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Experimental Challenge of Mallards (*Anas platyrhynchos*) with *Brachyspira hyodysenteriae* and "*Brachyspira suanatina*" Isolated from Pigs and Mallards

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Summary

Brachyspira hyodysenteriae, the aetiological agent of swine dysentery, and a recently proposed and closely related enteropathogenic spirochaete "*Brachyspira suanatina*", originally isolated from pigs or mallards (*Anas platyrhynchos*), were used to inoculate week-old mallard ducklings orally or cloacally. The colonization rate, clinical outcome, faecal dry matter content, blood leucocyte counts and gross, microscopical and electron microscopical features 14–16 days post-inoculation were investigated at necropsy examination. Strains of "*B. suanatina*" of pig and mallard origin and *B. hyodysenteriae* of mallard origin colonized the ducklings by oral inoculation, and colonization was also established by cloacal inoculation with a "*B. suanatina*" strain of mallard origin. The porcine reference strain of *B. hyodysenteriae* ($B204^R$) failed to colonize the birds. Unchallenged contact birds in one of the challenge groups were readily colonized by a strain of "*B. suanatina*" of mallard origin. The proportion of colonized birds differed significantly between the challenge groups ($P < 0.0001$). For each challenge group, the inoculum and a randomly selected subset of recovered isolates had an identical biochemical profile and banding pattern by randomly amplified polymorphic DNA (RAPD) analysis. None of the birds developed clinical signs of gastrointestinal disease during the trial. The faecal dry weight contents, body weights and total leucocyte and heterophil counts did not differ between the various groups of birds. At the microscopical and electron microscopical levels, the caecal mucosa in some of the *Brachyspira* culture-positive birds had sharply demarcated epithelial cell changes and there were features of irreversible cell damage in crypt necks coinciding with spirochaetal infiltration of the mucosa. The crypts in *Brachyspira* culture-positive birds were deeper than in culture-negative birds (median: 237 μm and 218 μm, respectively, $P = 0.019$). This challenge model was well suited for use in mallards and consistent with previous findings that strongly haemolytic *Brachyspira* spp. may cross the species barrier between pigs and birds.

Keywords: *Brachyspira* spp.; intestinal spirochaete; mallard; swine dysentery

Introduction

Brachyspira hyodysenteriae is the aetiological agent of swine dysentery (SD), a severe enteric disease of pigs that is characterized by mucohaemorrhagic to fibrinonecrotic typhlitis and colitis (Hampson *et al.*, 2006). Despite the substantial losses caused by SD, the pathophysiology of this disease is incompletely understood. Colonic malabsorption, rather than increased mucosal permeability, is thought to be the cause of diarrhoea (Argenzio *et al.*, 1980; Schmall *et al.*, 1983). Putative virulence factors have been suggested based on observations from studies in animal models. These include haemolysins, lipooligosaccharides, endotoxins, NADH oxidase activity, motility and mucin chemotaxis (Hampson *et al.*, 2006). Similarly, there is little information available on the epidemiology of this infection, particularly regarding avian reservoirs.

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In birds, *B. hyodysenteriae* has been associated with severe necrotizing typhlocolitis of high mortality in farmed common rheas (*Rhea americana*) (Sagartz *et al.*, 1992; Jensen *et al.*, 1996; Kutzer *et al.*, 2007). The bacterium has also been isolated from farmed and free-living wild mallards (*Anas platyrhynchos*) (Jansson *et al.*, 2001, 2004) and from commercial laying hens (Feberwee *et al.*, 2008), but its enteropathogenic potential in these two latter species is unknown. *B. hyodysenteriae* of porcine origin has, however, been shown to cause reduced weight gain, diarrhoea, and gross and histopathological lesions in young chickens in a challenge model (Adachi *et al.*, 1985; Sueyoshi *et al.*, 1986, 1987; Sueyoshi and Adachi, 1990; Trott and Hampson, 1998).

Recently, a closely related spirochaete, ''*B. suanatina*'', has been provisionally described from Swedish and Danish pig herds (Råsbäck *et al.*, 2007a). Differentiation between *B. hyodysenteriae* and ''*B. suanatina*'', which are both strongly haemolytic and indole positive, relies on a combination of selective anaerobic culture and molecular analysis (Råsbäck *et al.*, 2007a). ''*B. suanatina*'' has also been isolated from free-living and apparently healthy mallards (Jansson *et al.*, 2004; Råsbäck *et al.*, 2007a). By using a porcine challenge model, an isolate of ''*B. suanatina*'' of porcine origin was shown to cause clinical disease that could not be differentiated from SD, whereas an isolate originating from a mallard caused watery diarrhoea (Råsbäck *et al.*, 2007a).

To our knowledge, clinical disease caused by *Brachyspira* spp. in mallards has never been described. However, diarrhoea and typhlohepatitis of unknown aetiology have been reported from domestic duck farms in France (Callait-Cardinal *et al.*, 2006), and necrotizing typhlocolitis has been diagnosed in *Brachyspira* culture-positive mallards on game farms in Sweden (Jansson *et al.*, 2001). Given the frequency of spirochaete colonization of free-living wild and farmed mallards (Jansson *et al.*, 2004), it is of clinical interest to assess the enteropathogenic potential of various *Brachyspira* spp. isolated from this species of bird.

Free-living wild mallards could be of epidemiological importance as carriers of *Brachyspira* spp. (Oxberry *et al.*, 1998; Jansson *et al.*, 2004; Råsbäck *et al.*, 2007a). The mallard is a migratory species that is both numerous and widespread, with breeding grounds covering the temperate and sub-tropical areas of the Northern hemisphere, Australia and New Zealand (del Hoyo *et al.*, 1992). Therefore, there may be ample opportunity for spread of intestinal spirochaetes to domestic animals such as pigs.

The aim of the present study was to develop an experimental model for *Brachyspira* colonization in mallards. We hypothesized that mallards would become colonized by experimental challenge without developing clinical disease. Characterized strains of *B. hyodysenteriae* and ''*B. suanatina*'' of pig and mallard origin were used to challenge 1-week-old mallard ducklings. The bacteria were administered orally or cloacally, and the birds were followed clinically and bacteriologically for approximately two weeks. The birds were then killed and examined *post mortem*.

Materials and Methods

Animals and Husbandry

Sixty-five newly hatched male and female mallard ducklings were supplied by a commercial game bird breeder. Upon arrival at the National Veterinary Institute (SVA), the birds were subject to veterinary inspection and were randomly allocated to 5 groups of 10 birds each (groups 1, 3–6, Table 1) and 1 group of 15 birds (group 2, Table 1). Throughout the acclimatization period (days −7 to −1) and the study period, (0–16 days post-inoculation; dpi), the room temperature was kept at 20°C and the birds were maintained on a 12 h light/dark cycle. The groups of birds were housed in 1.2 × 1.2 m enclosures in separate rooms equipped with individual lock areas (i.e. separate anterooms with washing facilities and a barrier point for change of protective clothing and footwear). A heat lamp was present in one corner of each enclosure. The concrete floor was covered by autoclaved wood shavings, with water baths available. Wood shavings and bathing water were replaced on a daily basis. Drinking water and non-medicated commercial poultry starter diet containing 185 g protein per kg feed were offered *ad libitum*. At 12 days of age, the feed was changed to a commercially available non-medicated poultry grower diet containing 160 g protein per kg feed. The feeds used in this trial were purchased from a feed mill that does not produce feeds containing coccidiostats or antimicrobials at therapeutic levels. Growth promoters were banned in Sweden in 1986.

The negative control group (group 1, Table 1) was placed nearest to the entrance door and the other groups further along the corridor according to group number (groups 2–6, Table 1). In group 2, five of the 15 ducklings were randomly selected as unchallenged contact birds and were identified by colour marking of neck feathers and dorsal aspect of the beak (group 2b, Table 1). Bird health (general appearance, condition of plumage, cloacal soiling) and faeces (colour and presence of diarrhoea, blood and mucus) were assessed twice daily. The body weights of the birds were recorded on arrival and on 0, 3, 6, 9 and 12 dpi by using an electronic scale (Mettler