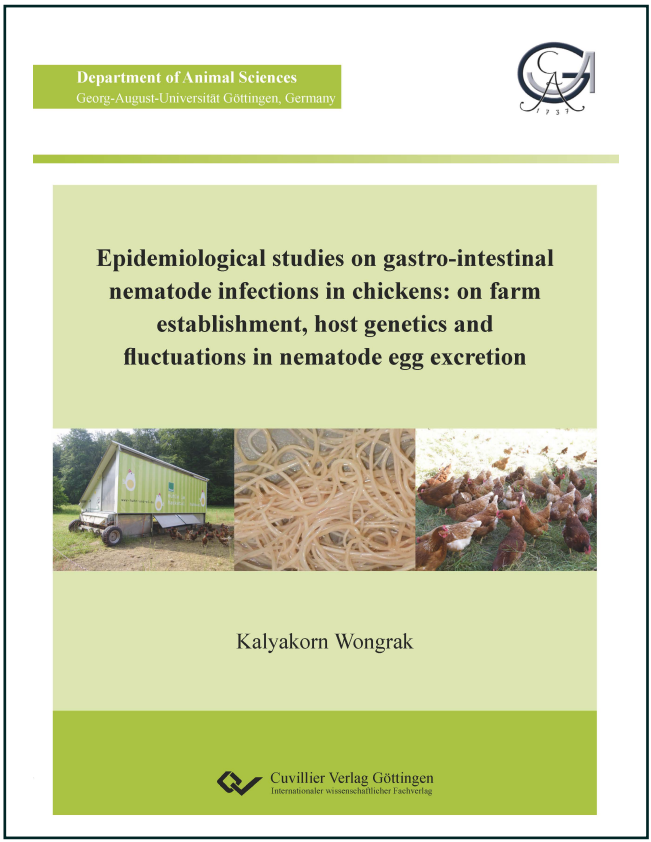
Kalyakorn Wongrak (Autor)

Epidemiological studies on gastro-intestinal nematode infections in chickens

On farm establishment, host genetics and fluctuations in nematode egg excretion



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Chapter 1

General introduction

1.1 Foreword

Since January 2012, the conventional cage system for laying hens has been prohibited in the European Union (European Union Council Directive 1999/74/EC; CEC, 1999). Since then, cage systems were widely replaced by alternative housing systems for laying hens, e.g., free-range systems, with the aim of improving animal health and welfare. These alternative systems allow hens to exhibit their natural behaviours, as well as offering more space per hen (Jendral, 2005; Fanatico, 2006). Furthermore, the demand for organic or free-range eggs is steadily increasing (Anonymous, 2013), despite higher costs per egg (Hermansen, 2003; Pottgüter and Schmutz, 2012). The term free-range refers to poultry systems in which the birds have continuous daytime access to open air, sunlight, runs or pasture (4 m^2/hen) (Gordon and Charles, 2002; Thear, 2002). Thus, it is suggested that chickens provided with free-range conditions are healthier and less stressed (Fanatico, 2006). However, the access to runs or pastures implies new challenges, including biosecurity and safety issues for the hens. One of those issues is a higher risk of parasitic infections (Permin et al., 1999; Häne et al., 2000; Kaufmann et al., 2011a). Parasite eggs are excreted to the external environment through faeces, and thus their presence and/or concentration in faeces are of diagnostic importance. Moreover, faecal egg counts (FEC) is often regarded as an indication of the severity of parasitic disease and size of the adult worm burden (Amarante, 2000). Therefore, the detection of the number of eggs when rearing chicken can be a possible warning sign of the magnitude of a parasite infection. However, there are various factors which can affect the FEC, such as the amount of faeces (De Bont et al., 2002; Daş et al., 2011a) and faeces consistency (Le Jambre et al., 2007). Additionally, the FEC may be influenced by environmental factors, e.g., seasons (Kaufmann et al., 2011a), weather (Vicente et al., 2005), as well as the storage temperature and storage time of faeces (Nielsen et al., 2010). Eggs of certain nematodes may periodically be released by day-to-day fluctuations (Yu et al., 1998; Giver et al., 2000).

Three parasitic nematodes are often encountered in free-range laying hens, with the caecal worm *Heterakis gallinarum* being the most prevalent, followed by the roundworm *Ascaridia galli* and the hairworms *Capillaria* spp. (Permin et al., 1999; Kaufmann et al., 2011a). A recent study revealed that the vast majority of hens in organic free-range systems are infected with *H. gallinarum* (98%), *A. galli* (88%) and *Capillaria* spp. (75%) (Kaufmann et al., 2011a).

High biosecurity standards are difficult to maintain in free-range systems because the hens are regularly in close contact with their faeces which enables the completion of the parasitic life cycles. Furthermore, the use of drugs, e.g., anthelmintics are limited in organic systems (CEC, 1999), where free-range is inherent. However, in cases where animals have to be treated for infections, there is an increased risk for the development of drug resistance. Thus, alternative approaches to control diseases, e.g., parasites, are needed in free-range poultry production systems (Schou et al., 2003). The selection of animals with an increased resistance to parasites is therefore desirable.

1.2 Laying hens in Germany

Up until 2008, there were approximately 1,355 chicken farms and 38,437,939 laying hens in Germany (including only enterprises with more than 3,000 hens). Due to regulations by CEC (1999) after 2008, the use of conventional cages decreased dramatically. By the year 2009, approximately 20% of the farms were using both free-range and conventional cage systems, while, deep litter systems were used by 60%. From 2010 to 2013, the picture was very similar with approximately 63.8% of the chicken farms rearing their animals in a barn or in a deep litter and 27.8% of the farms housed their animals in free-range systems. The proportion of hens kept in free-range systems increased from 20.9% in 2008 to 27.8% in 2013. On the other hand, the amount of farms using the cage system decreased sharply between 2008 and 2013, from 49.4% to 9.9%. The graph (Figure 1) shows that the percentage of bird housed in the cage system obviously decreased, while the free-range systems are growing and will be continue to be of interest in the future. Conventional battery cages were banned in Germany as January 1, 2010. However, so-called ‘enriched cages’ which are designed for keeping several hens in one cage are still permitted. The enriched cages are typically equipped with perches, nesting areas, a scratch pad, and substrate or litter on the floor area, thereby providing more behavioural outlets than conventional cages (Farm animal welfare council, 2007).

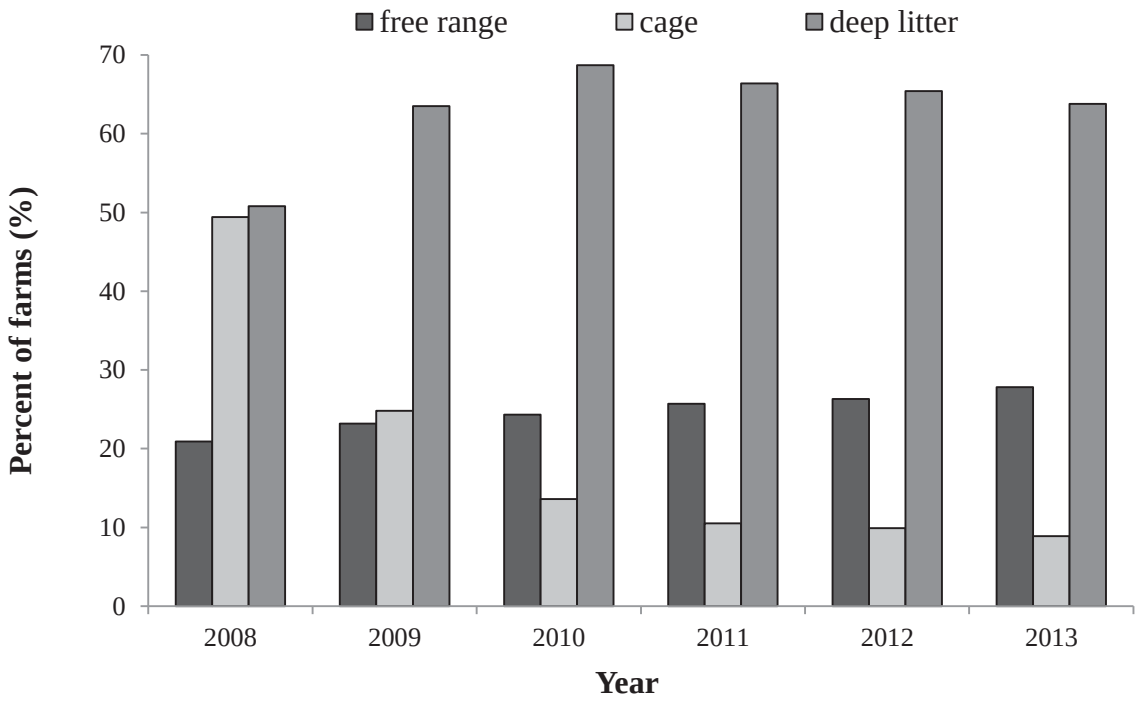

Figure 1. Percentage of housing systems for laying hens on German chicken farms for the years 2008 to 2013 (including only farms with > 3000 hens / farm; Adaptation from www.destatis.de). * From 2010 permitted caging: hen-keeping in groups and enriched cages.

1.3 Free-range chicken systems

Free-range systems in poultry consist of a housing structure and pastures or runs allowing animals to exhibit their natural behaviours more freely compared to closed systems (Shimmura et al., 2010; Castellini et al., 2006). The term free-range refers to poultry production systems in which the birds have continuous daytime access to pasture and thereby, sunlight and fresh air (Gordon and Charles, 2002; Thear, 2002). In the EU, each hen is supposed to have access to an outdoor area of at least 4 m^2, along with sufficient sunlight and natural ventilation in the housing structure (CEC, 1999). There are two main housing setups for free-range poultry production systems, fixed stalls and portable or mobile stalls (Fanatico, 2006).

Fixed stalls have an adjacent yard to provide outdoor access during the day, while at night birds are enclosed in the stall. Such systems are usually larger than mobile systems due to infrastructural properties. Portable or mobile stalls are smaller due to the necessity

to move the stalls when needed (Fanatico, 2006). Since hens use the same outdoor area over an extended period of time in fixed stalls and, thus, are in close contact to their faeces, they are at a higher risk of being infected by parasites (Permin et al., 1999; Häne et al., 2000; Kaufmann et al., 2011a). Mobile stall systems instead have been reported to reduce parasite infections in hens especially in combination with mixed grazing systems (Bassler et al., 2000).

It is generally believed that free-range chickens are more health, have a stronger immune system and have improved bone strength compared to caged chickens (Fanatico, 2006; Shimmura et al., 2010). There is, however, also a number of problems encountered in free-range systems, e.g., dirty eggs, grassland damage, production losses by predators such as foxes, dogs and birds of prey, and most importantly disease and parasitic infections (Bassler et al., 1999; Permin et al., 1999; Sommer and Vasicek, 2000; Pennycott and Steel, 2001; Thear, 2002; Fanatico, 2006; Shimmura et al., 2010; Kaufmann et al., 2011a).

For example, a study conducted in Sweden from 2001 to 2004 showed that free-range laying hens face more health problems in general, including cannibalism compared to laying hens kept in cages (Fossum et al., 2009). Furthermore, the same study showed that laying hens in free-range systems have a higher risk of being infected by bacteria and parasites and, thus, are more likely to contract more bacterial and parasitic related diseases compared to laying hens kept in cages. A recent study also revealed that the vast majority of organic free-range hens were infected with *H. gallinarum* (98%), *A. galli* (88%) and *Capillaria* spp. (75%) (Kaufmann et al., 2011a). In other studies, it has been shown that parasite related subclinical and clinical diseases lead to economic losses (Pinckney et al., 2008; Ruff and Norton, 1997; Permin and Ranvig, 2001). Therefore, it may be proposed that one of the most common disadvantage of free-range systems is the parasite infections (Permin et al., 1999; Häne et al., 2000; Kaufmann et al., 2011a).

1.4 Importance of poultry nematodes

The signs of a worm infection in hens may include diarrhoea, decrease in egg size and numbers, as well as a possible change in yolk colour (Thear, 2002). There is a very high prevalence of nematodes in free-range systems (Kaufmann et al., 2011a). Three important nematodes that are commonly encountered in free-range laying hens, are

Heterakis gallinarum which is the most prevalent, followed by *Ascaridia galli* and *Capillaria* spp. (Permin et al., 1999; Kaufmann et al., 2011a; Wongrak et al., 2012).

1.4.1 Ascaridia galli

Ascaridia galli is the most pathogenic nematode residing in the small intestine in chickens (Ruff and Norton, 1997; Gauly et al., 2001). *A. galli*, which has a direct life cycle, is a large roundworm that yellowish white in colour (Figure 2b). Adult worms are semi-transparent and measure 6 to 11.6 cm and 4.2 to 7.6 cm in males and females, respectively (Baker, 2007; Ramadan and Abou Znada, 1992). Adult male and female *A. galli* worms can be differentiated by their anterior and posterior ends (Taylor et al., 2007; Baker, 2007). A recent study on free-range hen systems in Germany showed that the prevalence of *A. galli* is 88% in laying hens (Kaufmann et al., 2011a). Compared to the two other common nematodes encountered in laying hens (i.e. *H. gallinarum* and *Capillaria* spp.), *A. galli* has a higher fecundity (Fine, 1975; Tompkins and Hudson, 1999; Permin et al., 1997) and a higher egg resistance to external environmental conditions (Ruff and Norton, 1997; Permin and Hansen, 1998). Infections with *A. galli* can cause growth depressions and lower nutrient utilization, which may be related to damages in the intestinal mucosa (Ramadan and Abou Znada, 1991; Gauly et al., 2005; Daş et al., 2010) that adversely affects the absorption of nutrients in the intestine, leading to blood loss, secondary infections (Ackert and Herrick, 1928; Soulsby, 1982) and behavioural changes (Gauly et al., 2007). It has been shown that these effects are more severe when animals are infected not only by *A. galli* but also by other pathogens (Chadfield et al., 2001; Dahl et al., 2002). Severity of the intestinal lesions may depend on the number of worms established in the intestine (Ikeme, 1971).

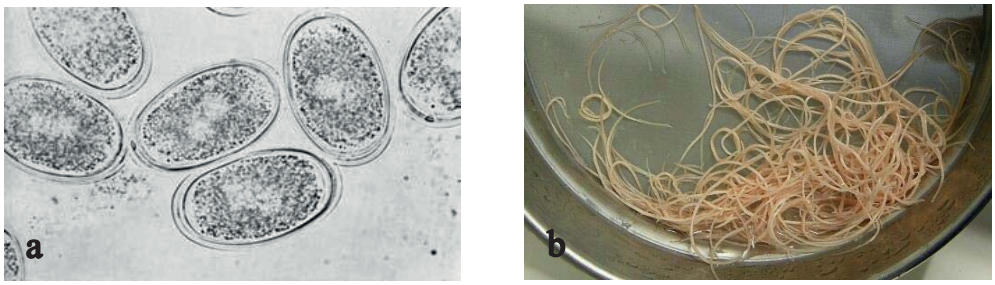

Figure 2. *Ascaridia galli* eggs (a) at 400x magnification (source: Baker, 2007) and adult worms (b) from samples of the small intestine of infected chicken.

As show in Figure 3 (without the indirect pathway), infection occurs when chickens ingest embryonated eggs containing the second or third larvae stage (Ramadan and Abou Znada, 1992; Araujo and Bressan, 1997) from the soil, litter or contaminated water or feed. The eggs have three different layers to protect the larvae inside until they reach the duodenum or jejunum (Hansen et al., 1956). The eggs were then transported to the proventriculus or the duodenum, where the larvae hatch within 24 hours (Ackert, 1923). The larvae then travel to the lumen of the duodenum where they stay for approximately 9 days. Once the larvae have hatched, they embed into the mucosal layer of the small intestine and enter the mucosal phase, with the highest number of larvae being located in the profound crypt zone of the mucosa of the lumen (Luna-Olivares et al., 2012). The duration of this phase varies between 3 days to 54 days before the final maturation in the lumen, depending on the level of infection (Herd and McNaught, 1975). The prepatent period (time from infection to the first egg being produced) is between 4 and 8 weeks depending on host age (Taylor et al., 2007).

1.4.2 Heterakis gallinarum

H. gallinarum, or the caecal worm also has a direct life cycle similar to *A. galli* and is a white worm which resides in the lumen of the caeca. It is the most prevalent species of nematodes in free-range systems at 98% (Kaufmann et al., 2011a). The nematode itself is not a serious life-threatening parasite for chickens, although it can also stunt growth to some extent (Daş et al., 2011b). However, it contributes to blackhead disease which causes necrosis of the caecal mucosa, swelling of the caecum and liver necrosis or infectious enterohepatitis due to its role as the vector in transporting the pathogenic protozoan *Histomonas meleagridis* (McDougald, 1998). *H. gallinarum* females produce 34,000 to 86,000 eggs in a lifetime or approximately 936 eggs per day (Fine, 1975). Due to morphological similarities, *H. gallinarum* egg and *A. galli* eggs (Figure 2a) cannot be distinguished from one another (Thienpoint et al., 1986). The nematode measures 7 to 13 mm in length for the male and 10 to 15 mm for the female (Baker, 2007). The life cycle is similar to that of *A. galli*, except that the chickens ingest infected eggs either directly or indirectly by an infected earthworm as an intermediate host (Figure 3). The larvae hatch in the small intestine and pass down to the caeca. With embryonation eggs develop into the third infective larvae stage within two weeks (Baker, 2007), and following the 21 to 34 days of the prepatent period, mature females start to lay eggs (Fine, 1975).

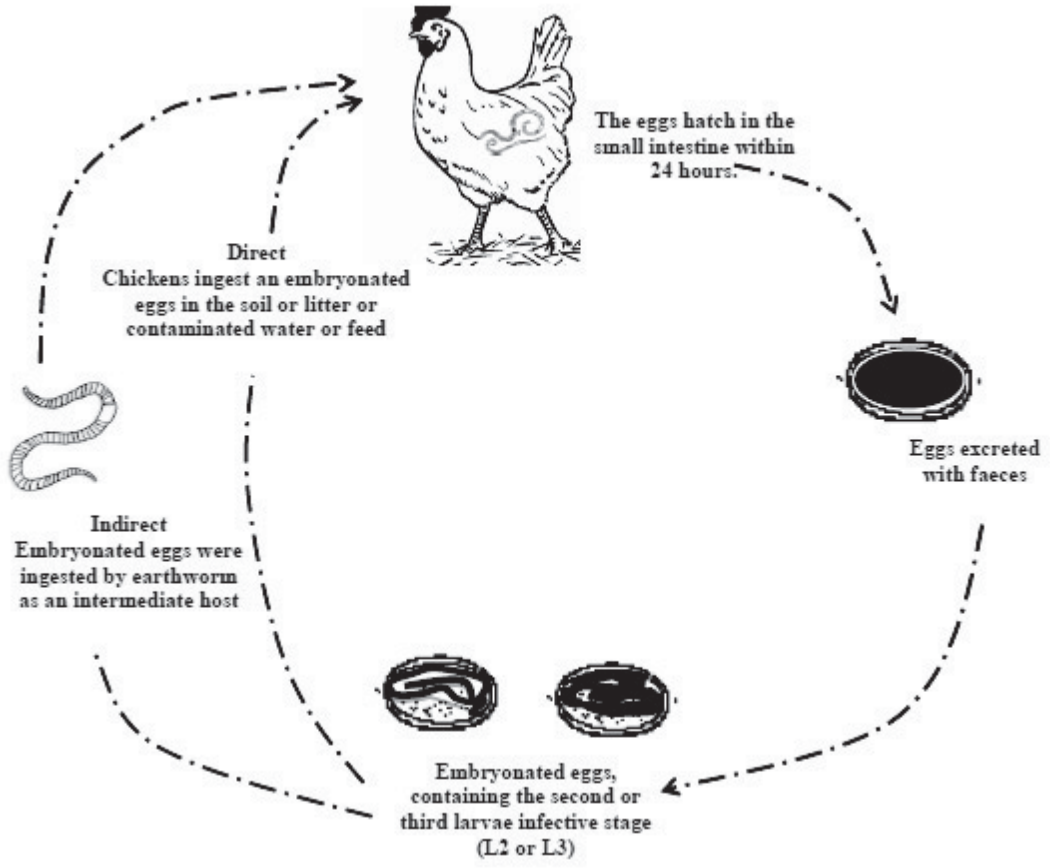

Figure 3. The life cycle of *Ascaridia galli* (without the indirect pathway) and *Heterakis gallinarum* worms.

1.4.3 Capillaria spp.

Capillaria species are hairworms which reside in the small intestine and in the caeca, depending on the species (Soulsby, 1982; Permin and Hansen, 1998). The eggs are barrel-shaped, colourless and have a thick shell that is slight striated with bipolar plugs (Taylor, 2007; Figure 4a). *Capillaria* spp. can cause emaciation, diarrhoea, haemorrhagic enteritis and can also be fatal (Wakelin, 1965). There are six common species of *Capillaria*, namely *C. annulata* and *C. contorta* which are found in the crop and in the esophagus; *C. caudinflata*, *C. bursata* and *C. obsignata* inhabit the small intestine, whereas *C. anatis* occurs in the caeca (Permin and Hansen, 1998). The females are differentiated by their vulva characteristics (Figure 5). *C. obsignata* is the most frequently diagnosed *Capillaria* spp. in chickens (Friedhoff and Ehlers-Bhodigen, 1965;

Permin et al., 1999; Wongrak et al., 2012). The prepatent time for *Capillaria* spp. is 3 weeks (Permin and Hansen, 1998).

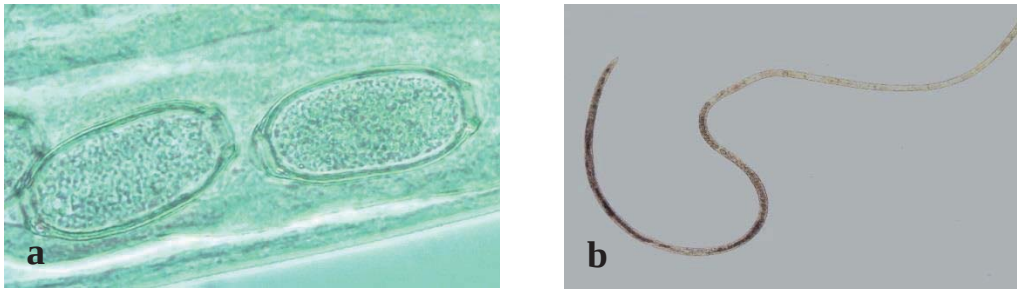

Figure 4. *Capillaria* spp. eggs (a) and adult female worm (b) from samples of the small intestine of infected chicken.

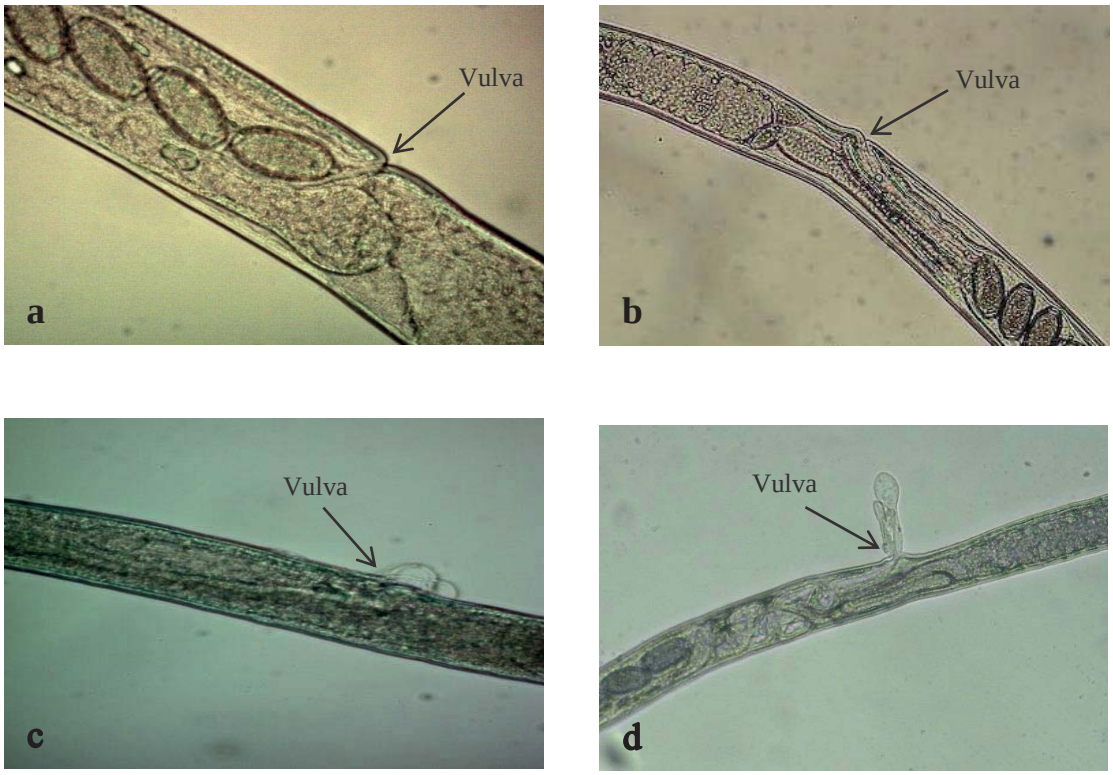

Figure 5. Vulva characteristics in *Capillaria* species in chickens; *C. anatis* (a), *C. obsignata* (b), *C. bursata* (c) and *C. caudinflata* (d).

1.5 Genetic variation in parasite infestations

Genetic resistance is a biological mechanism, innate and acquired resistance mechanisms to parasites are regulated by the sequential operation of genes, and resistance is therefore, by definition always genetically determined (Stear and Wakelin, 1998). Genetic variation in host response has been found in almost all host-parasite systems and have been the subject of intensive study for a variety of reasons (Gray and Grill, 1993), primarily:

1. evolutionary biologists seeking to understand the biodiversity of hosts and parasites.
2. applied biologists attempting to make use of genetic variation to increase resistance to parasitic diseases of economically important hosts.

Several studies have been conducted, investigating approaches for genetic selection on gastrointestinal nematodes resistance in chickens (Permin and Ranvig, 2001; Gauly et al., 2002; Schou et al., 2003; Gauly et al., 2008; Kaufmann et al., 2011b). Based on their results, they come to the conclusion that genetic resistance against parasites is heritable and could therefore be included into breeding programs.

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