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Phenotypic and molecular characterization of *Brachyspira* spp. isolated from laying hens in different housing systems

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Abstract

Several species of intestinal spirochaetes, *Brachyspira* (*B.*) *alvinipulli*, *B. intermedia* and *B. pilosicoli*, may cause reduced egg production and faecal staining of eggshells in chickens. The aim of this study was to characterize potentially pathogenic and presumably non-pathogenic *Brachyspira* spp. from commercial laying hens. Selective culture, phenotyping, PCR and 16S rRNA gene sequencing were used and clinical data were collected. Phenotypic profiles were obtained for 489 isolates and 351 isolates obtained after subculture, and 30 isolates were selected for molecular characterization. Seven isolates were positive by a *B. intermedia*-specific PCR based on the *nox* gene, and two were positive in a *B. hyodysenteriae*-specific 23S rRNA gene based PCR. By comparative phylogenetic analysis in combination with PCR and phenotyping, seven isolates were identified as *B. intermedia*, eight isolates as *B. innocens*, five as *B. murdochii*, and three isolates each as *B. alvinipulli* and ''*B. pulli*''. The remaining four isolates could not be assigned to any presently recognized species. Co-infection with several species or genetic variants of *Brachyspira* spp. were detected in some flocks and samples, suggesting a high level of diversity. Organic flocks with access to outdoor areas were at higher risk (RR = 2.3; 95% CI 1.5–3.6) for being colonized than chickens in other housing systems. No significant differences between colonized and non-colonized flocks were found regarding clinical parameters, i.e. mortality, egg production, faecally contaminated eggshells, and wet

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litter. Our results show that a combination of traditional laboratory diagnostics, molecular tests and phylogeny is needed for identification of *Brachyspira* sp. from chickens.

Keywords: Avian intestinal spirochaetes; *Brachyspira*; Chicken; Housing system; PCR; Phenotype; 16S rRNA gene sequencing

1. Introduction

The genus *Brachyspira* currently consists of seven recognized species; *B. aalborgi* (Hovind-Hougen et al., 1982), *B. alvinipulli* (Swayne et al., 1995; Stanton et al., 1998), *B. hyodysenteriae* (Taylor and Alexander, 1971; Ochiai et al., 1997), *B. innocens* (Stanton, 1992; Ochiai et al., 1997), *B. intermedia* (Stanton et al., 1997; Hampson and La, 2006), *B. murdochii* (Stanton et al., 1997; Hampson and La, 2006) and *B. pilosicoli* (Taylor et al., 1980; Trott et al., 1996; Ochiai et al., 1997). Two additional species have been officially proposed; "*B. canis"* (Duhamel et al., 1998) and "*B. suanatina*" (Råsbäck et al., 2007), and the name "*B. pulli"* has been assigned to a distinct group of chicken isolates based on data from multilocus enzyme electrophoresis (MLEE) (Stephens and Hampson, 1999; Stephens et al., 2005) and 16S rRNA gene sequence data (Phillips et al., 2005). All these genotypes except *B. aalborgi,* and *"B. canis"* have, so far, been isolated from at least one bird species. In chickens, *B. alvinipulli*, *B. intermedia* and *B. pilosicoli* are considered as potentially pathogenic species causing reduced egg production, delayed start of lay, increased water content in faeces and faecal staining of eggshells, while *B. innocens*, *B. murdochii* and "*B. pulli*" are presumed to be non-pathogenic species. Intestinal spirochaetes isolated from chickens are, with occasional exceptions, weakly haemolytic, and their growth pattern on agar plates does not allow reliable differentiation between species. Presently, differentiation between potentially pathogenic and presumably non-pathogenic *Brachyspira* spp. of chicken origin requires molecular diagnostic methods such as PCR, MLEE, pulsed field gel electrophoresis (PFGE), *nox* restriction fragment length polymorphism (RFLP) and/or sequencing of the 16S rRNA gene.

The aim of the current study was to characterize representative isolates of *Brachyspira* spp. from commercial laying hens, and to increase the understanding of *Brachyspira* sp. diversity among laying hens in different housing systems.

2. Materials and methods

2.1. Study population and sampling

The population under study in May 2003 to June 2004 consisted of five to six million Swedish commercial laying hens (number estimated by J. Bengtsson, Swedish Board of Agriculture, and J. Yngvesson, Swedish Welfare Agency, personal communication). Due to welfare concerns and national legislation the industry was in the process of replacing conventional battery cages with furnished cages (cages with perches, nests and litter boxes), single-tiered floor systems (with litter area, perches and manure bin or a manure removal system), multi-tiered aviary systems (with litter area, perches and manure removal system) and organic production (litter-based housing indoors, organic feed, comparatively low stocking density and access to outdoor pens and pasture) (Tauson, 2005).

In the spring of 2003, 104 commercial laying hen farms and one flock on each of these farms were randomly selected from data sets of the Swedish Board of Agriculture and KRAV® Incorporated Association (the Swedish certification body for organic production) to include the different housing systems in use except conventional battery cages which were being phased out. An equal proportion of flocks representing each housing system were included. Between May 2003 and June 2004 twenty individual and undisturbed samples of caecal droppings were collected from each flock. To minimize age and seasonal effects, the sampling was carried out when selected chickens reached the approximate age of 65 weeks. Depending on the housing system, droppings were collected from the litter area or from furniture and equipment such as manure conveyor belts, tiers, perches and/or on and under slats. The samples were transported in Amies medium (Venturi Transystem®, Copan innovation, Italy) at ambient temperature by surface mail. The following data on the