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SHORT REPORT
Survey on the occurrence of *Brachyspira* species and *Lawsonia intracellularis* in children living on pig farms

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SUMMARY

The occurrence of *Brachyspira* species and *Lawsonia intracellularis* was investigated by PCR analyses of faeces from 60 children living on European pig farms. In addition, 60 other children were included as controls. Two samples were positive for *B. aalborgi* but *B. pilosicoli* and *L. intracellularis* were not demonstrated.

Two species of the genus *Brachyspira*, *B. aalborgi* and *B. pilosicoli*, have been associated with human intestinal spirochaetosis (HIS) [1, 2]. The clinical significance of HIS is largely unknown. In Western countries, *B. aalborgi* seems to be more common with reported prevalences of for example, 7·9% [3] or 70% of submitted cases of HIS [4]. *Brachyspira pilosicoli* is more frequent in developing countries with reported prevalences of 15–23% [2, 3, 5]. Further, *B. pilosicoli* and the intracellular rod *Lawsonia intracellularis* are major causes of enteric disease in young, growing pigs with 32% and 48%, respectively, of the herds being infected [6, 7]. The infection causes similar clinical signs and the microbes often occur concomitantly. *L. intracellularis* have been isolated in a wide range of other animal species, e.g. pig, hamster, horse, guinea pig, dog, lamb, calf, ferret, fox, deer, rabbit, rat, mouse, ratites, wild boar, wolf, giraffe, hedgehog, and primates. The broad host range raises the question whether natural cross-species transmission might occur. *L. intracellularis* belongs to the Desulfovibrionaceae family and in patients with ulcerative colitis an increased carriage of closely related bacteria has been demonstrated. The microbe causes proliferative enteropathy, characterized by crypt hyperplasia of immature cells. The lesions are morphologically similar to those found in patients with coeliac disease and hypotheses regarding a similar aetiology have been proposed. However, the bacterium has never been reported in humans [8–10].

The aim of the present study was to investigate the occurrence of *Brachyspira* species and *L. intracellularis* in faeces from children living on European pig farms and compare the findings to those in children not living on pig farms.

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The study was conducted as part of a cross-sectional study on factors characteristic of anthroposophic and farming populations, focusing on children from these groups. The study was approved by the Regional Ethical Review Board, Stockholm, Sweden. Five European countries participated and 60 of the children included in the study were living on pig farms. Seven children were from Austria, 14 from Germany, 14 from The Netherlands, nine from Sweden and 16 from Switzerland. The mean age was 9 years (range 6–14 years). On average, each farm kept 12 pigs. All children had been in contact with the pigs. Moreover, 60 children of similar age and from the same geographic areas, but not living on pig farms, were included as controls in the present study. Of these, 30 were living on farms keeping other livestock than pigs, and 30 children were living in the same geographic areas but not on farms. Stool samples were collected in insulated bags, transported on ice and stored in a refrigerator (−80 °C) until required for processing. DNA was prepared from faeces by the use of a commercial kit (QIAamp® DNA Stool Mini kit; Qiagen Inc., Valencia, CA, USA). The detection of *Brachyspira* spp. were performed as described by Kraatz *et al*. [4]. Genus-specific primers targeting 16S rDNA were used in PCR and species determination was performed by sequencing of the PCR amplicons. Demonstration of *L. intracellularis* was carried out by PCR using specific primers directed to chromosomal DNA according to Jones *et al*. [11]. An internal control (mimic) was included in each tube to detect PCR inhibition. The sensitivity to detect the mimic in faecal sample preparations was 10^2 mimics per PCR [11–13].

Two samples, one from a 12-year-old boy living on a pig farm and one from a 9-year-old girl not living on a farm, both from Switzerland, were found positive for *B. aalborgi* by PCR and sequencing. In both children, strains similar to the type strain, strain W1 and a clone designated Hcc33 (GenBank accession numbers Z22781, AF200693, and AF228813, respectively) were identified. None of the children had been absent from school because of illness or treated with antibiotics during the last 3 months prior to sampling. Neither *Brachyspira pilosicoli* or *L. intracellularis* was detected in any sample. In one sample from a child living on a pig farm and in three samples from children in the control group the PCR was inhibited, as judged by the absence of the mimic amplicon. The mimic was visualized in the negative controls included.

B. aalborgi is generally the most common *Brachyspira* species found in Western societies [14]. This is in accordance with the present study, where only *B. aalborgi* was isolated. The clinical significance of the infection in humans has not been clarified [3, 15]. In the present study, no illness was recorded in the infected children. Further, it was not possible to relate the infection to husbandry. Isolates of *B. aalborgi* has been demonstrated in humans, non-human primates and opossums [16] and the ability to colonize might be determined by indigenous factors such as body temperature. Although commonly found in several animal species, it has not been possible to relate findings of *B. pilosicoli* to the keeping of pigs [2]. Human isolates have, however, previously been shown to cause disease in pigs and chickens in experimental challenge studies and cross-species transmission may occur naturally [17, 18]. In the present study, no samplings were performed in the pig herds and the occurrence of these bacteria in the herds is therefore unknown. Hence, it is not possible to draw any conclusions regarding the risk for children living on pig farms to contract these diseases.

The microbe *L. intracellularis* have a very broad host range, including non-human primates, and the infection does not seem to be related to the host's body temperature. Experimentally, cross-species transmission has been demonstrated in pig, horse, hamster and mice [19]. Hence, it does not seem unlikely that this microbe would also be capable of infecting humans. However, in the present study the bacterium was not detected. It is possible that children living under poor hygienic circumstances, in close contact with highly infected faeces, would have been a more suitable target in this respect.

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DECLARATION OF INTEREST

None.

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