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SHORT COMMUNICATION

Ruminants are not a reservoir of enteroaggregative *Escherichia coli*

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ABSTRACT. Enteroaggregative *Escherichia coli* (EAEC) causes both acute and persistent diarrhoea among children and adults in developing and developed countries. In addition, the large outbreak of food-borne illness in Europe in the summer of 2011 was caused by a verotoxin-producing EAEC O104:H4 strain. The public health threat posed by EAEC makes it important to identify animal reservoirs that might lead to epidemics in humans. Based on only one study in sheep and a few in cattle, researchers have suggested that EAEC are not found in ruminants. To expand the available data on this question, the present study applied polymerase chain reaction to faecal samples from 920 cattle, sheep and goats to detect EAEC. None of the samples tested positive for any of the EAEC genes tested, suggesting that goats, like cattle and sheep, are unlikely to be a significant reservoir of EAEC.

Key words: enteroaggregative *E. coli*, ruminants.

RESUMEN. Las *Escherichia coli* enteroagregativas (ECEA) causan tanto diarrea aguda como persistente en niños y adultos en países desarrollados y en vías de desarrollo. Además, el gran brote de transmisión alimentaria que sucedió en el verano de 2011 fue causado por una cepa de ECEA O104:H4 productora de verotoxinas. La amenaza a la Salud Pública debida a las ECEA hace importante identificar los reservorios animales a partir de los cuales se podrían originar epidemias o brotes en humanos. Basados en un único estudio en ovejas y unos pocos en vacas, algunos investigadores han sugerido que los ECEA no se aíslan de rumiantes. Para ampliar los datos disponibles acerca de esta cuestión, en este estudio se usó la reacción en cadena de la polimerasa para detectar ECEA en 920 muestras fecales de vacas, ovejas y cabras. Ninguna de las muestras resultó positiva a los genes de ECEA analizados, sugiriendo que las cabras, al igual que las vacas y ovejas, no son probablemente un reservorio importante de ECEA.

Palabras clave: *E. coli* enteroagregativos, rumiantes.

INTRODUCTION

Enteroaggregative *Escherichia coli* (EAEC), a subgroup of diarrhoeagenic *E. coli,* is receiving increasing attention as a cause of watery diarrhoea in humans, which is often persistent (Weintraub 2007). EAEC infection usually occurs sporadically, but some outbreaks involving both children and adults have been described (Scavia *et al* 2008). In addition, the large outbreak of food-borne illness in Europe in the summer of 2011 was caused by a verotoxin (VT)-producing EAEC O104:H4 strain (Bielaszewska *et al* 2011, EFSA 2011, Paddock *et al* 2013).

The defining characteristic of EAEC is their ability to adhere to epithelial cells such as HEp-2 in a "stacked-brick" pattern (Weintraub 2007). Certain EAEC strains carry a large plasmid associated with aggregative adherence (pAA). The pAA plasmid contains several virulence genes, including *aggR*, *aap* and *aat,* but not all EAEC carry the same complement of such genes. While "typical" EAEC strains, such as VT-producing EAEC O104:H4, carry the pAA plasmid or at least the *aggR* gene, "atypical" strains lack the pAA plasmid and the *aggR* gene, though they still adhere to the HEp-2 cells in a stacked-brick pattern (Uber *et al* 2006, Weintraub 2007, EFSA 2011). EAEC appear to have adapted specifically to the human host (Wieler *et al* 2011): typical EAEC have not been found in animals, and atypical EAEC, although identified in calves, piglets and horses (Uber *et al* 2006), are not considered zoonotic pathogens (Okhuysen and DuPont 2010). Nevertheless, the possibility remains that ruminants may be a reservoir of EAEC and a potential source of transmission to humans. In fact, two EAEC outbreaks of gastroenteritis in Italy have been tentatively attributed to a cheese made with unpasteurised sheep's milk (Scavia *et al* 2008).

Epidemiological studies on EAEC from ruminants are scarce (Cassar *et al* 2004, Wieler *et al* 2011, Auvray *et al* 2012, Paddock *et al* 2013) and have important limitations. Most of them identified the presence of EAEC based on only one gene, when several should have been analysed given that EAEC strains can harbor different combinations of genes (Chattaway *et al* 2011). Most studies have focused on VT-producing EAEC O104:H4 from cattle; in contrast, we are aware of only one epidemiological study on sheep as potential faecal carriers of EAEC (Cassar *et al* 2004) and we have not found any such study on goats. To expand the available data about ruminants as potential EAEC reservoirs, the present study analysed a large number of cattle, sheep and goat faecal samples. Our findings should help establish whether ruminants are a potential source of EAEC transmission to humans.

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MATERIAL AND METHODS

Faecal samples were collected between April 2012 and June 2013 from 920 ruminants (400 cattle, 340 sheep and 180 goats) on 46 farms (20 cattle farms, 17 sheep farms and 9 goat farms). Farms were located in Central, Eastern and Southeastern Spain, providing extensive coverage of the country's geography. Samples were collected from 20 animals per farm; the age of sampled cattle was between 1 month and 20 years, while that of sampled sheep and goats was between 1 month and 9 years. Faeces were obtained directly from the rectum using swabs, transferred to tubes containing Amies transport medium (Deltalab, Barcelona, Spain) and shipped on the day of sampling to the laboratory by express mail in refrigerated containers. Samples were processed within 24 h of arrival in the laboratory.

For EAEC detection, faecal samples were plated directly onto MacConkey agar and incubated overnight. Bacterial growth from the first streaked area of the culture plate was tested for the *aat*, *aap* and *aggR* genes by polymerase chain reaction according to Cerna *et al* (2003). As a positive control, the reference EAEC strain 17-2 (O3:H2) was processed in parallel.

RESULTS AND DISCUSSION

To our knowledge, this is the first study of EAEC epidemiology performed on goats and only the second one performed on sheep. None of the 920 faecal samples was positive for *aat*, *aap* or *aggR* genes. These results are consistent with those of Cassar *et al* (2004), who used a pAA EAEC-specific gene probe to test 807 *E. coli* isolates recovered from the faeces of cattle and sheep slaughtered in abattoirs across Great Britain; they failed to find any positive isolate. Similarly, several studies have analysed cattle faecal samples for the presence of several genes associated with the VT-producing EAEC O104:H4 strain and have failed to identify that strain or any other known EAEC strain (Wieler *et al* 2011, Auvray *et al* 2012, Paddock *et al* 2013). Our results confirm these previous studies in cattle and sheep, strongly suggesting that ECEA cannot colonize cattle or sheep. Our results extend the literature by indicating that goats are unlikely to be an EAEC reservoir either. While the available evidence suggests that ruminants are not a relevant reservoir of typical EAEC pathogenic to humans, it remains possible that EAEC can transiently colonize ruminants (Scavia *et al* 2008).

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