



Small ruminants as carriers of the emerging foodborne pathogen *Arcobacter* on small and medium farms

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ABSTRACT

In the past, the emerging pathogen *Arcobacter* has been associated with reproduction disorders and mastitis in livestock, but has also been isolated from healthy animals. Information on *Arcobacter* excretion by small ruminants is scarce. For this reason, the study reported in this paper aimed to assess the occurrence of arcobacters in healthy sheep and goats on farms. In total, 330 faecal samples were collected on three sheep, four goat farms, and one mixed farm. Drinking water, milk and urine samples were also collected on the same farms. Isolates, obtained by an *Arcobacter* selective method, were identified with a species-specific multiplex-PCR and characterized by enterobacterial intergenic consensus PCR. It was found that arcobacters were excreted in 43.1% of the faecal samples from sheep and out of 10.7% of those from goats. The percentages varied between the farms, animals and the sampling occasions. In both goats and sheep, *Arcobacter butzleri* and *Arcobacter cryaerophilus* were the dominant species, and the majority of the strains were only excreted once. This study indicates that healthy sheep and goats, in particular the former, are important carriers of *Arcobacter* species. The fact that arcobacters are asymptotically present in the intestinal tract of healthy small ruminants poses an important risk for faecal contamination of carcasses during slaughter and possibly of milk on farms.

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1. Introduction

Arcobacters, formerly known as ‘aerotolerant campylobacters’, are Gram-negative, slender-curved, non-sporeforming motile bacteria. They differ from the closely related campylobacters by their ability to grow at temperatures below 30°C and their aerotolerance (Vandamme et al., 1991). Since the description of the genus *Arcobacter* in 1991, 10 species have been identified, of which six have been found in humans and/or animals (De Smet et al., 2011).

Four of these six, *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter skirrowii* and *Arcobacter thereius*, are associated with reproduction disorders, mastitis and

enteritis in livestock (Ho et al., 2006; Houf et al., 2009). However, together with *Arcobacter trophiarum*, they are also frequently excreted in the faeces of healthy farm animals (Van Driessche et al., 2004, 2005; De Smet et al., 2011). The annotation of the complete *A. butzleri* genome suggests however that this bacterium is predominantly a free-living, waterborne organism (Miller et al., 2007).

In humans, it is predominantly *A. butzleri* which has been associated with enteritis and occasionally septicaemia, but *A. cryaerophilus* and *A. skirrowii* have also been isolated in the stools of diarrheic patients (Samie et al., 2007; Vandenberg et al., 2004; Wybo et al., 2004). Symptoms of an *Arcobacter* infection are similar to campylobacteriosis, though a more persistent and watery diarrhoea has been reported (Vandenberg et al., 2004).

The presence of *Arcobacter* in the faeces of healthy livestock at slaughter constitutes a significant risk of carcass and meat contamination (De Smet et al., 2010; Van

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Driessche et al., 2003; Van Driessche and Houf, 2007b). In contrast, arcobacters have only rarely been isolated from intestinal content of poultry, so that the origin of the carcass contamination remains unclear (Van Driessche and Houf, 2007a). Consumption and handling of raw and undercooked meat, such as poultry, pork, beef and lamb are probable routes of foodborne infection (Vyřřasov et al., 2003; Rivas et al., 2004; Ho et al., 2006; Van Driessche and Houf, 2007a,b). Furthermore, contact with pets and person-to-person transmission are also identified as potential risk factors for human infection (Fera et al., 2009; Ho et al., 2006; Houf et al., 2008).

The associations of *Arcobacter*-like organisms with ovine, bovine and porcine abortions were first described in the late 1970s (Ellis et al., 1977, 1978; Vandamme et al., 1991). However, it was not until the turn of the century that the presence of arcobacters in cattle and pigs on farms was further studied. To date, information about *Arcobacter* excretion by small ruminants remains scarce. Except for the detection of *A. skirrowii* and *A. butzleri* in the diarrheic faeces of three lambs and the isolation of *A. butzleri* and *A. cryaerophilus* from faecal samples of sheep at the slaughterhouse, no information on the frequency of occurrence of *Arcobacter* in sheep and goat is available (Aydin et al., 2007; Pejchalová et al., 2008; Vandamme et al., 1992; Van Driessche et al., 2003).

The aims of the study reported below were to assess the *Arcobacter* occurrence in the faeces of different aged sheep and goats at farm level. Subsequently, the heterogeneity was examined by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) to determine whether the same strain diversity previously detected in cattle and pig faeces (Van Driessche et al., 2004, 2005) was also present in small ruminants.

2. Materials and methods

2.1. Study design

Between November 2006 and March 2007, a total of 330 faecal samples from 122 sheep and 177 goats were collected on three unrelated sheep farms (A–C), four goat farms (E–H) and one mixed sheep and goat flock (D) situated in the northern part of Belgium (Table 1). On farms A and B, samples were collected on four and two sampling occasions respectively. All other farms were visited once. Samplings took place in November (beginning and end), December and January (farm A), January and March (farm B), November (farm C), January (farm D), February (farm E) and March (farms F–H).

Faeces were taken rectally from randomly chosen animals using sterile gloves. In addition, samples of non-chlorinated drinking water, urine, milk and one faecal dog sample were collected (Table 2). Drinking water was collected from the drinking trough. The urine samples were received directly in a sterile recipient (120-ml sterile recipient with screw cap, ANI18APS, Novolab, Geraardsbergen, Belgium) during urinating. Milk was taken aseptically from each teat and collected in sterile containers (120-ml sterile recipient with screw cap, ANI18APS, Novolab). The teat ends were prepared by disinfection with ethanol, followed by drying of the teat with an individual disposable paper towel. The dog's faeces were taken immediately after defaecation. All samples were transported at 7 °C to the laboratory and processed within 6 h.

2.2. Isolation of *Arcobacter* spp.

Arcobacters were isolated by a selective isolation method for animal faeces (Van Driessche et al., 2003). Each 5 g of the sheep, goat or dog faecal sample was homogenized in 45 ml *Arcobacter* selective isolation broth [containing 24 g l^{-1} *Arcobacter* broth (CM 965, Oxoid,

Table 1
Farm data.

Farm	A	B	C	D	D	E	F	G	H
Farm type	Sheep	Sheep	Sheep	Mixed (sheep)	Mixed (goat)	Goat	Goat	Goat	Goat
Breed	Ouessant	Texel	Belgian milksheep (13), Texel (2) and Swifter (4)	Suffolk (9) and Hampshire (1)	Boergoats	Saanen × Toggenburg	Saanen and Saanen × Toggenburg	Saanen and Saanen × Toggenburg	Goat Saanen
No. of female animals	6	300	14	9	7	300	460	250	70
No. of male animals	1	12	2	1	1	12	16	9	3
No. of young animals (<1 year)	4	60	3	2	2	150	155	150	20
Pasture/indoor	Pasture	Pasture and indoor	Pasture	Pasture	Pasture	Indoor	Indoor	Indoor	Indoor

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