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SHORT COMMUNICATION

Report of first isolation of the zoonotic *Arcobacter* species from swine fecal samples in Ecuador

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ABSTRACT. *Arcobacter butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. thereius* are recognised as emerging zoonotic agents, recovered from animals and human beings. The information available about *Arcobacter* species in Latin America is scarce. Among domestic animals, swine are considered important reservoir and source of contamination of different *Arcobacter* species. This communication reports the first simultaneous isolation of the four zoonotic *Arcobacter* species in Ecuador. Preliminary identification was done by phenotypic characteristics and definitive species identification was made by multiplex PCR method. Further investigation about the prevalence, distribution, ecology and interactions with human beings of these species is required.

Key words: *Arcobacter*, zoonotic species, reservoir, Ecuador.

INTRODUCTION

The genus *Arcobacter*, included into the family Campylobacteraceae, class Proteobacteria, subclass Gracillicutes, comprises aero-tolerant, curved or spiral rod shaped and polar flagellated bacteria, firstly described as *Vibrio*/*Spirillum* organisms and later as aero-tolerant *Campylobacter*-like organisms (Vandamme 2000, Fernández *et al* 1995, Collado and Figueras 2011).

Nowadays, genus *Arcobacter* comprises 27 validated species[1] showing diverse habitats, being *A. canalis* the last described (Perez-Cataluña *et al* 2018). Some species live in association with a wide diversity of animals while others are found in environmental sources (Collado and Figueras 2011, Ferreira *et al* 2017). Only four of these species, *A. butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. thereius* are recognised as emerging zoonotic agents, being recovered from animals and human beings (Vandenberg *et al* 2004, Lehner *et al* 2005, Šilha *et al* 2015, van den Abeele *et al* 2015, Ferreira *et al* 2017).

It is important to highlight that among domestic animals, swine are considered important hosts, reservoir and source of contamination of different *Arcobacter* species (Ho *et al* 2006), and that in Latin America the information available about *Arcobacter* species is scarce. These bacteria have been isolated only in Chile (Fernández *et al* 1995), Argentina (Giacoboni et al 1997), Brazil (de Oliviera *et al* 1999), Mexico (Villarruel-López *et al* 2003), Peru (Zerpa *et al* 2015) and Costa Rica (Bogantes *et al* 2015). Thus, we conducted a pilot study in order to establish the presence of *Arcobacter* species in faecal samples obtained of healthy pigs at slaughterhouse.

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[1] http://www.bacterio.net/arcobacter.html

MATERIAL AND METHODS

Twenty faecal samples were randomly taken by rectal swabs from healthy pigs, before the beginning of the slaughter at the slaughterhouse of Loja city, Southern Ecuador (3°59' Lat S; 79°12' Long W).

To isolate *Arcobacter* sp., each sample was seeded into CAT (cefaperazone, amphotericin B and teicoplanin) enrichment broth and incubated at 30 °C for 72 h under aerobic conditions (Collado *et al* 2013), followed by passive filtration through 0.45 µm membrane filter placed on blood agar plates. After 30 min filtration, the filters were removed and the plates were incubated under the same conditions described above (Collado *et al* 2013, Fernández *et al* 2015). Preliminary identification of isolates was carried out by phenotypic characteristics [curved Gram negative rod, motility +, aerobic growth +, oxidase + and catalase + (Fernández *et al* 2015)]. Definitive species identification was done by the multiplex PCR (mPCR) method proposed by Douidah *et al* (2010) that characterizes the 5 most common *Arcobacter* species.

RESULTS AND DISCUSSION

Among the 20 faecal samples studied, three of them (15%) were positive for *Arcobacter* sp. One yielded *A. cryaerophilus*, the second *A. skirrowii* and the third, *A. butzleri* and *A. thereius* simultaneously. These four species have been isolated from pigs in different parts of the world, including in some Latin American countries (Collado *et al* 2013, Fernández *et al* 2015, Barboza *et al* 2017, Ferreira *et al* 2017). However, so far as we know, in Latin America these four zoonotic have been reported simultaneously only in Costa Rica, especially in samples from poultry and food of animal origin (Barboza *et al* 2017).

The isolation rate (15%) found in this pilot study falls within the ranges reported by De Smet *et al* (2011)

in four Belgian pig farms where the isolation rates were 11.3, 15.6, 15.0 and 50.0%, and the isolated species were *A. butzleri*, *A. cryaerophilus*, *A. skirrowii*, *A. thereius* and *A. trophiarum*. However, it is higher than the 4% reported by Gobby *et al* (2018) in Brazil. The diversity of species found by these authors was restricted only to *A. butzleri* and *A. cryaerophilus*.

Arcobacter butzleri, *A. cryaerophilus*, *A. skirrowii* and *A. thereius* are currently recognised as emerging zoonotic agents isolated from different animals and human beings (Vandenberg *et al* 2004, Lehner *et al* 2005, Šilha *et al* 2015, van den Abeele *et al* 2015, Ferreira *et al* 2017). Since their main routes of transmission to humans are the consumption and handling of raw or undercooked foods of animal origin like meats, milk, seafood and water, they are also considered as potential foodborne pathogens (Vandenberg *et al* 2004, Lehner *et al* 2005, Collado *et al* 2013, Fernández *et al* 2015, Šilha *et al* 2015, van den Abeele *et al* 2015, Ferreira *et al* 2017). From these species, *A. butzleri* is the most frequently isolated from animal, environmental and human samples, followed by *A. cryaerophilus*, being both associated with abortion and enteritis in animals as well as with diarrhea and bacteremia in children and adults (Collado *et al* 2013, Šilha et al 2015, van den Abeele *et al* 2015, Ferreira *et al* 2017, Barboza *et al* 2017). *Arcobacter skirrowii* has been isolated from sheep and cattle with diarrhea, aborted pig foetus, and chronic and acute diarrhea in humans (Lehner *et al* 2005, Collado *et al* 2013, van den Abeele *et al* 2015, Ferreira *et al* 2017) whereas *A. thereius* has been isolated from pig's faecal samples, duck's cloacal samples and from livers and kidneys of pigs with spontaneous abortions. More recently, it was isolated from stool of hospitalised patients with diarrhoea (van den Abeele *et al* 2015, Ferreira *et al* 2017, Rovetto *et al* 2017).

In addition to these four zoonotic species of *Arcobacter*, other species of the genus have been isolated from pigs, such as *A. thereius*, *A. throphiarum*, *A. suis* and *A. lanthieri* (Whiteduck-Léveillée *et al* 2015). More recently Figueras *et al* (2017) established that *A. thereius* included a group strains (represented by strain LMG 24487) that clustered separately from the type strain (LMG 24486T) representing the new species *Arcobacter porcinus*. However, none of these species, except *A. thereius*, has been isolated from human beings until now. Bearing in mind that the zoonotic species of *Arcobacter* are considered as emerging species in human pathology, it is necessary to pay attention to the eventual presence of these other species in human clinical samples. Also, in the case of the isolation to *A. thereius*, it becomes necessary to differentiate it with *A porcinus*. The PCR method used in this work (Douidah *et al* 2010) cannot differentiate *A. porcinus* from *A. thereius*, because it was developed before the proposal of Figueras *et al* (2017).

This communication reports the first simultaneous isolation of the four zoonotic *Arcobacter* species in Ecuador allowing the identification of swine as one of their reservoir in this country.

Considering the zoonotic, emergent and foodborne character of these bacteria, we believe that it is important to establish their presence in other animal sources as well as in foods of animal origin, environmental waters and humans. This information will contribute to a better understanding of the epidemiology of these emerging foodborne enteropathogens as well as to evaluate the aplication of control actions from a food safety point of view.

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