22 (77.3)	2.69	0.98-7.40	0.05
	No	317/568 (55.8)	Ref.	-	-
	Drink nipples				
	Yes	144/236 (61.0)	1.35	0.97-1.89	0.08
	No	190/354 (53.7)	Ref.	-	-
	Drink nipples with cups				
	Yes	231/410 (56.3)	0.96	0.68-1.37	0.84
	No	103/180 (57.2)	Ref.	-	-
	Other type of drinkers				
	Yes	8/11 (72.7)	2.07	0.54-7.88	0.28
	No	326/579 (56.3)	Ref.	-	-
What water regimen was used during the last crop					
	Ad libitum	12/18 (66.7)	Ref.	-	-
	Controlled	316/562 (56.2)	0.64	0.24-1.74	0.38
What was the source of water supply during the last crop					
	Mains				
	Yes	262/434 (60.4)	1.78	1.23-2.59	0.002
	No	70/152 (46.1)	Ref.	-	-
	Well				
	Yes	19/39 (48.7)	0.71	0.37-1.36	0.30
	No	313/547 (57.2)	Ref.	-	-
	Borehole				
	Yes	65/148 (43.9)	0.50	0.34-0.73	<0.001
	No	267/438 (61.0)	Ref.	-	-
	Stream				
	Yes	2/2 (100.0)	-	-	-
	No	330/584 (56.5)	Ref.	-	-
	Other source of water supply				
	Yes	5/7 (71.4)	1.93	0.37-10.01	0.43
	No	327/579 (56.5)	Ref.	-	-
Has the water been analysed					
	Yes	162/319 (50.8)	0.59	0.41-0.85	0.004
	No	126/198 (63.6)	Ref.	-	-

Variable	Risk of wet litter (%)	OR	95% C.I.	p-value
Water (continued)				
What kind of water analysis				
Bacteriological				
Yes	117/238 (49.2)	0.59	0.42-0.84	0.004
No	171/276 (62.0)	Ref.	-	-
Chemical				
Yes	28/60 (46.7)	0.65	0.38-1.12	0.12
No	260/454 (57.3)	Ref.	-	-
Were there any water supply failures during the last crop				
Yes	4/8 (50.0)	0.76	0.19-3.08	0.70
No	326/575 (56.7)	Ref.	-	-
Was a water sanitizer used during the last crop				
Yes	206/351 (58.7)	1.17	0.83-1.65	0.36
No	120/219 (54.8)	Ref.	-	-
Treatment/Prevention				
Which vaccinations were used during the last crop				
NCD				
Yes	76/132 (57.6)	1.07	0.68-1.67	0.78
No	107/191 (56.0)	Ref.	-	-
IBD				
Yes	309/548 (56.4)	0.78	0.28-2.16	0.63
No	10/16 (62.5)	Ref.	-	-
IB				
Yes	230/381 (60.4)	1.58	0.90-2.79	0.11
No	27/55 (49.1)	Ref.	-	-
TRT				
Yes	8/16 (50.0)	0.69	0.25-1.92	0.48
No	127/215 (59.1)	Ref.	-	-
Other types of vaccination				
Yes	1/3 (33.3)	0.30	0.02-4.91	0.39
No	5/8 (62.5)	Ref.	-	-
Were preventive antibiotics used				
Yes	181/301 (60.1)	1.28	0.90-1.81	0.16
No	125/231 (54.1)	Ref.	-	-
Number of times preventive antibiotics were given during the last crop				
once	137/229 (59.8)	Ref.	-	-
2-4 times	45/74 (60.8)	1.04	0.61-1.78	0.88
Were curative antibiotics used during the last crop				
Yes	149/217 (68.7)	2.31	1.62-3.31	<0.0001
No	161/331 (48.6)	Ref.	-	-
Number of times curative antibiotics were given during the last crop				
once	104/145 (71.7)	Ref.	-	-
2-4 times	45/72 (62.5)	0.66	0.36-1.20	0.17
Were coccidiostatic agents used during the last crop				
Yes	246/415 (59.3)	1.46	0.78-2.71	0.24
No	22/44 (50.0)	Ref.	-	-
Were competitive exclusion products (CE) used during the last crop				
Yes	31/67 (46.3)	0.63	0.38-1.06	0.08
No	254/440 (57.7)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Treatment/Prevention (continued)					
Which type of CE product was used					0.24[1]
	Aviquard	24/56 (42.9)	Ref.	-	-
	Broilact	4/5 (80.0)	5.33	0.56-50.82	0.15
	Other	1/3 (33.3)	0.67	0.06-7.79	0.75
Method of CE product administration					
	Via water				
	Yes	17/47 (36.2)	0.26	0.09-0.78	0.01
	No	15/22 (68.2)	Ref.	-	-
	Via feed				
	Yes	2/3 (66.7)	2.40	0.21-27.78	0.47
	No	30/66 (45.5)	Ref.	-	-
	Via spray				
	Yes	13/19 (68.4)	3.54	1.15-10.87	0.02
	No	19/50 (38.0)	Ref.	-	-
Flock performance					
Feed conversion of the last crop					0.46[1]
	1.60-1.80	62/123 (50.4)	Ref	-	-
	1.81-1.87	80/136 (58.8)	1.41	0.86-2.30	0.18
	1.88-1.92	61/102 (59.8)	1.46	0.86-2.49	0.16
	1.93-2.26	67/120 (55.8)	1.24	0.75-2.06	0.40
Feed conversion of the last crop *(continuous, per 0.1 point increase)*		-	1.11	0.90-1.39	0.33
Total mortality (%) during the last crop					0.63[1]
	0.20-2.63	75/135 (55.6)	Ref.	-	-
	2.65-3.44	70/132 (53.0)	0.90	0.56-1.46	0.68
	3.45-4.24	82/135 (60.7)	1.24	0.76-2.01	0.39
	4.25-11.80	74/133 (55.6)	1.00	0.62-1.62	0.99
Total mortality (%) during the last crop *(continuous)*		-	1.02	0.90-1.16	0.72
EPEF during the last crop					<0.001[1]
	180-270	70/95 (73.7)	Ref.	-	-
	271-285	55/94 (58.5)	0.50	0.27-0.93	0.03
	286-300	51/93 (54.8)	0.43	0.24-0.80	0.008
	301-379	37/91 (40.7)	0.24	0.13-0.45	<0.001
EPEF during the last crop *(continuous, per 10 points increase)*		-	0.81	0.74-0.89	<0.001

[1] Likelihood ratio test statistic

Appendix 8: Complete results of the univariate analysis of the associations between the occurrence of wet litter with a reported disease aetiology and explanatory variables.

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Disease					
Necrotic enteritis in the last crop					
	Yes	42/61 (68.9)	7.68	4.15-14.20	<0.001
	No	59/264 (22.3)	Ref.	-	-
Coccidiosis in the last crop					
	Yes	23/25 (92.0)	28.67	6.85-255.94	<0.001
	No	89/313 (28.4)	Ref.	-	-
Respiratory diseases in the last crop					
	Yes	9/16 (56.3)	3.02	0.98-9.39	0.03
	No	95/318 (29.9)	Ref.	-	-
Immunosuppressive diseases in the last crop					
	Yes	5/5 (100.0)	-	-	-
	No	99/331 (29.9)	Ref.	-	
Environment					
Number of houses on the farm					<0.001[1]
	1-2	17/103 (16.5)	Ref.	-	-
	3-4	23/97 (23.7)	1.57	0.78-3.17	0.21
	5-7	37/72 (51.4)	5.35	2.67-10.72	<0.001
	8-20	40/84 (47.6)	4.60	2.34-9.02	<0.001
Number of houses on the farm *(continuous)*		-	1.17	1.10-1.25	<0.001
Fractional polynomial: (-0.5)		-	0.29	0.19-0.47	<0.001
Age of the newest house					0.02[1]
	<6 months-5 years	41/118 (34.8)	Ref.	-	-
	6-11 years	12/65 (18.5)	0.43	0.20-0.88	0.02
	12-26 years	27/79 (34.2)	0.98	0.54-1.78	0.93
	27-60 years	35/83 (42.2)	1.37	0.77-2.44	0.29
Age of the newest house (years) *(continuous)*		-	1.01	0.99-1.03	0.24
Age of the eldest house					0.006[1]
	6 months-12 years	24/94 (25.5)	Ref.	-	-
	13-28 years	23/83 (27.7)	1.12	0.57-2.18	0.74
	29-37 years	30/82 (36.6)	1.68	0.88-3.21	0.11
	38-60 years	37/75 (49.3)	2.84	1.49-5.43	0.002
Age of the eldest house (years) *(continuous)*		-	1.03	1.01-1.04	0.003
Average age of the houses					0.30[1]
	6 months-9 years	24/86 (27.9)	Ref.	-	-
	10-18 years	24/74 (18.3)	1.24	0.63-2.44	0.53
	19-30 years	27/77 (35.1)	1.40	0.72-2.71	0.33
	31-60 years	30/71 (42.3)	1.89	0.97-3.68	0.06
Average age of the houses (years) *(continuous)*		-	1.02	1.00-1.04	0.07
Minimum distance between houses					0.26[1]
	1-16 feet	35/77 (45.5)	Ref.	-	-
	18-22 feet	22/70 (31.4)	0.55	0.28-1.08	0.08
	23-30 feet	31/93 (33.3)	0.60	0.32-1.12	0.11
	32-10560 feet	24/71 (33.8)	0.61	0.31-1.19	0.15
Minimum distance between houses *(continuous, per 10 feet)*		-	0.99	0.98-1.01	0.22

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Environment (continued)					
Maximum distance between houses					0.02[1]
	8-20 feet	34/85 (40.0)	Ref.	-	-
	21-30 feet	24/98 (24.5)	0.49	0.26-0.92	0.03
	33-40 feet	23/50 (46.0)	1.28	0.63-2.59	0.50
	41-10560 feet	31/72 (43.1)	1.13	0.60-2.14	0.70
Maximum distance between houses *(continuous, per 10 feet)*		-	1.00	1.00-1.00	0.81
Wooden house walls					
	Yes	80/215 (37.2)	1.59	0.97-2.61	0.05
	No	38/140 (27.1)	Ref.	-	-
Concrete house walls					
	Yes	44/116 (37.9)	1.36	0.83-2.24	0.19
	No	74/239 (31.0)	Ref.	-	-
Brick house walls					
	Yes	19/64 (29.7)	0.82	0.43-1.54	0.51
	No	99/291 (34.0)	Ref.	-	-
Breeze block house walls					
	Yes	58/156 (37.2)	1.37	0.85-2.20	0.16
	No	60/199 (30.2)	Ref.	-	-
Asbestos house walls					
	Yes	42/115 (36.5)	1.24	0.75-2.04	0.36
	No	76/240 (31.7)	Ref.	-	-
Plaster board house walls					
	Yes	3/20 (15.0)	0.34	0.08-1.27	0.07
	No	115/335 (34.3)	Ref.	-	-
Metal lining & wood house walls					
	Yes	31/76 (40.8)	1.52	0.87-2.66	0.12
	No	87/279 (31.2)	Ref.	-	-
Metal lining & plastic house walls					
	Yes	15/41 (36.6)	1.18	0.56-2.46	0.63
	No	103/314 (32.8)	Ref.	-	-
Other type of house walls					
	Yes	16/54 (29.6)	0.83	0.42-1.63	0.55
	No	102/302 (33.8)	Ref.	-	-
Wooden roof					
	Yes	26/95 (27.4)	0.71	0.41-1.24	0.20
	No	89/257 (34.6)	Ref.	-	-
Asbestos roof					
	Yes	60/178 (33.7)	1.10	0.68-1.77	0.67
	No	55/174 (31.6)	Ref.	-	-
Metal lining & wood roof					
	Yes	54/153 (35.3)	1.23	0.77-1.99	0.36
	No	61/199 (30.7)	Ref.	-	-
Metal lining & plastic roof					
	Yes	10/43 (23.3)	0.59	0.26-1.31	0.16
	No	105/309 (34.0)	Ref.	-	-
Other type of roof					
	Yes	27/77 (35.1)	1.14	0.64-2.02	0.63
	No	89/277 (32.1)	Ref.	-	-
Concrete floor					
	Yes	118/356 (33.1)	-	-	-
	No	0/2 (0.0)	Ref.	-	-
Earth floor					
	Yes	0/3 (0.0)	-	-	-
	No	118/355 (33.2)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Environment (continued)					
Other type of floor					
	Yes	3/5 (60.0)	3.10	0.34-37.51	0.20
	No	115/353 (32.6)	Ref.	-	-
Type of litter used during the last crop					
Straw as litter					
	Yes	49/124 (39.5)	1.56	0.96-2.54	0.05
	No	69/234 (29.5)	Ref.	-	-
Wood shavings as litter					
	Yes	76/246 (30.9)	0.75	0.45-1.23	0.22
	No	42/112 (37.5)	Ref.	-	-
Saw dust as litter					
	Yes	14/48 (29.2)	0.82	0.39-1.67	0.55
	No	104/310 (33.5)	Ref.	-	-
Wood bark as litter					
	Yes	3/3 (100.0)	-	-	-
	No	115/355 (32.4)	Ref.	-	-
Other type of litter					
	Yes	2/5 (40.0)	1.37	0.11-12.10	0.73
	No	115/351 (32.8)	Ref.	-	-
Roof ventilation					
	Yes	67/225 (29.8)	0.67	0.41-1.08	0.08
	No	51/131 (38.9)	Ref.	-	-
Side ventilation					
	Yes	62/135 (45.9)	2.50	1.54-4.07	<0.001
	No	56/221 (25.3)	Ref.	-	-
Automatic controlled natural ventilation					
	Yes	10/56 (17.9)	0.39	0.17-0.84	0.008
	No	108/300 (36.0)	Ref.	-	-
Other types of ventilation					
	Yes	7/11 (63.6)	3.69	0.91-17.48	0.03
	No	111/345 (32.2)	Ref.	-	-
Ventilation failure					
	Yes	1/7 (14.3)	0.33	0.01-2.86	0.27
	No	116/347 (33.4)	Ref.	-	-
Cooling devices used					
	Yes	3/7 (42.9)	1.51	0.22-9.09	0.59
	No	115/347 (33.1)	Ref.	-	-
Canopy brood heating					
	Yes	34/108 (31.5)	0.90	0.53-1.50	0.66
	No	84/248 (33.9)	Ref.	-	-
Whole house heating					
	Yes	100/290 (34.5)	1.40	0.74-2.67	0.26
	No	18/66 (27.3)	Ref.	-	-
Other type of heating					
	Yes	1/2 (50.0)	2.03	0.03-159.66	0.61
	No	117/354 (33.1)	Ref.	-	-
Did heating failures occur during the last crop					
	Yes	19/31 (61.3)	3.67	1.61-8.46	<0.001
	No	98/325 (30.2)	Ref.	-	-
Use of light bulbs					
	Yes	27/100 (27.0)	0.68	0.39-1.17	0.13
	No	90/255 (35.3)	Ref.	-	-
Use of fluorescent lights					
	Yes	66/213 (31.0)	0.80	0.50-1.29	0.33
	No	51/142 (35.9)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Environment (continued)					
Use of low energy light bulbs					
	Yes	58/144 (40.3)	1.74	1.08-2.80	0.02
	No	59/211 (28.0)	Ref.	-	-
Other types of light					
	Yes	3/4 (75.0)	6.23	0.49-330.14	0.07
	No	114/352 (32.4)	Ref.	-	-
Use of a light regime during the last crop					
	Yes	90/275 (32.7)	0.95	0.54-1.70	0.86
	No	26/77 (33.8)	Ref.	-	-
Change in light regime/intensity during the last crop					
	Yes	16/39 (41.0)	1.53	0.73-3.19	0.22
	No	98/313 (31.3)	Ref.	-	-
Did light failures occur during the last crop					
	Yes	4/8 (50.0)	2.06	0.38-11.26	0.25
	No	112/343 (32.7)	Ref.	-	-
Husbandry/Management					
Number of people working on the farm					
	≤2	91/286 (31.8)	Ref.	-	-
	>2	27/69 (39.1)	1.38	0.80-2.37	0.25
Number of people working on the farm *(continuous)*		-	1.01	0.93-1.10	0.81
Number of crops per year					<0.001[1]
	2-5	15/80 (18.8)	Ref.	-	-
	6	58/195 (29.7)	1.83	0.97-3.48	0.06
	7-8	45/82 (54.9)	5.27	2.59-10.72	<0.001
Number of crops per year *(continuous)*		-	2.15	1.55-2.99	<0.001
Month of placement of the last crop					
	January – March	26/84 (31.0)	Ref.	-	-
	April – June	0/2 (0)	-	-	-
	July – September	0/1 (0)	-	-	-
	October - December	88/255 (34.5)	1.18	0.69-2.00	0.55
Number of chickens placed in last crop					<0.001[1]
	8300-46500	9/87 (10.3)	Ref.	-	-
	47000-90000	30/91 (33.0)	4.26	1.88-9.65	0.001
	90200-144000	34/87 (39.1)	5.56	2.47-12.54	<0.001
	144200-582260	41/85 (48.2)	8.08	3.59-18.16	<0.001
Number of chickens placed in the last crop *(continuous, per 10000)*		-	1.09	1.06-1.12	<0.001
Number of parent flocks that supplied the chicks for the last crop					0.02[1]
	1-2	12/53 (22.6)	Ref.	-	-
	3-4	29/85 (34.1)	1.77	0.81-3.88	0.15
	5-6	22/62 (35.5)	1.88	0.82-4.30	0.14
	7-24	35/70 (50.0)	3.42	1.54-7.57	0.002
Number of parent flocks that supplied the chicks for the last crop *(continuous)*		-	1.14	1.05-1.23	0.001
Number of hatcheries that supplied the chicks for the last crop					
	1	59/213 (27.7)	Ref.	-	-
	2-9	57/126 (45.2)	2.16	1.36-3.42	0.001
Number of hatcheries that supplied the chicks for the last crop *(continuous)*		-	1.91	1.35-2.70	<0.001

Variable	Risk of wet litter (%)	OR	95% C.I.	p-value
Husbandry/Management (continued)				
Maximum density (kg/m^2) during the last crop				0.50[1]
16.86-35.00	20/61 (32.8)	Ref.	-	-
35.10-37.60	17/55 (30.9)	0.92	0.42-2.01	0.83
37.63-38.12	20/56 (35.7)	1.14	0.53-2.45	0.74
38.20-51.30	23/52 (44.2)	1.63	0.76-3.49	0.21
Maximum density (kg/m^2) during the last crop *(continuous)*	-	1.02	0.97-1.07	0.44
Age at slaughter (days) of last crop				0.001[1]
37-42	21/69 (30.4)	Ref.	-	-
43-47	48/101 (47.5)	2.07	1.07-3.94	0.03
48-52	17/80 (21.3)	0.62	0.29-1.29	0.20
53-72	27/100 (27.0)	0.85	0.43-1.66	0.63
Age at slaughter of the last crop *(continuous)*	-	0.96	0.92-1.00	0.04
Weight at slaughter (grams) of the last crop				0.003[1]
1680-2195	33/72 (45.8)	Ref.	-	-
2200-2520	31/78 (39.7)	0.78	0.41-1.49	0.45
2525-3390	16/78 (20.5)	0.30	0.15-0.63	0.001
3400-5100	23/83 (27.7)	0.45	0.23-0.88	0.02
Weight at slaughter of the last crop *(continuous, per 100 gram increase)*	-	0.95	0.91-0.98	0.006
Number of times per day chickens are examined				
≤ 3	71/233 (30.5)	Ref.	-	-
> 3	41/117 (35.0)	1.23	0.77-1.97	0.39
Number of times per day chickens are examined *(continuous)*	-	0.95	0.78-1.17	0.65
Changes in management or employees during the last crop				
Yes	12/26 (46.2)	1.82	0.75-4.38	0.14
No	106/331 (32.0)	Ref.	-	-
Breed of chickens used during the last crop				
Breed A				
Yes	112/297 (37.7)	6.54	2.52-21.49	<0.001
No	5/59 (8.5)	Ref.	-	-
Breed B				
Yes	10/15 (66.7)	4.37	1.32-16.64	0.004
No	107/341 (31.4)	Ref.	-	-
Breed C				
Yes	65/180 (36.1)	1.35	0.84-2.16	0.19
No	52/176 (29.5)	Ref.	-	-
Breed D				
Yes	1/4 (25.0)	0.68	0.01-8.56	0.74
No	116/352 (33.0)	Ref.	-	-
Flock was thinned				
Yes	105/313 (33.5)	1.79	0.78-4.24	0.14
No	9/41 (22.0)	Ref.	-	-
Chickens were grown separated by sex				
Yes	70/246 (28.5)	0.52	0.32-0.87	0.008
No	47/109 (43.1)	Ref.	-	-
Number of years having been a broiler farmer				0.69[1]
1-8 years	38/105 (36.2)	Ref.	-	-
9-15 years	30/100 (30.0)	0.76	0.42-1.36	0.35
16-25 years	25/69 (36.2)	1.00	0.53-1.88	1.00
26-52 years	24/79 (30.4)	0.77	0.41-1.43	0.41
Number of years having been a broiler farmer *(continuous)*	-	1.00	0.98-1.02	0.89

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Husbandry/Management (continued)					
Number of years having farmed for the company					0.20[1]
	<6 months-4 years	39/99 (39.4)	Ref.	-	-
	5-9 years	32/85 (37.7)	0.93	0.51-1.69	0.81
	10-15 years	24/82 (29.3)	0.64	0.34-1.19	0.16
	16-45 years	23/86 (26.7)	0.56	0.30-1.05	0.07
Number of years having farmed for the company *(continuous)*		-	0.98	0.95-1.01	0.13
Hygiene & biosecurity					
Use of all in/all out					
	Yes	103/323 (31.9)	0.64	0.29-1.41	0.22
	No	14/33 (42.4)	Ref.	-	-
Length of turn-around (days) before last harvested crop					<0.001[1]
	1-6	38/78 (48.7)	Ref.	-	-
	7-9	34/101 (33.7)	0.53	0.29-0.98	0.04
	10-12	30/94 (31.9)	0.49	0.27-0.92	0.03
	14-62	16/85 (18.8)	0.24	0.12-0.49	<0.001
Length of turn-around (days) before the last crop *(continuous)*		-	0.93	0.88-0.97	0.003
Minimum time between disinfection and restocking last crop (days)					<0.001[1]
	0.5-2	27/71 (38.0)	Ref.	-	-
	3-4	47/107 (43.9)	1.28	0.69-2.36	0.44
	4.5-6	24/75 (32.0)	0.77	0.39-1.52	0.45
	7-48	19/101 (18.8)	0.38	0.19-0.75	0.006
Minimum time between disinfection and restocking last crop (days) *(continuous)*		-	0.88	0.81-0.95	0.001
Fractional polynomial: 3		-	0.24	0.11-0.50	<0.001
3		-	5.00	1.52-16.43	0.008
Were the houses were cleaned before last crop					
	Yes	117/238 (49.2)	-	-	-
	No	1/1 (100.0)	Ref.	-	-
Who cleaned the houses					<0.001[1]
	Contract cleaner	84/220 (38.2)	Ref.	-	-
	Company cleaner	13/28 (46.4)	1.40	0.64-3.09	0.40
	Farm manager	11/76 (14.5)	0.27	0.14-0.55	<0.001
How were the houses cleaned					0.59[1]
	With water and detergent	100/291 (34.4)	Ref.	-	-
	With water	7/28 (25.0)	0.64	0.26-1.55	0.32
	Dry brush and/or compressed air	1/3 (33.3)	0.96	0.09-10.66	0.97
Were the houses were disinfected before last crop					
	Yes	118/353 (33.4)	-	-	-
	No	0/0	Ref.	-	-
Who disinfected the houses					<0.001[1]
	Contract cleaner	83/219 (37.9)	Ref.	-	-
	Company cleaner	14/32 (43.8)	1.27	0.60-2.70	0.53
	Farm manager	14/87 (16.1)	0.31	0.17-0.59	<0.001
How were the houses disinfected					0.57[1]
	Spray disinfectant	56/163 (34.4)	Ref.	-	-
	Fumigation	0/4 (0)	-	-	-
	Both	55/175 (31.4)	0.88	0.56-1.38	0.57
Use of ammonia as a disinfectant before the last crop					
	Yes	20/36 (55.6)	2.90	1.35-6.25	0.002
	No	85/282 (30.1)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Were bacterial counts taken from inside the house					
	Yes	49/138 (35.5)	1.24	0.76-2.03	0.36
	No	60/195 (30.8)	Ref.	-	-
Was the inside water system cleaned					
	Yes	106/321 (33.0)	1.08	0.47-2.58	0.84
	No	10/32 (31.3)	Ref.	-	-
Are hand wash facilities available					
	Yes	115/349 (33.0)	0.98	0.21-6.18	0.98
	No	3/9 (33.3)	Ref.	-	-
Are hand wash facilities available in each house					
	Yes	42/129 (32.6)	0.91	0.55-1.49	0.68
	No	74/213 (34.7)	Ref.	-	-
Do you wash your hands before entering the house					0.25[1]
	Never	17/62 (27.4)	Ref.	-	-
	Occasionally	59/184 (32.1)	1.25	0.66-2.36	0.49
	Always	35/88 (39.8)	1.75	0.87-3.53	0.12
Are hand sanitizers available					
	Yes	105/312 (33.7)	1.57	0.72-3.46	0.22
	No	11/45 (24.4)	Ref.	-	-
Are hand sanitizersavailable in each house					
	Yes	89/249 (35.7)	1.55	0.90-2.67	0.09
	No	27/102 (26.5)	Ref.	-	-
Do you sanitise your hands before entering the house					0.02[1]
	Never	11/56 (19.6)	Ref.	-	-
	Occasionally	54/166 (32.5)	1.97	0.95-4.11	0.07
	Always	52/129 (40.3)	2.76	1.31-5.83	0.008
Are shower facilities available					
	Yes	25/61 (41.0)	1.52	0.83-2.80	0.14
	No	93/297 (31.3)	Ref.	-	-
Are shower facilities available in each house					
	Yes	0/1 (0.0)	-	-	-
	No	118/357 (33.1)	Ref.	-	-
Do you shower before entering the house					0.18[1]
	Never	83/258 (32.2)	Ref.	-	-
	Occasionally	11/24 (45.8)	1.78	0.77-4.15	0.18
	Always	0/0 (-)	-	-	-
Is farm clothing available					
	Yes	114/344 (33.1)	1.49	0.36-8.69	0.40
	No	3/12 (25.0)	Ref.	-	-
Is separate clothing available for each house					
	Yes	18/108 (16.7)	0.28	0.15-0.51	<0.001
	No	98/233 (42.1)	Ref.	-	-
Do you change clothes before entering the house					0.003[1]
	Never	74/184 (40.2)	Ref.	-	-
	Occasionally	20/82 (24.4)	0.48	0.27-0.86	0.014
	Always	17/78 (21.8)	0.41	0.22-0.76	0.005
Are there barriers before entrance to the house					
	Yes	78/250 (31.2)	0.75	0.45-1.24	0.23
	No	40/106 (37.7)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Are there barriers before entrance to each house					
	Yes	72/239 (30.1)	0.64	0.38-1.05	0.06
	No	44/109 (40.4)	Ref.	-	-
Are boot dip facilities available					
	Yes	118/354 (33.3)	-	-	-
	No	0/4 (0)	Ref.	-	-
Are there boot dip facilities available for each house					
	Yes	112/326 (34.4)	0.52	0.04-7.32	0.51
	No	2/4 (50.0)	Ref.	-	-
Do you dip your boots before entering the house					0.15[1]
	Never	110/340 (32.4)	Ref.	-	-
	Occasionally	8/16 (50.0)	2.09	0.76-5.72	0.15
	Always	0/3 (0)	-	-	-
Are farm boots available					
	Yes	110/330 (33.3)	1.31	0.53-3.37	0.53
	No	8/29 (27.6)	Ref.	-	-
Are farm boots available for each house					
	Yes	44/152 (28.9)	0.67	0.41-1.09	0.08
	No	68/179 (38.0)	Ref.	-	-
Do you change boots before entering the house					0.11[1]
	Never	57/148 (38.5)	Ref.	-	-
	Occasionally	13/41 (31.7)	0.74	0.35-1.55	0.42
	Always	44/161 (27.3)	0.60	0.37-0.97	0.04
Are plastic over boots available					
	Yes	95/282 (33.7)	1.19	0.66-2.15	0.53
	No	23/77 (29.9)	Ref.	-	-
Are plastic over boots available for each house					
	Yes	71/219 (32.4)	1.03	0.63-1.68	0.91
	No	43/135 (31.9)	Ref.	-	-
Do you use plastic over boots before entering the house					0.51[1]
	Never	71/205 (34.6)	Ref.	-	-
	Occasionally	22/80 (27.5)	0.72	0.41-1.26	0.25
	Always	20/62 (32.3)	0.90	0.49-1.65	0.73
Are rodents seen on the farm					0.54[1]
	Never	24/92 (26.1)	Ref.	-	-
	Every six months	53/143 (37.1)	1.67	0.94-2.97	0.08
	Every crop	20/51 (39.2)	1.83	0.88-3.79	0.11
	Every month	10/34 (29.4)	1.18	0.49-2.82	0.71
	Every week	3/12 (25.0)	0.94	0.24-3.78	0.94
	Every few days	2/9 (22.2)	0.81	0.16-4.17	0.80
	Daily	2/6 (33.3)	1.42	0.24-8.23	0.70
Are rodents seen in- or outside the shed					0.11[1]
	Outside	66/188 (35.1)	Ref.	-	-
	Inside	12/47 (25.5)	0.63	0.31-1.30	0.22
	Both	13/26 (50.0)	1.85	0.81-4.22	0.14
Are there rodent control measures in place					
	Yes	118/357 (33.1)	-	-	-
	No	0/1 (0)	Ref.	-	-
What type of rodent control measures					0.96[1]
	Poison	91/275 (33.1)	Ref.	-	-
	Other (i.e. cats, traps, dogs)	1/3 (33.3)	1.01	0.09-11.30	0.99
	Poison and other	21/67 (31.3)	0.92	0.52-1.64	0.79

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Number of rodents baits used					<0.001[1]
	1-9	11/92 (12.0)	Ref.	-	-
	10-16	29/87 (33.3)	3.68	1.70-7.96	0.001
	17-30	28/81 (34.6)	3.89	1.79-8.48	0.001
	31-150	39/71 (54.9)	8.97	4.10-19.66	<0.001
Number of rodents baits used *(continuous)*		-	1.04	1.02-1.05	<0.001
Fractional polynomial: 0		-	2.32	1.66-3.25	<0.001
Presence of litter beetles in the house					
	Yes	52/149 (34.9)	1.19	0.74-1.92	0.45
	No	64/206 (31.1)	Ref.	-	-
Are there litter beetle control measures in place					
	Yes	57/156 (36.5)	1.27	0.78-2.06	0.31
	No	55/176 (31.3)	Ref.	-	-
Are there domestic animals on the farm					
	Yes	81/242 (33.5)	1.14	0.68-1.90	0.60
	No	35/114 (30.7)	Ref.	-	-
Are there cattle on the farm					
	Yes	19/86 (22.1)	0.51	0.27-0.93	0.02
	No	97/270 (35.9)	Ref.	-	-
Are there sheep on the farm					
	Yes	21/72 (29.2)	0.82	0.44-1.50	0.49
	No	95/284 (33.5)	Ref.	-	-
Are there pigs on the farm					
	Yes	4/8 (50.0)	2.10	0.38-11.50	0.29
	No	112/348 (32.2)	Ref.	-	-
Are there horses on the farm					
	Yes	10/40 (25.0)	0.66	0.29-1.48	0.27
	No	106/315 (33.7)	Ref.	-	-
Are there dogs on the farm					
	Yes	67/194 (34.5)	1.22	0.76-1.96	0.39
	No	49/162 (30.2)	Ref.	-	-
Are there cats on the farm					
	Yes	46/143 (32.2)	0.97	0.60-1.57	0.89
	No	70/213 (32.9)	Ref.	-	-
Are there other types of animals on the farm					
	Yes	3/7 (42.9)	1.56	0.22-9.39	0.56
	No	110/339 (32.4)	Ref.	-	-
Are (is) there (a) poultry farm(s) within a 2 mile radius					
	Yes	76/217 (35.0)	1.23	0.76-2.01	0.37
	No	42/138 (30.4)	Ref.	-	-
Are (is) there (a) broiler farm(s) within a 2 mile radius					
	Yes	58/172 (33.7)	1.04	0.65-1.67	0.85
	No	60/183 (32.8)	Ref.	-	-
Are (is) there (a) broiler breeder farm(s) within a 2 mile radius					
	Yes	14/33 (42.4)	1.54	0.70-3.41	0.24
	No	104/322 (32.3)	Ref.	-	-
Are (is) there (a) breeder rearing farm(s) within a 2 mile radius					
	Yes	8/17 (47.1)	1.84	0.62-5.43	0.22
	No	110/338 (32.5)	Ref.	-	-
Are (is) there (a) layer farm(s) within a 2 mile radius					
	Yes	6/27 (22.2)	0.55	0.19-1.50	0.20
	No	112/327 (34.3)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Are (is) there (a) layer breeder farm(s) within a 2 mile radius					
	Yes	1/4 (25.0)	0.66	0.01-8.38	0.72
	No	117/350 (33.4)	Ref.	-	-
Are (is) there (a) layer rearing farm(s) within a 2 mile radius					
	Yes	1/8 (12.5)	0.28	0.01-2.32	0.19
	No	117/346 (33.8)	Ref.	-	-
Are (is) there (a) turkey farm(s) within a 2 mile radius					
	Yes	10/24 (41.7)	1.47	0.58-3.69	0.37
	No	108/330 (32.7)	Ref.	-	-
Are (is) there (a) turkey breeder farm(s) within a 2 mile radius					
	Yes	1/6 (16.7)	0.40	0.01-3.59	0.38
	No	117/348 (33.6)	Ref.	-	-
Are (is) there (a) turkey breeder rearing farm(s) within a 2 mile radius					
	Yes	0/0 (0)	-	-	-
	No	118/354 (33.3)	Ref.	-	-
Are (is) there (a) free-range broiler farm(s) within a 2 mile radius					
	Yes	2/7 (28.6)	0.80	0.07-4.96	0.79
	No	116/347 (33.4)	Ref.	-	-
Are (is) there (a) free-range layer farm(s) within a 2mile radius					
	Yes	11/25 (44.0)	1.63	0.66-4.00	0.24
	No	107/329 (32.5)	Ref.	-	-
Are (is) there (an) other poultry farm(s) within a 2 mile radius					
	Yes	6/12 (50.0)	2.05	0.53-7.85	0.21
	No	112/342 (32.7)	Ref.	-	-
Are there domestic animals on adjoining fields					
	Yes	77/242 (31.8)	0.93	0.56-1.55	0.78
	No	38/114 (33.3)	Ref.	-	-
Are there cattle on adjoining fields					
	Yes	42/156 (26.9)	0.64	0.39-1.03	0.05
	No	73/199 (36.7)	Ref.	-	-
Are there sheep on adjoining fields					
	Yes	45/145 (31.0)	0.90	0.55-1.46	0.65
	No	70/210 (33.3)	Ref.	-	-
Are there pigs on adjoining fields					
	Yes	2/9 (22.2)	0.59	0.08-3.19	0.51
	No	113/346 (32.7)	Ref.	-	-
Are there horses on adjoining fields					
	Yes	31/81(38.3)	1.40	0.81-2.44	0.20
	No	84/274 (30.7)	Ref.	-	-
Are there dogs on adjoining fields					
	Yes	25/74 (33.8)	1.08	0.60-1.94	0.77
	No	90/281 (32.0)	Ref.	-	-
Are there cats on adjoining fields					
	Yes	22/66 (33.3)	1.05	0.57-1.94	0.86
	No	93/289 (32.2)	Ref.	-	-
Are there other domestic animals on adjoining fields					
	Yes	1/3 (33.3)	1.04	0.02-20.24	0.69
	No	114/352 (32.4)	Ref.	-	-

Variable	Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)				
Are other animals, domestic or wild, seen in the poultry house				
Yes	6/31 (19.4)	0.46	0.16-1.24	0.09
No	112/327 (34.3)	Ref.	-	-
When are other animals seen in the house				
During occupation				
Yes	1/8 (12.5)	0.51	0.02-6.51	0.57
No	5/23 (21.7)	Ref.	-	-
When empty				
Yes	5/25 (20.0)	1.24	0.10-70.79	0.85
No	1/6 (16.7)	Ref.	-	-
Are wild birds seen in the poultry house				
Yes	37/103 (35.9)	1.21	0.72-2.02	0.45
No	80/252 (31.7)	Ref.	-	-
When are wild birds seen in the house				
During occupation				
Yes	10/28 (35.7)	0.97	0.35-2.64	0.94
No	27/74 (36.5)	Ref.	-	-
When empty				
Yes	30/84 (35.7)	0.87	0.27-2.85	0.80
No	7/18 (38.9)	Ref.	-	-
Litter is disposed by spreading on the field				
Yes	33/123 (26.8)	0.67	0.40-1.11	0.10
No	83/234 (35.5)	Ref.	-	-
Litter is deep stacked and then spread on the field				
Yes	41/134 (30.6)	0.87	0.53-1.42	0.55
No	75/223 (33.6)	Ref.	-	-
Litter is disposed to a power plant				
Yes	56/127 (44.1)	2.23	1.37-3.64	0.001
No	60/230 (26.1)	Ref.	-	-
Litter is used for mushroom composting				
Yes	18/60 (30.0)	0.87	0.45-1.66	0.65
No	98/297 (33.0)	Ref.	-	-
Other types of litter disposal				
Yes	8/18 (44.4)	1.70	0.59-4.86	0.27
No	109/340 (32.1)	Ref.	-	-
If the litter is spread on the field, the field is located:				
On the farm				
Yes	24/101 (23.8)	0.62	0.33-1.18	0.12
No	40/120 (33.3)	Ref.	-	-
On adjacent premises				
Yes	16/45 (35.6)	1.47	0.69-3.13	0.27
No	48/176 (27.3)	Ref.	-	-
Elsewhere				
Yes	47/140 (33.6)	1.90	0.95-3.82	0.05
No	17/81 (21.0)	Ref.	-	-
Was the used litter removed from the farm prior to disinfection of the house				
Yes	108/333 (32.4)	0.85	0.34-2.18	0.71
No	9/25 (36.0)	Ref.	-	-
Was the used litter removed from the farm prior to the arrival of the new chicks				
Yes	113/342 (33.0)	1.23	0.35-5.50	0.73
No	4/14 (28.6)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Is manure spread on fields of the farm					
	Yes	20/108 (18.5)	0.36	0.20-0.65	<0.001
	No	93/241 (38.6)	Ref.	-	-
Is cow manure spread on fields of the farm					
	Yes	17/92 (18.5)	0.37	0.20-0.70	<0.001
	No	95/252 (37.7)	Ref.	-	-
Is pig manure spread on fields of the farm					
	Yes	3/10 (30.0)	0.88	0.18-3.93	0.86
	No	109/334 (32.6)	Ref.	-	-
Is human sludge spread on fields of the farm					
	Yes	2/4 (50.0)	2.09	0.15-29.13	0.45
	No	110/340 (32.4)	Ref.	-	-
Is other type of manure spread on fields of the farm					
	Yes	1/5 (20.0)	0.51	0.01-5.28	0.55
	No	111/339 (32.7)	Ref.	-	-
Is manure spread on fields adjoining the farm					
	Yes	60/207 (29.0)	0.69	0.42-1.13	0.12
	No	47/126 (37.3)	Ref.	-	-
Is cow manure spread on fields adjoining the farm					
	Yes	39/160 (24.4)	0.49	0.30-0.82	0.003
	No	66/167 (39.5)	Ref.	-	-
Is pig manure spread on fields adjoining the farm					
	Yes	13/30 (43.3)	1.70	0.74-3.91	0.17
	No	92/297 (31.0)	Ref.	-	-
Is poultry manure spread on fields adjoining the farm					
	Yes	19/45 (42.2)	1.67	0.83-3.34	0.12
	No	86/282 (30.5)	Ref.	-	-
Is human sludge spread on fields adjoining the farm					
	Yes	7/13 (53.8)	2.56	0.72-9.49	0.09
	No	98/314 (31.2)	Ref.	-	-
Is other type of manure spread on fields adjoining the farm					
	Yes	1/4 (25.0)	0.70	0.01-8.87	0.76
	No	104/323 (32.2)	Ref.	-	-
Did people other than the farm manager, employees or area manager enter the house during the last crop					
	Yes	66/168 (39.3)	1.76	1.10-2.84	0.01
	No	51/190 (26.8)	Ref.	-	-
Did a veterinarian enter the house during the last crop					
	Yes	16/40 (40.0)	1.44	0.69-2.99	0.29
	No	100/316 (31.6)	Ref.	-	-
Did a feed representative enter the house during the last crop					
	Yes	7/13 (53.8)	2.48	0.69-9.15	0.09
	No	109/341 (32.0)	Ref.	-	-
Did an electrician enter the house during the last crop					
	Yes	52/126 (41.3)	1.81	1.11-2.95	0.01
	No	64/229 (27.9)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Did a service engineer enter the house during the last crop					
	Yes	24/59 (40.7)	1.51	0.81-2.79	0.16
	No	92/294 (31.3)	Ref.	-	-
Did an other person enter the house during the last crop					
	Yes	13/41 (31.7)	0.94	0.44-2.00	0.87
	No	103/312 (33.0)	Ref.	-	-
Did commercial vehicles come on the farm during the last crop					
	Yes	118/358 (33.0)	-	-	-
	No	0/0 (-)	Ref.	-	-
Did feed lorries come unto the farm during the last crop					
	Yes	118/356 (33.1)	-	-	-
	No	0/2 (0)	Ref.	-	-
Did hatchery lorries come onto the farm during the last crop					
	Yes	114/344 (33.1)	1.24	0.35-5.53	0.72
	No	4/14 (28.6)	Ref.	-	-
Did collection lorries come onto the farm during the last crop					
	Yes	117/344 (34.0)	6.68	0.98-287.14	0.04
	No	1/14 (7.1)	Ref.	-	-
Did gas lorries come onto the farm during the last crop					
	Yes	104/311 (33.4)	1.08	0.52-2.25	0.83
	No	14/44 (31.8)	Ref.	-	-
Did oil lorries come onto the farm during the last crop					
	Yes	45/127 (35.4)	1.14	0.70-1.87	0.57
	No	71/219 (32.4)	Ref.	-	-
Did straw/shavings lorries come onto the farm during the last crop					
	Yes	106/329 (32.2)	0.51	0.21-1.26	0.11
	No	12/25 (48.0)	Ref.	-	-
Did other types of lorries come onto the farm during the last crop					
	Yes	26/73 (35.6)	1.13	0.63-2.03	0.66
	No	87/265 (32.8)	Ref.	-	-
Feed					
Number of feed bins on the farm					<0.001[1]
	1-3	11/70 (15.7)	Ref.	-	-
	4-6	19/73 (27.7)	1.89	0.82-4.32	0.133
	7-9	48/124 (43.6)	3.39	1.62-7.09	0.001
	10-40	40/88 (44.0)	4.471	2.07-9.64	<0.001
Number of feed bins on the farm *(continuous)*		-	1.12	1.07-1.17	<0.001
Are single or double feed bins used					0.80[1]
	Single	39/125 (31.2)	Ref.	-	-
	Double	61/190 (32.1)	1.04	0.64-1.69	0.87
	Both	15/39 (38.5)	1.38	0.65-2.91	0.40
	Treble	1/2 (50.0)	2.21	0.13-36.17	0.58

Variable	Risk of wet litter (%)	OR	95% C.I.	p-value
Feed (continued)				
How often are feed bins cleaned inside				0.39[1]
Never	61/174 (35.1)	Ref.	-	-
Once per year	14/62 (22.6)	0.54	0.28-1.06	0.07
Once per 6 months	8/34 (23.5)	0.57	0.24-1.34	0.20
Once per crop	23/69 (33.3)	0.93	0.51-1.67	0.80
More often than once per crop	1/2 (50.0)	1.85	0.11-30.14	0.67
Other	1/2 (50.0)	1.85	0.11-30.14	0.67
How often are feed bins disinfected inside				0.73[1]
Never	87/257 (33.9)	Ref.	-	-
Once per year	12/45 (26.7)	0.71	0.35-1.44	0.35
Once per 6 months	8/28 (28.6)	0.78	0.33-1.85	0.57
Once per crop	4/17 (23.5)	0.60	0.19-1.90	0.39
Other	1/2 (50.0)	1.95	0.12-31.62	0.64
Type of feeders				
Flat chain/auger feeder				
Yes	67/183 (36.6)	1.42	0.88-2.27	0.12
No	51/176 (29.0)	Ref.	-	-
Pan feeder				
Yes	69/217 (31.8)	0.88	0.55-1.43	0.59
No	49/142 (34.5)	Ref.	-	-
Tube feeder				
Yes	3/14 (21.4)	0.55	0.12-2.19	0.35
No	115/345 (33.3)	Ref.	-	-
Other type of feeder				
Yes	2/2 (100.0)	-	-	-
No	116/357 (32.5)	Ref.	-	-
What feeding regimen was used during the last crop				0.95[1]
Ad libitum	83/250 (33.2)	Ref.	-	
Controlled	33/99 (33.3)	1.01	0.61-1.65	0.98
Both	2/5 (40.0)	1.34	0.22-8.18	0.75
Were there any feeding equipment break-downs during the last crop				
Yes	65/124 (52.4)	3.97	2.40-6.57	<0.001
No	50/230 (21.7)	Ref.	-	-
Number of feed equipment break-downs during last crop				
≤3	33/65 (50.8)	Ref.	-	-
>3	19/38 (50.0)	0.97	0.44-2.16	0.94
Number of feed equipment break-downs during the last crop *(continuous)*	-	0.97	0.90-1.04	0.42
Number of feed lorries coming on the farm during the last crop				<0.001[1]
1-10	15/90 (16.7)	Ref.	-	-
11-17	25/75 (33.3)	2.50	1.20-5.21	0.01
18-26	32/90 (35.6)	2.76	1.37-5.57	0.005
27-96	41/79 (51.9)	5.39	2.66-10.96	<0.001
Number of feed lorries coming on the farm during the last crop *(continuous)*	-	1.04	1.03-1.06	<0.001
Was whole wheat added to the feed during the last crop				
Yes	106/280 (37.9)	2.30	1.00-5.43	0.03
No	9/43 (20.9)	Ref.	-	-
Minimum (%) amount of whole wheat added				
≤5	47/152 (30.9)	Ref.	-	-
>5	35/72 (48.6)	2.11	1.19-3.76	0.01

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Feed (continued)					
Minimum (%) amount of whole wheat added *(continuous)*		-	1.06	0.99-1.13	0.11
Maximum (%) amount of whole wheat added	≤20	63/178 (35.4)	Ref.	-	-
	>20	29/62 (46.8)	1.60	0.89-2.88	0.11
Maximum (%) amount of whole wheat added *(continuous)*		-	0.99	0.95-1.04	0.70
Who supplied the wheat	Own farm				
	Yes	16/41 (39.0)	1.09	0.52-2.28	0.80
	No	89/241 (36.9)	Ref.	-	-
	Other farm				
	Yes	10/21 (47.6)	1.59	0.59-4.24	0.31
	No	95/261 (36.4)	Ref.	-	-
	Feed mill				
	Yes	84/229 (36.7)	0.88	0.46-1.71	0.69
	No	21/53 (39.6)	Ref.	-	-
Where was the whole wheat added:	Blended on the farm				
	Yes	28/60 (46.7)	1.61	0.86-3.01	0.10
	No	77/219 (35.2)	Ref.	-	-
	Blended in the mill				
	Yes	27/94 (28.7)	0.55	0.31-0.98	0.03
	No	78/185 (42.2)	Ref.	-	-
	Blended in the wagon				
	Yes	20/51 (39.2)	1.09	0.55-2.13	0.80
	No	85/228 (37.3)	Ref.	-	-
	Dumped on the wagon				
	Yes	37/84 (44.0)	1.47	0.84-2.57	0.15
	No	68/195 (34.9)	Ref.	-	-
Was the wheat treated	Yes	58/167 (34.7)	0.60	0.26-1.39	0.19
	No	15/32 (46.9)	Ref.	-	-
With what was the wheat treated	Organic acids				
	Yes	20/57 (35.1)	1.05	0.50-2.20	0.89
	No	35/103 (34.0)	Ref.	-	-
	Virkon				
	Yes	1/1 (100.0)	-	-	-
	No	54/159 (34.0)	Ref.	-	-
	Salcurb				
	Yes	20/50 (40.0)	1.43	0.67-3.05	0.31
	No	35/110 (31.8)	Ref.	-	-
	Other				
	Yes	4/9 (44.4)	1.56	0.30-7.62	0.51
	No	51/151 (33.8)	Ref.	-	-
Water					
Type of drinkers used	Bell drinkers				
	Yes	9/14 (64.3)	3.88	1.13-15.04	0.01
	No	109/344 (31.7)	Ref.	-	-
	Drink nipples				
	Yes	59/144 (41.0)	1.82	1.13-2.94	0.008
	No	59/214 (27.6)	Ref.	-	-
	Drink nipples with cups				
	Yes	73/242 (30.2)	0.68	0.42-1.12	0.10
	No	45/116 (38.8)	Ref.	-	-

Variable	Risk of wet litter (%)	OR	95% C.I.	p-value
Water (continued)				
Other type of drinkers				
Yes	2/5 (40.0)	1.36	0.11-12.05	0.53
No	116/353 (32.9)	Ref.	-	-
What water regimen was used during the last crop				
Ad libitum	113/342 (33.04)	Ref.	-	-
Controlled	4/10 (40.0)	1.35	0.37-4.88	0.65
What was the source of water supply during the last crop				
Mains	95/260 (36.5)	1.91	1.07-3.42	0.02
Yes	22/95 (23.2)	Ref.	-	-
No				
Well				
Yes	3/22 (13.6)	0.30	0.07-1.13	0.05
No	114/333 (34.2)	Ref.	-	-
Borehole				
Yes	23/98 (23.5)	0.53	0.30-0.94	0.02
No	94/257 (36.6)	Ref.	-	-
Stream				
Yes	0/0 (-)	-	-	-
No	117/355 (33.0)	Ref.	-	-
Other source of water supply				
Yes	3/4 (75.0)	6.20	0.49-328.76	0.07
No	114/351 (32.5)	Ref.	-	-
Has the water been analysed				
Yes	66/211 (31.3)	0.98	0.57-1.68	0.94
No	33/104 (31.7)	Ref.	-	-
What kind of analysis				
Bacteriological				
Yes	45/158 (28.5)	0.77	0.46-1.28	0.28
No	53/155 (34.2)	Ref.	-	-
Chemical				
Yes	9/40 (22.5)	0.60	0.25-1.40	0.20
No	89/273 (32.6)	Ref.	-	-
Were there any water supply failures during the last crop				
Yes	3/7 (42.9)	1.52	0.22-9.13	0.59
No	115/348 (33.0)	Ref.	-	-
Was a water sanitizer used during the last crop				
Yes	80/215 (37.2)	1.51	0.91-2.49	0.09
No	37/131 (28.2)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Treatment/Prevention					
Which vaccinations were used during the last crop					
	NCD				
	Yes	26/77 (33.8)	1.03	0.53-2.00	0.92
	No	38/115 (33.0)	Ref.	-	-
	IBD				
	Yes	110/334 (32.9)	1.23	0.20-13.08	0.81
	No	2/7 (28.6)	Ref.	-	-
	IB				
	Yes	87/229 (38.0)	2.18	0.87-6.23	0.08
	No	7/32 (21.9)	Ref.	-	-
	TRT				
	Yes	3/11 (27.3)	0.64	0.13-2.87	0.52
	No	48/130 (36.9)	Ref.	-	-
	Other types of vaccination				
	Yes	0/2 (0.0)	-	-	-
	No	1/4 (25.0)	Ref.	-	-
Where preventive antibiotics used during the last crop					
	Yes	70/181 (38.7)	1.63	0.98-2.72	0.05
	No	38/136 (27.9)	Ref.	-	-
Number of times preventive antibiotics were given during the last crop					
	once	49/133 (36.8)	Ref.	-	-
	2-4 times	21/49 (42.9)	1.29	0.66-2.50	0.46
Where curative antibiotics used during the last crop					
	Yes	81/147 (55.1)	8.03	4.51-14.37	<0.0001
	No	24/181 (13.3)	Ref.	-	-
Number of times curative antibiotics were given during the last crop					
	once	53/94 (56.4)	Ref.	-	-
	2-4 times	28/53 (52.8)	0.87	0.44-1.70	0.68
Were coccidiostatic agents used during the last crop					
	Yes	100/259 (38.6)	2.77	0.98-9.630	0.04
	No	5/27 (18.5)	Ref.	-	-
Were competitive exclusion products (CE) used during the last crop					
	Yes	6/39 (15.4)	0.35	0.12-0.92	0.02
	No	92/268 (34.3)	Ref.	-	-
Which type of CE product was used					0.04[1]
	Aviquard	4/33 (12.1)	Ref.	-	-
	Broilact	2/3 (66.7)	14.50	1.06-198.80	0.05
	Other	0/2 (0)	-	-	-
Method of CE product administration					
	Via water				
	Yes	1/29 (3.4)	0.04	0.00-0.53	0.001
	No	5/11 (45.5)	Ref.	-	-
	Via feed				
	Yes	0/1 (0)	-	-	-
	No	6/39 (15.4)	Ref.	-	-
	Via spray				
	Yes	5/10 (50.0)	25.43	2.24-1416.03	<0.001
	No	1/30 (3.3)	Ref.	-	-

Variable		Risk of wet litter (%)	OR	95% C.I.	p-value
Flock performance					
Feed conversion of last crop					0.28[1]
	1.60-1.80	22/78 (28.2)	Ref.	-	-
	1.81-1.87	38/92 (41.3)	1.79	0.94-3.41	0.08
	1.88-1.92	16/54 (29.6)	1.07	0.50-2.30	0.86
	1.93-2.26	26/75 (34.7)	1.35	0.68-2.68	0.39
Feed conversion of the last crop *(continuous, per 0.1 point increase)*		-	2.38	0.15-39.07	0.54
Total mortality (%) during last crop					0.86[1]
	0.20-2.63	24/80 (30.0)	Ref.	-	-
	2.65-3.44	27/85 (31.8)	1.09	0.56-2.10	0.81
	3.45-4.24	27/77 (35.1)	1.26	0.65-2.46	0.50
	4.25-11.80	30/85 (35.3)	1.27	0.66-2.45	0.47
Total mortality (%) during the last crop *(continuous)*		-	1.08	0.91-1.27	0.38
EPEF during last crop					<0.001[1]
	180-270	35/59 (59.3)	Ref.	-	-
	271-285	24/59 (40.7)	0.47	0.23-0.98	0.04
	286-300	16/58 (27.6)	0.26	0.12-0.57	0.001
	301-379	17/70 (24.3)	0.22	0.10-0.47	<0.001
EPEF during the last crop *(continuous, per 10 points increase)*		-	0.97	0.96-0.99	<0.001

[1]. Likelihood ratio test statistic

Appendix 9: Complete results of the univariate analysis of the associations between the occurrence of clinical coccidiosis and explanatory variables.

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Disease					
Wet litter in the last crop					
	Yes	32/310 (10.3)	9.14	2.81-47.25	<0.001
	No	3/242 (1.2)	Ref.	-	-
Necrotic enteritis in the last crop					
	Yes	12/71 (16.9)	6.63	2.66-16.51	<0.001
	No	13/437 (3.0)	Ref.	-	-
Respiratory diseases in the last crop					
	Yes	2/16 (12.5)	2.35	0.00-11.82	0.26
	No	29/507 (5.7)	Ref.	-	-
Immunosuppressive diseases in the last crop					
	Yes	1/9 (11.1)	2.06	0.00-17.41	0.49
	No	30/524 (5.7)	Ref.	-	-
Environment					
Number of houses on the farm					<0.001[1]
	1-2	2/143 (1.4)	Ref	-	-
	3-4	6/147 (4.1)	3.00	0.60-15.12	0.18
	5-7	8/113 (7.1)	5.37	1.12-25.82	0.04
	8-20	19/151 (12.6)	10.15	2.32-44.41	0.002
Number of houses on the farm *(continuous)*		-	1.17	1.07-1.27	<0.001
Age of the newest house					0.04[1]
	<6 months-5 years	9/164 (5.5)	Ref.	-	-
	6-11 years	2/109 (1.8)	0.32	0.07-1.52	0.15
	12-26 years	13/137 (9.5)	1.81	0.75-4.36	0.19
	27-60 years	11/131 (8.4)	1.58	0.63-3.93	0.33
Age of the newest house *(continuous)*		-	1.01	0.99-1.04	0.41
Age of the eldest house					0.26[1]
	6 months-12 years	4/133 (3.0)	Ref.	-	-
	13-28 years	10/134 (7.5)	2.60	0.79-8.51	0.11
	29-37 years	11/140 (7.9)	2.75	0.85-8.86	0.09
	38-60 years	9/122 (7.4)	2.57	0.77-8.57	0.13
Age of the eldest house *(continuous)*		-	1.02	0.99-1.04	0.25
Average age of the houses					0.007[1]
	6 months-9 years	2/124 (1.6)	Ref.	-	-
	10-18 years	14/126 (11.1)	7.63	1.70-34.30	0.008
	19-30 years	9/127 (7.1)	4.65	0.98-21.98	0.05
	31-60 years	4/113 (3.5)	2.24	0.40-12.46	0.36
Average age of the houses *(continuous)*		-	1.01	0.98-1.04	0.64
Minimum distance between houses					0.16[1]
	1 -16 feet	12/128 (9.4)	Ref.	-	-
	18-22 feet	11/121 (9.1)	0.97	0.41-2.28	0.94
	23-30 feet	5/142 (3.5)	0.35	0.12-1.03	0.06
	32-10560 feet	6/105 (5.7)	0.59	0.21-1.62	0.30
Minimum distance between houses *(continuous, per 10 feet)*		-	0.81	0.61-1.09	0.16
Maximum distance between houses					0.09[1]
	8-20 feet	16/138 (11.6)	Ref.	-	-
	21-30 feet	6/149 (4.0)	0.32	0.12-0.84	0.02
	33-40 feet	5/76 (6.6)	0.54	0.19-1.53	0.24
	41-10560 feet	7/123 (5.7)	0.46	0.18-1.16	0.10

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Environment (continued)					
Maximum distance between houses (*continuous, per 10 feet*)		-	1.00	0.96-1.03	0.79
Wooden house walls					
	Yes	29/356 (8.1)	2.82	1.12-8.46	0.02
	No	6/197 (3.0)	Ref.	-	-
Concrete house walls					
	Yes	12/167 (7.2)	1.22	0.55-2.66	0.59
	No	23/386 (6.0)	Ref.	-	-
Brick house walls					
	Yes	5/102 (4.9)	0.72	0.24-2.04	0.51
	No	30/451 (6.7)	Ref.	-	-
Breeze block house walls					
	Yes	18/263 (6.8)	1.18	0.56-2.48	0.64
	No	17/290 (5.9)	Ref.	-	-
Asbestos house walls					
	Yes	17/196 (8.7)	1.79	0.85-3.77	0.09
	No	18/357 (5.0)	Ref.	-	-
Plaster board house walls					
	Yes	1/34 (2.9)	0.43	0.02-3.14	0.40
	No	34/519 (6.6)	Ref.	-	-
Metal lining & wood house walls					
	Yes	5/102 (4.9)	0.72	0.24-2.04	0.51
	No	30/451 (6.7)	Ref.	-	-
Metal lining & plastic house walls					
	Yes	3/55 (5.5)	0.84	0.20-3.03	0.78
	No	32/498 (6.4)	Ref.	-	-
Other type of house walls					
	Yes	6/81 (7.4)	1.22	0.44-3.26	0.66
	No	29/473 (6.1)	Ref.	-	-
Wooden roof					
	Yes	9/155 (5.8)	0.91	0.38-2.11	0.81
	No	25/393 (6.4)	Ref.	-	-
Asbestos roof					
	Yes	20/293 (6.8)	1.26	0.59-2.72	0.52
	No	14/255 (5.5)	Ref.	-	-
Metal lining & wood roof					
	Yes	14/218 (6.4)	1.06	0.49-2.28	0.86
	No	20/330 (6.1)	Ref.	-	-
Metal lining & plastic roof					
	Yes	3/67 (4.5)	0.68	0.16-2.44	0.53
	No	31/481 (6.4)	Ref.	-	-
Other type of roof					
	Yes	7/120 (5.8)	0.94	0.36-2.35	0.88
	No	27/435 (6.2)	Ref.	-	-
Concrete floor					
	Yes	35/553 (6.3)	-	-	-
	No	0/5 (0)	Ref.	-	-
Earth floor					
	Yes	0/4 (0)	-	-	-
	No	35/554 (6.3)	Ref.	-	-
Other type of floor					
	Yes	2/14 (14.3)	2.58	0.00-13.12	0.21
	No	33/544 (6.1)	Ref.	-	-
Type of litter used during the last crop					
	Straw as litter				
	Yes	8/174 (4.6)	0.63	0.26-1.51	0.27
	No	27/382 (7.1)	Ref.	-	-

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Environment (continued)					
Wood shavings as litter					
	Yes	28/399 (7.0)	1.62	0.67-4.48	0.26
	No	7/157 (4.5)	Ref.	-	-
Saw dust as litter					
	Yes	3/71 (4.2)	0.62	0.15-2.23	0.44
	No	32/485 (6.6)	Ref.	-	-
Wood bark as litter					
	Yes	1/1 (100.0)	-	-	-
	No	34/555 (6.1)	Ref.	-	-
Other type of litter					
	Yes	1/10 (10.0)	1.66	0.00-13.65	0.63
	No	34/542 (6.3)	Ref.	-	-
Roof ventilation					
	Yes	18/372 (4.8)	0.50	0.24-1.05	0.04
	No	17/183 (9.3)	Ref.	-	-
Side ventilation					
	Yes	19/204 (9.3)	2.15	1.02-4.55	0.03
	No	16/351 (4.6)	Ref.	-	-
Automatic controlled natural ventilation					
	Yes	1/96 (1.0)	0.13	0.01-0.92	0.02
	No	34/459 (7.4)	Ref.	-	-
Other types of ventilation					
	Yes	2/13 (15.4)	2.80	0.00-14.45	0.17
	No	33/542 (6.1)	Ref.	-	-
Did ventilation failures occur during the last crop					
	Yes	0/14 (0)	-	-	-
	No	35/540 (6.5)	Ref.	-	-
Were cooling devices used during the last crop					
	Yes	4/21 (19.0)	3.80	1.00-13.18	0.01
	No	31/532 (5.8)	Ref.	-	-
Canopy brood heating					
	Yes	8/170 (4.7)	0.65	0.27-1.56	0.30
	No	27/385 (7.0)	Ref.	-	-
Whole house heating					
	Yes	30/455 (6.6)	1.34	0.50-4.54	0.55
	No	5/100 (5.0)	Ref.	-	-
Other type of heating					
	Yes	1/4 (25.0)	5.04	0.09-64.70	0.12
	No	34/551 (6.2)	Ref.	-	-
Did heating failures occur during the last crop					
	Yes	5/53 (9.4)	1.64	0.53-4.75	0.32
	No	30/502 (6.0)	Ref.	-	-
Use of light bulbs					
	Yes	6/160 (3.8)	0.49	0.18-1.28	0.11
	No	29/395 (7.3)	Ref.	-	-
Use of fluorescent lights					
	Yes	25/325 (7.7)	1.83	0.82-4.21	0.11
	No	10/230 (4.3)	Ref.	-	-
Use of low energy light bulbs					
	Yes	21/235 (8.9)	2.14	1.01-4.59	0.03
	No	14/320 (4.4)	Ref.	-	-
Other types of light					
	Yes	1/6 (16.7)	3.03	0.06-28.15	0.29
	No	34/550 (6.2)	Ref.	-	-

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Environment (continued)					
Use of a light regime during the last crop					
	Yes	28/440 (6.4)	0.98	0.40-2.74	0.96
	No	7/108 (6.5)	Ref.	-	-
Change in light regime/intensity during the last crop					
	Yes	3/62 (4.8)	0.72	0.17-2.60	0.60
	No	32/488 (6.6)	Ref.	-	-
Did lighting failures occur during the last crop					
	Yes	0/13 (0)	-	-	-
	No	34/539 (6.3)	Ref.	-	-
Husbandry/Management					
Month of placement of the last crop					0.91[1]
	January – March	9/132 (6.8)	Ref.	-	-
	April – June	0/2 (0)	-	-	-
	July – September	0/3 (0)	-	-	-
	October - December	26/398 (6.5)	0.96	0.44-2.09	0.91
Breed of chicken used during the last crop					
	Breed A				
	Yes	34/458 (7.4)	-	-	-
	No	0/94 (0)	Ref.	-	-
	Breed B				
	Yes	1/18 (5.6)	0.89	0.00-6.79	0.91
	No	33/534 (6.2)	Ref.	-	-
	Breed C				
	Yes	11/247 (4.5)	0.57	0.25-1.27	0.13
	No	23/305 (7.5)	Ref.	-	-
	Breed D				
	Yes	0/7 (0)	-	-	-
	No	34/545 (6.2)	Ref.	-	-
Number of people working on the farm					0.53[1]
	≤2	26/443 (5.9)	Ref.	-	-
	>2	8/106 (7.6)	1.31	0.58-2.98	0.52
Number of people working on the farm (*continuous*)		-	1.00	0.86-1.16	0.99
Number of crops per year					0.59[1]
	2-5	6/134 (4.5)	Ref.	-	-
	6	20/291 (6.9)	1.57	0.62-4.02	0.34
	7-8	9/131 (6.9)	1.57	0.54-4.55	0.40
Number of crops per year (*continuous*)		-	1.26	0.82-1.94	0.30
Number of chickens placed in last crop					0.02[1]
	8300-46500	4/137 (2.9)	Ref.	-	-
	47000-90000	7/129 (5.4)	1.91	0.55-6.68	0.31
	90200-144000	7/138 (5.1)	1.78	0.51-6.21	0.37
	144200-582260	17/142 (12.0)	4.52	1.48-13.81	0.008
Number of chickens placed in last crop (*continuous, per 10000*)		-	1.06	1.03-1.10	<0.001
Flock was thinned					
	Yes	29/496 (5.8)	0.78	0.26-3.16	0.65
	No	4/54 (7.4)	Ref.	-	-
Chickens were grown separated by sex					
	Yes	23/383 (6.0)	1.01	0.44-2.35	0.98
	No	10/168 (6.0)	Ref.	-	-

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Husbandry/Management (continued)					
Number of parent flocks that produced the chicks for the last crop					0.64[1]
	1-2	5/75 (6.7)	Ref.	-	-
	3-4	6/121 (5.0)	0.73	0.21-2.48	0.62
	5-6	9/107 (8.4)	1.29	0.41-4.00	0.66
	7-24	10/114 (8.8)	1.35	0.44-4.11	0.60
Number of parent flocks that produced the chicks for the last crop		-	1.07	0.98-1.17	0.15
Number of hatcheries that supplied the chicks for the last crop					
	1	16/327 (4.9)	Ref.	-	-
	2-9	19/201 (9.5)	2.03	1.02-4.04	0.04
Number of hatcheries that supplied the chicks for the last crop (*continuous*)		-	1.78	1.15-2.75	0.01
Maximum density (kg/m^2) during the last crop					0.14[1]
	16.86-35.00	2/91 (2.2)	Ref.	-	-
	35.10-37.60	6/83 (7.2)	3.47	0.68-17.68	0.14
	37.63-38.12	9/90 (10.0)	4.94	1.04-23.56	0.05
	38.20-51.30	5/90 (5.6)	2.62	0.49-13.86	0.26
Maximum density (kg/m^2) during the last crop (*continuous*)		-	1.03	0.95-1.12	0.46
Fractional polynomial: 3		-	-	-	0.06
3		-	-	-	0.06
Age at slaughter (days) of the last crop					0.003[1]
	37-42	2/107 (1.9)	Ref.	-	-
	43-47	18/163 (11.0)	6.52	1.48-28.70	0.01
	48-52	9/112 (8.0)	4.59	0.97-21.75	0.06
	53-72	5/159 (3.1)	1.70	0.32-8.95	0.53
Age at slaughter (days) of the last crop (*continuous*)		-	0.99	0.93-1.06	0.81
Fractional polynomial: -2		-	-	-	0.01
-2		-	-	-	0.01
Weight at slaughter (grams) of last crop					0.07[1]
	1680-2195	12/119 (10.1)	Ref.	-	-
	2200-2520	7/123 (5.7)	0.54	0.20-1.42	0.21
	2525-3390	9/120 (7.5)	0.72	0.29-1.79	0.48
	3400-5100	3/125 (2.4)	0.22	0.06-0.80	0.02
Average weight at slaughter (grams) of last crop (*continuous, per 100 gram increase*)		-	0.94	0.88-1.00	0.05
Number of years having been a broiler farmer					0.65[1]
	1-8 years	8/136 (5.9)	Ref.	-	-
	9-15 years	12/169 (7.1)	1.22	0.49-3.08	0.67
	16-25 years	9/112 (8.0)	1.40	0.52-3.75	0.51
	26-52 years	6/136 (4.4)	0.74	0.25-2.19	0.58
Number of years having been a broiler farmer (*continuous*)		-	0.99	0.96-1.02	0.65
Number of years having farmed for the company					0.99[1]
	<6 months-4 years	10/154 (6.5)	Ref.	-	-
	5-9 years	8/121 (6.6)	1.02	0.39-2.67	0.97
	10-15 years	8/137 (5.8)	0.89	0.34-2.33	0.82
	16-45 years	8/140 (5.7)	0.87	0.33-2.28	0.78
Number of years having farmed for the company (*continuous*)		-	0.99	0.96-1.03	0.73

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Husbandry/Management (continued)					
Are you a member of quality assurance scheme					
	Yes	35/528 (6.6)	-	-	-
	No	0/21 (0)	Ref.		
Hygiene & biosecurity					
Use of all in/all out					
	Yes	30/502 (6.0)	0.60	0.22-2.07	0.30
	No	5/52 (9.6)	Ref.	-	-
Were the houses cleaned before the last crop					
	Yes	35/553 (6.3)	-	-	-
	No	0/1 (0)	Ref.	-	-
Who cleaned the houses					0.006[1]
	Contract cleaner	30/357 (8.4)	Ref.	-	-
	Company cleaner	1/37 (2.7)	0.30	0.04-2.29	0.25
	Farm manager	2/127 (1.6)	0.17	0.04-0.74	0.02
How were the houses cleaned					0.41[1]
	With water and detergent	31/463 (6.7)	Ref.	-	-
	With water	2/51 (3.9)	0.57	0.13-2.45	0.45
	Dry brush and/or compressed air	0/5 (0)	-	-	-
Were the houses disinfected before the last crop					
	Yes	34/550 (6.2)	-	-	-
	No	0/0	Ref.	-	-
Who disinfected the houses					0.01[1]
	Contract cleaner	28/348 (8.0)	Ref.	-	-
	Company cleaner	1/42 (2.4)	0.28	0.04-2.10	0.22
	Farm manager	3/141 (2.1)	0.25	0.07-0.83	0.02
How were the houses disinfected					0.04[1]
	Spray disinfectant	9/235 (3.8)	Ref.	-	-
	Fumigation	0/5 (0)	-	-	-
	Both	24/294 (8.2)	2.23	1.02-4.90	0.05
Use of ammonia as a disinfectant before the last crop					
	Yes	5/51 (9.8)	1.72	0.55-5.04	0.28
	No	27/454 (5.9)	Ref.	-	-
Were bacterial counts taken from inside the house					
	Yes	7/223 (3.1)	0.36	0.14-0.89	0.01
	No	25/300 (8.3)	Ref.	-	-
Was the inside of the water system cleaned					
	Yes	33/499 (6.6)	1.73	0.42-15.36	0.45
	No	2/51 (3.9)	Ref.	-	-
Length of turn-around (days) before the last crop					0.43[1]
	1-6	11/140 (7.9)	Ref.	-	-
	7-9	9/153 (5.9)	0.73	0.29-1.83	0.51
	10-12	10/127 (7.9)	1.00	0.41-2.45	1.00
	14-62	5/133 (3.8)	0.46	0.15-1.36	0.16
Length of turn-around (days) before the last crop (*continuous*)		-	0.95	0.88-1.02	0.17
Minimum time between disinfection and restocking last crop (days)					0.49[1]
	0.5-2	5/105 (4.8)	Ref.	-	-
	3-4	14/179 (7.8)	1.70	0.59-4.85	0.32
	4.5-6	9/115 (7.8)	1.70	0.55-5.24	0.36
	7-48	7/154 (4.6)	0.95	0.29-3.09	0.94

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Minimum time between disinfection and restocking last crop (days) (*continuous*)		-	0.94	0.85-1.05	0.30
Are hand sanitizers available in each house					
	Yes	28/398 (7.0)	1.45	0.53-4.94	0.45
	No	5/101 (5.0)	Ref.	-	-
Do you sanitise your hands before entering the house					0.41[1]
	Never	4/95 (4.2)	Ref.	-	-
	Occasionally	15/254 (5.9)	1.43	0.46-4.42	0.54
	Always	16/200 (8.0)	1.98	0.64-6.09	0.23
Are shower facilities available					
	Yes	5/77 (6.5)	1.04	0.34-2.97	0.93
	No	30/481 (6.2)	Ref.	-	-
Are shower facilities available in each house					
	Yes	0/1 (0.0)	-	-	-
	No	35/556 (6.3)	Ref.	-	-
Do you shower before entering the house					0.93[1]
	Never	26/405 (6.4)	Ref.	-	-
	Occasionally	2/33 (6.1)	0.94	0.21-4.15	0.94
	Always	0/1 (0)	-	-	-
Is farm clothing available					
	Yes	34/541 (6.3)	0.94	0.13-40.85	0.95
	No	1/15 (6.7)	Ref.	-	-
Is separate clothing available for each house					
	Yes	9/172 (5.2)	0.71	0.30-1.65	0.39
	No	26/361 (7.2)	Ref.	-	-
Do you change clothes before entering the house					0.24[1]
	Never	22/278 (7.9)	Ref.	-	-
	Occasionally	5/131 (3.8)	0.46	0.17-1.25	0.13
	Always	7/128 (5.5)	0.67	0.28-1.62	0.38
Are there barriers before entrance to the house					
	Yes	28/405 (6.9)	1.53	0.63-4.23	0.33
	No	7/151 (4.6)	Ref.	-	-
Are there barriers before the entrance to each house					
	Yes	27/390 (6.9)	1.60	0.66-4.46	0.27
	No	7/158 (4.4)	Ref.	-	-
Are there boot dip facilities available					
	Yes	35/554 (6.3)	-	-	-
	No	0/4 (20.0)	Ref.	-	-
Are there boot dip facilities available for each house					
	Yes	31/516 (6.0)	0.19	0.01-10.39	0.12
	No	1/4 (25.0)	Ref.	-	-
Do you dip your boots before entering the house					0.43[1]
	Always	32/524 (6.1)	Ref.	-	-
	Occasionally	3/30 (10.0)	1.71	0.49-5.93	0.40
	Never	0/5 (0)	-	-	-
Are farm boots available					
	Yes	32/522 (6.1)	0.74	0.21-3.97	0.63
	No	3/37 (8.1)	Ref.	-	-
Are farm boots available for each house					
	Yes	16/256 (6.3)	1.02	0.47-2.22	0.95
	No	16/261 (6.1)	Ref.	-	-

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Do you change boots before entering the house					0.94[1]
	Never	12/207 (5.8)	Ref.		-
	Occasionally	5/72 (6.9)	1.21	-0.41-3.57	0.73
	Always	16/266 (6.0)	1.04	0.48-2.25	0.92
Are plastic over boots available					
	Yes	31/446 (7.0)	2.00	0.68-7.95	0.19
	No	4/111 (3.6)	Ref.	-	-
Are plastic over boots available for each house					
	Yes	23/354 (6.5)	1.14	0.51-2.57	0.73
	No	11/191 (5.8)	Ref.	-	-
Do you use plastic over boots before entering the house					0.12[1]
	Never	16/304 (5.3)	Ref.	-	-
	Occasionally	7/128 (5.5)	1.04	0.42-2.60	0.93
	Always	12/108 (11.1)	2.25	1.03-4.92	0.04
Are rodents seen on the farm					0.95[1]
	Never	8/134 (6.0)	Ref.	-	-
	Every six months	16/220 (7.3)	1.24	0.51-2.97	0.64
	Every crop	6/84 (7.1)	1.21	0.41-3.62	0.73
	Every month	2/49 (4.1)	0.67	0.14-3.27	0.62
	Every week	1/24 (4.2)	0.68	0.08-5.74	0.73
	Every few days	1/15 (6.7)	1.13	0.13-9.67	0.92
	Daily	0/10 (0)	-	-	-
Are rodents seen in- or outside the shed					0.24[1]
	Outside	20/292 (6.8)	Ref.	-	-
	Inside	2/79 (2.5)	0.35	0.08-1.54	0.17
	Both	4/48 (8.3)	1.24	0.40-3.79	0.71
Are there rodent control measures in place					
	Yes	35/556 (6.3)	-	-	-
	No	0/1 (0)	Ref.	-	-
What type of rodent control measures					0.38[1]
	Poison	29/425 (6.8)	Ref.		-
	Other (i.e. cats, traps, dogs)	0/4 (0)	-	-	-
	Poison and other	5/109 (4.6)	0.66	0.25-1.74	0.40
Number of rodents baits used					0.22[1]
	1-9	5/137 (3.7)	Ref.	-	-
	10-16	5/123 (4.1)	1.12	0.32-3.96	0.86
	17-30	10/137 (7.3)	2.08	0.69-6.25	0.19
	31-150	11/124 (8.9)	2.57	0.87-7.62	0.09
Number of rodents baits used (*continuous*)		-	1.01	0.99-1.03	0.19
Presence of litter beetles in the house					
	Yes	8/221 (3.6)	0.46	0.19-1.11	0.06
	No	25/333 (7.5)	Ref.	-	-
Are there litter beetle control measures in place					
	Yes	15/224 (6.7)	1.10	0.51-2.36	0.80
	No	18/293 (6.1)	Ref.	-	-
Are there domestic animals on the farm					
	Yes	23/371 (6.2)	0.94	0.43-2.06	0.86
	No	12/182 (6.6)	Ref.	-	-
Are there cattle on the farm					
	Yes	3/139 (2.2)	0.26	0.06-0.93	0.02
	No	32/414 (7.7)	Ref.	-	-

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Are there sheep on the farm					
	Yes	4/100 (4.0)	0.57	0.16-1.75	0.29
	No	31/453 (6.8)	Ref.	-	-
Are there pigs on the farm					
	Yes	2/18 (11.1)	1.90	0.00-9.30	0.40
	No	33/535 (6.2)	Ref.	-	-
Are there horses on the farm					
	Yes	3/72 (4.2)	0.61	0.14-2.18	0.42
	No	32/480 (6.7)	Ref.	-	-
Are there dogs on the farm					
	Yes	21/290 (7.2)	1.39	0.65-2.97	0.35
	No	14/263 (5.3)	Ref.	-	-
Are there cats on the farm					
	Yes	15/224 (6.7)	1.11	0.52-2.34	0.77
	No	20/329 (6.1)	Ref.	-	-
Are there other types of animals on the farm					
	Yes	1/12 (8.3)	1.35	0.00-10.81	0.77
	No	33/524 (6.3)	Ref.	-	-
Are (is) there (a) poultry farm(s) within a two mile radius					
	Yes	17/351 (4.8)	0.52	0.25-1.09	0.06
	No	18/201 (9.0)	Ref.	-	-
Are (is) there (a) broiler farm(s) within a two mile radius					
	Yes	14/276 (5.1)	0.65	0.30-1.38	0.22
	No	21/276 (7.6)	Ref.	-	-
Are (is) there (a) broiler breeder farm(s) within a two mile radius					
	Yes	2/48 (4.2)	0.62	0.10-2.81	0.52
	No	33/504 (6.5)	Ref.	-	-
Are (is) there (a) breeder rearing farm(s) within a two mile radius					
	Yes	1/25 (4.0)	0.60	0.03-4.46	0.62
	No	34/527 (6.5)	Ref.	-	-
Are (is) there (a) layer farm(s) within a two mile radius					
	Yes	2/44 (4.5)	0.68	0.11-3.11	0.61
	No	33/507 (6.5)	Ref.	-	-
Are (is) there (a) layer breeder farm(s) within a two mile radius					
	Yes	0/11 (0)	-	-	-
	No	35/540 (6.5)	Ref.	-	-
Are (is) there (a) layer rearing farm(s) within a two mile radius					
	Yes	1/13 (7.7)	1.24	0.00-9.74	0.84
	No	34/538 (6.3)	Ref.	-	-
Are (is) there (a) turkey farm(s) within a two mile radius					
	Yes	1/39 (2.6)	0.37	0.02-2.67	0.31
	No	34/512 (6.6)	Ref.	-	-
Are (is) there (a) turkey breeder farm(s) within a two mile radius					
	Yes	0/10 (0)	-	-	-
	No	35/541 (6.5)	Ref.	-	-

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Are (is) there (a) turkey breeder rearing farm(s) within a two mile radius					
	Yes	0/2 (0)	-	-	-
	No	35/549 (6.4)	Ref.	-	-
Are (is) there (a) free-range broiler farm(s) within a two mile radius					
	Yes	0/15 (0)	-	-	-
	No	35/536 (6.5)	Ref.	-	-
Are (is) there (a) free-range layer farm(s) within a two mile radius					
	Yes	4/35 (11.4)	2.02	0.56-6.58	0.20
	No	31/516 (6.0)	Ref.	-	-
Are (is) there (an) other poultry farm(s) within a two mile radius					
	Yes	1/19 (5.3)	0.81	0.00-6.15	0.84
	No	34/532 (6.4)	Ref.	-	-
Are there domestic animals on adjoining fields					
	Yes	22/379 (5.8)	0.83	0.38-1.84	0.61
	No	12/173 (6.9)	Ref.	-	-
Are there cattle on adjoining fields					
	Yes	12/253 (4.7)	0.62	0.28-1.36	0.20
	No	22/297 (7.4)	Ref.	-	-
Are there sheep on adjoining fields					
	Yes	11/223 (4.9)	0.69	0.30-1.52	0.32
	No	23/327 (7.0)	Ref.	-	-
Are there pigs on adjoining fields					
	Yes	0/11 (0)	-	-	-
	No	34/539 (6.3)	Ref.	-	-
Are there horses on adjoining fields					
	Yes	6/114 (5.3)	0.81	0.29-2.14	0.65
	No	28/436 (6.4)	Ref.	-	-
Are there dogs on adjoining fields					
	Yes	5/117 (4.3)	0.62	0.20-1.75	0.33
	No	29/433 (6.7)	Ref.	-	-
Are there cats on adjoining fields					
	Yes	5/102 (4.9)	0.74	0.24-2.11	0.55
	No	29/448 (6.5)	Ref.	-	-
Are there other domestic animals on adjoining fields					
	Yes	0/5 (0)	-	-	-
	No	34/545 (6.2)	Ref.	-	-
Are other animals, domestic or wild, seen in the poultry house					
	Yes	1/49 (2.0)	0.29	0.01-2.07	0.20
	No	34/506 (6.7)	Ref.	-	-
When are other animals seen in the house					
	During occupation				
	Yes	0/11 (0)	-	-	-
	No	1/38 (2.6)	Ref.	-	-
	When empty				
	Yes	1/42 (2.4)	-	-	-
	No	0/7 (0)	Ref.	-	-
Are wild birds seen in the poultry house					
	Yes	7/150 (4.7)	0.68	0.26-1.70	0.37
	No	27/402 (6.7)	Ref.	-	-

Variable	Risk of coccidiosis (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)				
When are wild birds seen in the house				
During occupation				
Yes	2/31 (6.5)	1.54	0.14-10.01	0.61
No	5/117 (4.3)	Ref.	-	-
When empty				
Yes	6/126 (4.8)	1.05	0.12-50.57	0.96
No	1/22 (4.5)	Ref.	-	-
Litter is disposed by spreading on the field				
Yes	10/186 (5.4)	0.78	0.34-1.76	0.52
No	25/369 (6.8)	Ref.	-	-
Litter is deep stacked and then spread on the field				
Yes	8/189 (4.2)	0.55	0.23-1.32	0.15
No	27/366 (7.4)	Ref.	-	-
Litter is disposed to a power plant				
Yes	19/212 (9.0)	2.01	0.96-4.25	0.04
No	16/343 (4.7)	Ref.	-	-
Litter is used for mushroom composting				
Yes	7/93 (7.5)	1.26	0.48-3.18	0.60
No	28/462 (6.1)	Ref.	-	-
Other types of litter disposal				
Yes	4/31 (12.9)	2.37	0.65-7.80	0.12
No	31/526 (5.9)	Ref.	-	-
If litter is spread on a field, the field is located:				
On the farm				
Yes	5/148 (3.4)	0.55	0.16-1.78	0.27
No	11/184 (6.0)	Ref.	-	-
On adjacent premises				
Yes	3/57 (5.3)	1.12	0.24-4.46	0.86
No	13/275 (4.7)	Ref.	-	-
Elsewhere				
Yes	12/214 (5.6)	1.69	0.50-7.36	0.37
No	4/118 (3.4)	Ref.	-	-
Was the used litter removed from the farm prior to disinfection of the house				
Yes	30/518 (5.8)	0.49	0.16-2.04	0.20
No	4/36 (11.1)	Ref.	-	-
Was the used litter removed from the farm prior to arrival of the new chicks				
Yes	32/527 (6.1)	0.43	0.12-2.39	0.18
No	3/23 (13.0)	Ref.	-	-
Is manure spread on fields of the farm				
Yes	7/180 (3.9)	0.50	0.19-1.25	0.10
No	27/361 (7.5)	Ref.	-	-
Is cow manure spread on fields of the farm				
Yes	4/154 (2.6)	0.31	0.09-0.96	0.02
No	30/381 (7.9)	Ref.	-	-
Is pig manure spread on fields of the farm				
Yes	3/18 (16.7)	3.14	0.67-12.57	0.09
No	31/517 (6.0)	Ref.	-	-
Is human sludge spread on fields of the farm				
Yes	0/4 (0)	-	-	-
No	34/531 (6.4)	Ref.	-	-

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Is other type of manure spread on fields of the farm					
	Yes	1/9 (11.1)	1.87	0.00-15.70	0.56
	No	33/526 (6.3)	Ref.	-	-
Is manure spread on fields adjoining the farm					
	Yes	16/324 (4.9)	0.56	0.26-1.22	0.11
	No	16/189 (8.5)	Ref.	-	-
Is cow manure spread on fields adjoining the farm					
	Yes	8/259 (3.1)	0.31	0.13-0.76	0.004
	No	23/249 (9.2)	Ref.	-	-
Is pig manure spread on fields adjoining the farm					
	Yes	4/48 (8.3)	1.46	0.41-4.70	0.50
	No	27/460 (5.9)	Ref.	-	-
Is poultry manure spread on fields adjoining the farm					
	Yes	5/71 (7.0)	1.20	0.38-3.47	0.72
	No	26/437 (5.9)	Ref.	-	-
Is human sludge spread on fields adjoining the farm					
	Yes	1/14 (7.1)	1.19	0.00-9.36	0.87
	No	30/494 (6.1)	Ref.	-	-
Is another type of manure spread on fields adjoining the farm					
	Yes	0/6 (0)	-	-	-
	No	31/502 (6.2)	Ref.	-	-
Number of times per day chickens are examined					
	≤ 3	22/384 (5.7)	Ref.	-	-
	> 3	13/167 (7.8)	1.39	0.68-2.83	0.37
Number of times per day chickens are examined (*continuous*)		-	1.05	0.77-1.43	0.74
Were there changes in management or employees during the last crop					
	Yes	5/34 (14.7)	2.83	0.88-8.48	0.04
	No	30/522 (5.7)	Ref.	-	-
Did people other than the farm manager, employees or area manager enter the house during the last crop					
	Yes	25/275 (9.1)	2.72	1.21-6.24	0.007
	No	10/282 (3.5)	Ref.	-	-
Did a veterinarian enter the house during the last crop					
	Yes	6/66 (9.1)	1.58	0.56-4.23	0.33
	No	29/486 (6.0)	Ref.	-	-
Did a feed representative enter the house during the last crop					
	Yes	4/15 (26.7)	5.91	1.47-21.98	0.001
	No	31/535 (5.8)	Ref.	-	-
Did a electrician enter the house during the last crop					
	Yes	22/207 (10.6)	3.03	1.41-6.57	0.001
	No	13/344 (3.8)	Ref.	-	-

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Hygiene & biosecurity (continued)					
Did a service engineer enter the house during the last crop					
	Yes	8/95 (8.4)	1.45	0.58-3.51	0.37
	No	27/453 (6.0)	Ref.	-	-
Did an other person enter the house during the last crop					
	Yes	4/69 (5.8)	0.89	0.26-2.79	0.83
	No	31/480 (6.5)	Ref.	-	-
Did commercial vehicles come on the farm during the last crop					
	Yes	35/557 (6.3)	-	-	-
	No	0/0 (-)	Ref.	-	-
Did feed lorries come on the farm during the last crop					
	Yes	35/552 (6.3)	-	-	-
	No	0/4 (0)	Ref.	-	-
Did hatchery lorries come on the farm during the last crop					
	Yes	31/537 (5.8)	0.25	0.07-1.07	0.01
	No	4/20 (20.0)	Ref.	-	-
Did collection lorries come on the farm during the last crop					
	Yes	33/526 (6.3)	0.94	0.22-8.46	0.93
	No	2/30 (6.7)	Ref.	-	-
Did gas lorries come on the farm during the last crop					
	Yes	29/480 (6.0)	0.72	0.28-2.20	0.48
	No	6/73 (8.2)	Ref.	-	-
Did oil lorries come on the farm during the last crop					
	Yes	19/211 (9.0)	2.03	0.95-4.36	0.04
	No	15/323 (4.6)	Ref.	-	-
Did straw/shavings lorries come on the farm during the last crop					
	Yes	32/516 (6.2)	0.75	0.22-4.02	0.65
	No	3/37 (8.1)	Ref.	-	-
Did another type of lorry come on the farm during the last crop					
	Yes	8/120 (6.7)	0.98	0.40-2.37	0.97
	No	27/399 (6.8)	Ref.	-	-
Feed					
Number of feed bins on the farm					0.02[1]
	1-3	3/105 (2.9)	Ref.	-	-
	4-6	2/107 (1.9)	0.65	0.11-3.96	0.64
	7-9	17/193 (8.8)	3.28	0.94-11.48	0.06
	10-40	12/148 (8.1)	3.00	0.83-10.91	0.10
Number of feed bins on the farm *(continuous)*			1.05	0.99-1.11	0.13
Fractional polynomial: -1			-	-	0.04
Are single or double feed bins used					0.24[1]
	Single	9/201 (4.5)	Ref.	-	-
	Double	18/282 (6.4)	1.45	0.64-3.31	0.37
	Both	7/67 (10.4)	2.49	0.89-6.97	0.08
	Treble	0/2 (0)	-	-	-

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Feed (continued)					
How often are feed bins cleaned inside					0.78[1]
	Never	17/271 (6.3)	Ref.	-	-
	Once per year	4/86 (4.7)	0.73	0.24-2.23	0.58
	Once per 6 months	5/55 (9.1)	1.49	0.53-4.24	0.45
	Once per crop	7/109 (6.4)	1.03	0.41-2.55	0.96
	More often than once per crop	0/7 (0)	-	-	-
	Other	0/4 (0)	-	-	-
How often are feed bins disinfected inside					0.85[1]
	Never	24/402 (6.0)	Ref.	-	-
	Once per year	5/65 (7.7)	1.31	0.48-3.57	0.60
	Once per 6 months	3/45 (6.7)	1.13	0.32-3.89	0.85
	Once per crop	2/27 (7.4)	1.26	0.28-5.64	0.76
	More often than once per crop	0/0 (-)	-	-	-
	Other	1/5 (20.0)	3.94	0.42-36.61	0.23
Type of feeders used					
	Flat chain/auger feeder				
	Yes	19/304 (6.3)	0.99	0.47-2.08	0.97
	No	16/253 (6.3)	Ref.	-	-
	Pan feeder				
	Yes	21/331 (6.3)	1.03	0.48-2.20	0.94
	No	14/226 (6.2)	Ref.	-	-
	Tube feeder				
	Yes	0/22 (0)	-	-	-
	No	35/535 (6.5)	Ref.	-	-
	Other type of feeder				
	Yes	2/4 (50.0)	15.54	1.10-153.20	<0.001
	No	33/553 (6.0)	Ref.	-	-
What feeding regimen was used during the last crop					
	Ad libitum	28/396 (7.1)	Ref.	-	-
	Controlled	6/146 (4.1)	0.56	0.23-1.39	0.21
Were there any feeding equipment break-downs during the last crop					
	Yes	20/207 (9.7)	2.54	1.18-5.48	0.008
	No	14/346 (4.0)	Ref.	-	-
Number of feed equipment break-downs during the last crop					
	≤3	11/101 (10.9)	Ref.	-	-
	>3	4/71 (5.6)	0.49	0.15-1.60	0.24
Number of feed equipment break-downs during the last crop *(continuous)*		-	1.03	0.95-1.11	0.49
Number of feed lorries entering farm during the last crop					0.04[1]
	1-10	4/125 (3.2)	Ref.	-	-
	11-17	9/130 (6.9)	2.25	0.67-7.50	0.19
	18-26	5/124 (4.0)	1.27	0.33-4.85	0.73
	27-96	15/133 (11.3)	3.85	1.24-11.92	0.02
Number of feed lorries entering the farm during the last crop *(continuous)*		-	1.03	1.01-1.06	0.001
Addition of whole wheat to the feed					
	Yes	33/435 (7.6)	5.41	0.87-223.69	0.06
	No	1/67 (1.5)	Ref.	-	-
Minimum (%) amount of whole wheat added					
	≤5	17/239 (7.1)	Ref.	-	-
	>5	10/120 (8.3)	1.19	0.53-2.68	0.68
Minimum (%) amount of whole wheat added *(continuous)*		-	0.98	0.87-1.10	0.72

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Feed (continued)					
Maximum (%) amount of whole wheat added					
	≤20	29/299 (9.7)	Ref.	-	-
	>20	1/81 (1.2)	0.12	0.02-0.87	0.04
Maximum (%) amount of whole wheat added *(continuous)*		-	0.89	0.84-0.95	<0.001
Who supplied the wheat					
	Own farm				
	Yes	3/59 (5.1)	0.62	0.14-2.24	0.44
	No	30/376 (8.0)	Ref.	-	-
	Other farm				
	Yes	1/31 (3.2)	0.39	0.02-2.84	0.34
	No	32/404 (7.9)	Ref.	-	-
	Feed mill				
	Yes	29/357 (8.1)	1.63	0.55-6.59	0.37
	No	4/78 (5.1)	Ref.	-	-
Where was the whole wheat added:					
	Blended on the farm				
	Yes	10/92 (10.9)	1.76	0.74-4.13	0.15
	No	22/340 (6.5)	Ref.	-	-
	Blended in the mill				
	Yes	13/162 (8.0)	1.15	0.52-2.55	0.70
	No	19/270 (7.0)	Ref.	-	-
	Blended in the wagon				
	Yes	4/66 (6.1)	0.78	0.22-2.47	0.65
	No	28/366 (7.7)	Ref.	-	-
	Dumped on the wagon				
	Yes	8/129 (6.2)	0.77	0.31-1.87	0.53
	No	24/303 (7.9)	Ref.	-	-
Was the wheat treated					
	Yes	22/250 (8.8)	1.51	0.43-8.20	0.51
	No	3/50 (6.0)	Ref.	-	-
With what was the wheat treated					
	Organic acids				
	Yes	10/79 (12.7)	1.99	0.73-5.38	0.13
	No	11/162 (6.8)	Ref.	-	-
	Virkon				
	Yes	0/1 (0)	-	-	-
	No	21/240 (8.8)	Ref.	-	-
	Salcurb				
	Yes	7/76 (9.2)	1.09	0.38-3.09	0.85
	No	14/165 (8.5)	Ref.	-	-
	Other				
	Yes	0/17 (0)	-	-	-
	No	21/224 (9.4)	Ref.	-	-
Water					
Type of drinkers used					
	Bell drinkers				
	Yes	5/22 (22.7)	4.93	1.46-15.70	0.001
	No	30/533 (5.6)	Ref.	-	-
	Drink nipples				
	Yes	15/222 (6.8)	1.13	0.53-2.40	0.72
	No	20/333 (6.0)	Ref.	-	-
	Drink nipples with cups				
	Yes	25/383 (6.5)	1.13	0.50-2.61	0.75
	No	10/172 (5.8)	Ref.	-	-

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Water (continued)					
Other type of drinkers					
	Yes	0/10 (0)	-	-	-
	No	35/545 (6.4)	Ref.	-	-
What water regimen was used during the last crop					
	Ad libitum	35/530 (6.6)	Ref.	-	-
	Controlled	0/15 (0)	-	-	-
What was the source of water supply during the last crop					
Mains					
	Yes	28/408 (6.9)	1.68	0.66-5.07	0.25
	No	6/143 (4.2)	Ref.	-	-
Well					
	Yes	1/35 (2.9)	0.43	0.02-3.12	0.40
	No	33/516 (6.4)	Ref.	-	-
Borehole					
	Yes	5/141 (3.5)	0.48	0.16-1.36	0.13
	No	29/410 (7.1)	Ref.	-	-
Stream					
	Yes	0/1 (0)	-	-	-
	No	34/550 (6.2)	Ref.	-	-
Other					
	Yes	1/7 (14.3)	2.58	0.00-23.07	0.37
	No	33/544 (6.1)	Ref.	-	-
Has the water been analysed					
	Yes	13/301 (4.3)	0.43	0.19-0.95	0.02
	No	18/188 (9.6)	Ref.	-	-
What kind of water analysis					
Bacteriological					
	Yes	8/227 (3.5)	0.37	0.15-0.91	0.02
	No	23/258 (8.9)	Ref	-	-
Chemical					
	Yes	1/57 (1.8)	0.24	0.01-1.70	0.13
	No	30/428 (7.0)	Ref.	-	-
Were there any water supply failures during the last crop					
	Yes	1/7 (14.3)	2.58	0.00-23.07	0.37
	No	33/544 (6.1)	Ref.	-	-
Was a water sanitizer used during the last crop					
	Yes	26/336 (7.7)	2.08	0.87-5.13	0.07
	No	8/206 (3.9)	Ref.	-	-

Variable	Risk of coccidiosis (%)	OR	95% C.I.	p-value
Treatment/Prevention				
Which vaccinations were used during the last crop				
NCD				
Yes	8/120 (6.7)	1.40	0.47-4.15	0.50
No	9/186 (4.8)	Ref.	-	-
IBD				
Yes	31/516 (6.0)	0.83	0.12-36.44	0.86
No	1/14 (7.1)	Ref.	-	-
IB				
Yes	25/370 (6.8)	1.74	0.41-15.60	0.46
No	2/50 (4.0)	Ref.	-	-
TRT				
Yes	3/16 (18.8)	3.48	0.68-15.80	0.06
No	13/209 (6.2)	Ref.	-	-
Other types of vaccination				
Yes	0/3 (0)	-	-	-
No	1/9 (11.1)	Ref.	-	-
Were preventive antibiotics used during the last crop				
Yes	21/296 (7.1)	1.73	0.73-4.21	0.18
No	9/213 (4.2)	Ref.	-	-
Were curative antibiotics used during the last crop				
Yes	18/208 (8.7)	2.03	0.93-4.46	0.05
No	14/314 (4.5)	Ref.	-	-
Were coccidiostatic agents used during the last crop				
Yes	29/406 (7.1)	1.61	0.38-14.44	0.52
No	2/44 (4.5)	Ref.	-	-
Were competitive exclusion products (CE) used during the last crop				
Yes	1/61 (1.6)	0.23	0.01-1.62	0.11
No	29/423 (6.9)	Ref.	-	-
Which type of CE product was used				
Aviquard	1/52 (1.9)	Ref.	-	-
Broilact	0/5 (0)	-	-	-
Other	0/3 (0)	-	-	-
Method of CE product administration				
Via water				
Yes	1/43 (2.3)	-	-	-
No	0/21 (0)	Ref.	-	-
Via feed				
Yes	0/3 (0)	-	-	-
No	1/61 (1.6)	Ref.	-	-
Via spray				
Yes	0/18 (0)	-	-	-
No	1/46 (2.2)	Ref.	-	-
Flock performance				
Feed conversion of last crop				0.77[1]
1.60-1.80	8/118 (6.8)	Ref.	-	-
1.81-1.87	11/127 (8.7)	1.30	0.51-3.36	0.58
1.88-1.92	7/98 (7.1)	1.06	0.37-3.03	0.92
1.93-2.26	6/115 (5.2)	0.76	0.25-2.25	0.62
Feed conversion of the last crop *(continuous, per 0.1 point increase)*	-	0.85	0.55-1.31	0.47

Variable		Risk of coccidiosis (%)	OR	95% C.I.	p-value
Flock performance (continued)					
Total mortality (%) during last crop					0.85[1]
	0.20-2.63	7/130 (5.4)	Ref.	-	-
	2.65-3.44	10/128 (7.8)	1.49	0.55-4.04	0.43
	3.45-4.24	7/124 (5.7)	1.05	0.36-3.09	0.93
	4.25-11.80	7/124 (5.7)	1.05	0.36-3.09	0.93
Total mortality (%) during the last crop *(continuous)*		-	1.01	0.78-1.30	0.94
EPEF during last crop					0.25[1]
	180-270	7/86 (8.1)	Ref.	-	-
	271-285	5/92 (5.4)	0.65	0.20-2.13	0.48
	286-300	2/89 (2.3)	0.26	0.05-1.29	0.10
	301-379	7/87 (8.1)	0.99	0.33-2.95	0.98
EPEF during the last crop *(continuous, per 10 points increase)*		-	0.95	0.81-1.12	0.55

[1]. Likelihood ratio test statistic

THE UNIVERSITY
of LIVERPOOL

Appendix 10: Sampling of necrotic enteritis cases – cover letter, protocol and submission form.

Department of Veterinary Clinical Science and Animal Husbandry

Faculty of Veterinary Science

Leahurst
Chester High Road
Neston
CH64 7TE

Telephone: 0151 794 7582
Facsimile: 0151 794 6028

Liverpool, 23rd September 2003.

RE: SAMPLING OF NECROTIC ENTERITIS CASES.

Dear colleague,

As part of an ongoing study, we are carrying out a sampling scheme for cases of necrotic enteritis in broilers diagnosed in the field. We are sending you the protocol for this scheme, submission forms, formalin bottles, and self-addressed freepost envelopes. We would be most grateful for your participation.

The most common method for diagnosing NE is by post-mortem examination. No diagnostic test is 100% accurate and we wish to get an estimate of the accuracy of post-mortem examination for diagnosing NE. Therefore, we are collecting post-mortem samples according to the protocol enclosed with this letter. The samples will be used for histological examination.

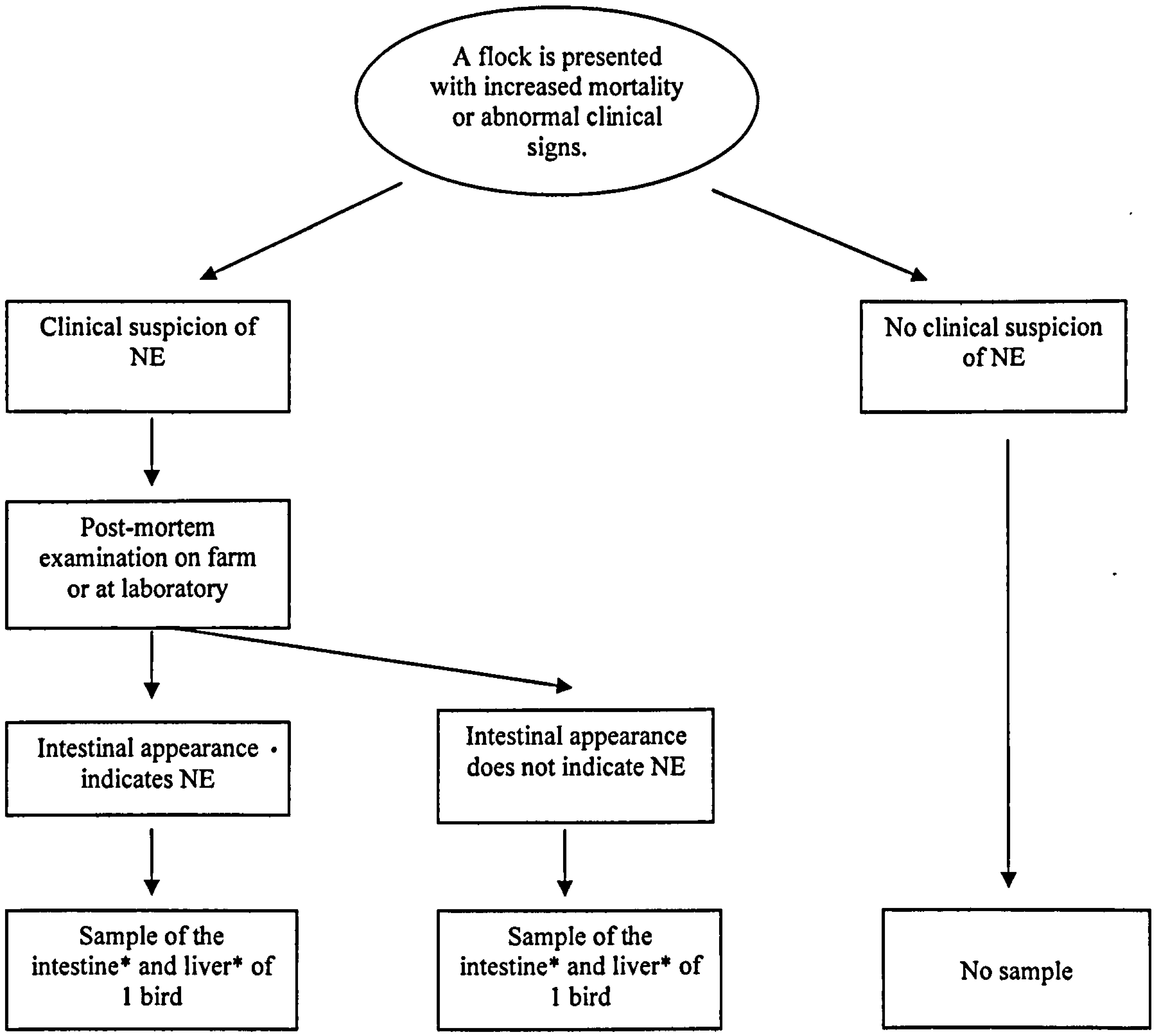
We realize that in some cases field managers assist veterinarians in the diagnoses, particularly on farms with recurrent NE problems. If this is the case, we would appreciate it if you distribute bottles, envelopes, submission forms and a protocol to these field managers as well. Please take some time to read the protocol carefully and note that we would like samples when gross pathology is consistent with NE and when it isn't. If you need further information, please do not hesitate to contact me on 0151-794-6027 (office) or 0151-794-7582 (mobile).

Many thanks in advance for your participation.

Yours sincerely,

Patrick Hermans.   Professor Kenton Morgan

University switchboard
Telephone: 0151 794 2000
Facsimile: 0151 708 6502



***Intestine:**

- Should be approximately 3 cm in length
- Samples should be taken at the transition of the affected portion of the gut into the unaffected portion of the gut so that the difference can be seen between healthy and affected tissue. In case of no lesions, samples can be taken from any portion of the gut.
- The inner surface should not be scraped or otherwise disturbed.

***Liver:**

- The liver sample should be taken from the bottom of the largest lobe (right lobe) and be approximately 1cm by 1cm in size.
- Take the liver sample from the same bird from which the intestinal sample was taken.

Use one bottle for both intestinal and liver sample. Place the bottle inside plastic bag (maximum of 1 bottle per bag), fold the top of the bag over three or four times and close by bending yellow taps inwards. Send together with the submission form in the self-addressed freepost jiffy bag.

(If you have any questions, please contact Patrick Hermans on 0151-7947582).

NECROTIC ENTERITIS - SUBMISSION FORM.

YOUR DIAGNOSIS.

Your diagnosis based on appearance of intestines: ☐ NE ☐ No NE

FLOCK DETAILS

Flock age: ....days

Flock size: ....

Clinical history of flock: ....
....

Treatment history of flock: ....
....

SPECIMEN DETAILS

Area of intestine submitted:

☐ Small intestine → ☐ Duodenum / ☐ Jejunum / ☐ Ileum

☐ Large intestine → ☐ Caecum / ☐ Colon

Approximate time between moment of death and post-mortem examination: ☐ < 6 hours ☐ 6-12 hours ☐ 12-24 hours ☐ >24 hours

SENDER'S DETAILS

Name: ....

Contact Telephone number: ....

(Place the formalin bottle with samples inside the plastic bag. Fold the top of the bag over three or four times and close by bending yellow taps inwards. Send in self-addressed freepost jiffy bag with this submission form.)