Research Note

The Presence of Arcobacter Species in Breeding Hens and Eggs from These Hens

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ABSTRACT The presence of Arcobacter spp. in 2 breeding hen flocks was determined by examination of the intestinal tract, oviduct magnum mucosa, and ovarian follicles of slaughtered chicken. The bacteria were detected by PCR and cultural isolation in 34 out of 40 intestinal tracts from one flock (A) and 6 out of 30 from the other (B). The strains were Arcobacter butzleri, Arcobacter cryaerophilus, and Arcobacter skirrowii. From flock A, arcobacters were recovered from 6 out of 40 oviduct magnum mucosa samples. The majority of isolated strains were A. butzleri. Arcobacter spp. could not be detected, by either PCR or isolation, from 20 eggs collected on the farm of flock A and from 20 eggs still remaining in the vagina of hens in flock B. Furthermore, none of the ovarian follicles from each flock were positive. The results indicate that breeding hens can be infected with Arcobacter spp. in the intestinal tract and oviduct. No evidence was obtained for transmission of Arcobacter spp. from hens to eggs.

Key words: Arcobacter spp., breeding hen, egg

INTRODUCTION

Arcobacter spp. members of the Campylobacteraceae have recently been considered to play a role as potential zoonotic microorganisms (Ho et al., 2006a). Arcobacter butzleri, Arcobacter cryaerophilus, and Arcobacter skirrowii have been detected in diseased as well as in healthy humans and animals. In humans, the bacteria were isolated from several cases of bacteraemia (On et al., 1995; Hsueh et al., 1997; Yan et al., 2000; Woo et al., 2001; Lau et al., 2002; Wybo et al., 2004) and were found both in stool specimens of healthy and diarrheal humans (Vandenberg et al., 2004; Samie et al., 2007; Houf and Stephan, 2007). Arcobacter spp. are isolated from healthy farm animals (Kabeya et al., 2003; Van Driessche et al., 2003, 2004, 2005) but also could be related to reproductive disorders in cattle and swine (Ellis et al., 1977, 1978; De Oliveira et al., 1997; On et al., 2002). Furthermore, Ho et al. (2006b) revealed vertical and horizontal transmission of Arcobacter spp. from sows to piglets.

Like Campylobacter, Arcobacter spp. have been detected worldwide in meats, especially in chicken products (Zanetti et al., 1996; Kabeya et al., 2004; Rivas et al., 2004). The bacteria are present on almost all broiler carcasses and at significantly greater recovery rates than Campylobacter spp. on the same samples (Houf et al., 2002). However, the organisms are rarely isolated from live chicken and from their gut contents (Atabay and Corry, 1997; Van Driessche and Houf, 2007). The prevalence of Arcobacter spp. increased with the bird age (Wesley and Baetz, 1999). The aim of this study was to investigate the presence of Arcobacter spp. in breeding hens and to check for possible transmission of Arcobacter spp. from these hens to eggs.

MATERIALS AND METHODS

Sampling and Sample Processing

In total, 70 viscera were taken from 2 broiler breeding flocks (A and B) at a slaughterhouse in the Netherlands. Gut contents and oviduct magnums were sampled. The sampling area was flushed with 75% ethanol. Hot flamed scissors were used to open the intestines (ileum and ceca), and cotton swabs were used to transfer the gut contents into 10 mL of enrichment broth (Arcobacter broth, Oxoid, CM965 supplemented with cefoperazone, teicoplanin, amphotericin B (CAT), Oxoid, SR174, Hampshire, UK). Ten-centimeter samples of the oviduct magnum mucosa were gently scalped with sterile scalpels, and cotton swabs were used to transfer the samples into 10 mL of enrichment broth.
From 60 viscera, 1 mL of a 2-cm ovarian egg was taken using a sterile syringe and added into 9 mL of enrichment broth (Ho et al., 2008).

In addition to the viscera, 20 freshly laid eggs from flock A were taken on the farm, and 20 eggs were collected from vaginas of birds in flock B. The eggs were randomly paired, the eggshell surface of each pair was wiped with a sterile cotton compress moistened in 20 mL of Arcobacter broth, and 1 mL of the juice was inoculated in 9 mL of enrichment medium. The egg contents of each pair were pooled, homogenized, and 1 mL was inoculated in 9 mL of enrichment medium. All enrichment samples were incubated for 48 h at 30°C under microaerophilic conditions (generated by BD CampyPak, Becton, Dickinson and Company, Franklin Lakes, NJ).

Detection of Arcobacter spp.

The enrichment samples were examined by both PCR and isolation methods for Arcobacter spp. The DNA from each enrichment culture was extracted by the boiled lysis method and used in a genus-specific PCR reaction (Harmon and Wesley, 1996). The PCR-positive samples were then examined for A. butzleri, A. cryaerophilus, and A. skirrowii by a multiplex PCR (m-PCR; Houf et al., 2000). The DNA from A. butzleri LMG 6620, A. cryaerophilus LMG 7537, and A. skirrowii LMG 6621 was used as positive control (Ho et al., 2008).

For bacterial isolation, 50 μL of each enrichment sample was dropped onto a cellulose-nitrate membrane filter (0.65 μm, Sartorius, Nieuwegein, the Netherlands), which was placed onto blood agar plates (brain heart infusion agar, Oxoid, plus 5% horse blood) supplemented with CAT. The plates were incubated for 1 h at 30°C in air, the filters were removed, and the filtrate was distributed evenly on the agar surface with a sterile spreader. The agar plates were incubated for 48 h at 30°C under microaerophilic conditions, and samples of no growth were incubated for another 48 h. Suspected colonies were transferred onto blood agar plates without CAT supplement and incubated for 48 h at 30°C under microaerophilic conditions. Arcobacter isolates were identified at species level by the m-PCR (Ho et al., 2008).

RESULTS AND DISCUSSION

From flock A, 34 out of 40 gut tracts (85%) were Arcobacter positive by PCR. By multiplex PCR, A. butzleri was identified from 30 tracts, A. cryaerophilus from 2 tracts, and both A. butzleri and A. skirrowii from another 2 tracts. Arcobacter spp. were isolated from 33 out of 40 hens, the majority being A. butzleri strains (Table 1). Six out of 40 oviducts from the chickens were PCR positive for Arcobacter spp. Multiplex PCR identified A. butzleri 4 times. The 3 isolated A. butzleri strains from the oviducts showed similar enterobacterial repetitive intergenic consensus PCR fingerprints as isolates from gut contents (data not shown). In flock B, Arcobacter spp. were detected by PCR in 6 out of 30 gut tracts (20%), of which 5 were shown by m-PCR to contain both A. butzleri and A. cryaerophilus, and only the latter was detected from the sixth tract. Four times A. cryaerophilus strains could be isolated. None of the oviduct magnum samples from this flock were positive either by PCR or isolation (Table 1). None of the 20 eggs taken at farm (flock A), 20 eggs in vagina (flock B), and ovarian follicles (40 from flock A and 20 from flock B) were positive for Arcobacter spp. by PCR and isolation.

The results demonstrate the presence of Arcobacter spp. in the gut of breeding hens. Prevalence rates between the 2 flocks differ enormously (85% versus 20%). No explanation can be given for this difference. Although oviducts of hens from flock A were positive for Arcobacter spp., egg infection could not be found.

For other members of the Campylobacteraceae (e.g., Campylobacter spp.), vertical transmission from breeding parents to eggs and chicks and the significance of this route of transmission in flock contamination is

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of samples</th>
<th>Number positive by PCR</th>
<th>Number positive by isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeder flock A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal tracts</td>
<td>40</td>
<td>34 (85%)</td>
<td>33(82.5%)</td>
</tr>
<tr>
<td>Magnum mucosa</td>
<td>40</td>
<td>6 (15%)</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Ovarian follicle</td>
<td>40</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Eggs (on farm)</td>
<td>20</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Surface wipe</td>
<td></td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Egg contents</td>
<td></td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Breeder flock B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal tracts</td>
<td>30</td>
<td>6 (20%)</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td>Magnum mucosa</td>
<td>30</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Ovarian follicle</td>
<td>20</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Eggs in vagina</td>
<td>20</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Surface wipe</td>
<td></td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Egg contents</td>
<td></td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Table 1. The presence of Arcobacter spp. in breeder hens and eggs
still controversial due to the inconsistency of results and data interpretation from different studies. Some research groups reported the existence of Campylobacter spp. in hen ovarian follicles and oviducts as well as in hatchery facilities and wastes, which conclusively indicates the potential of vertical transmission from breeders to broilers (Hiett et al., 2002; Cox et al., 2005; Byrd et al., 2007). In contrast, observations on parent breeders, broiler breeding eggs, young broilers, hatchery samples, and artificial infection to specific-pathogen-free eggs suggest that the vertical route of passing Campylobacter spp. from breeders to broilers may not be of importance compared with the transmission from surrounding environments (Petersen et al., 2001; Sahin et al., 2003; Callicott et al., 2006).

From the described experiments, it can be concluded that Arcobacter spp. can be found in breeding hens both in the gut as in the oviduct. No evidence was found for transmission of Arcobacter spp. from breeding hens to eggs.

REFERENCES


