LETTERS

DOI: 10.7589/2015-01-024

Journal of Wildlife Diseases, 51(4), 2015, pp. 000–000

Direct Detection of *Escherichia coli* Virulence Genes by Real-Time PCR in Fecal Samples from Bats in Brazil

Adriana Cabal,[1,2] Maria J Pereira,[3,4,5] Ludmilla MS Aguiar,[6] Lucas Domínguez,[1] Carlos Fonseca,[5] Julio Álvarez,[7] Jan F Drexler,[8] and Christian Gortázar[2,9] [1]VISAVET Health Surveillance Centre, Complutense University of Madrid, Avenida Puerta de Hierro s/n 28040 Madrid, Spain; [2]SaBio IREC (CSIC-UCLM-JCCM)Ronda de Toledo s/n 13071 Ciudad Real, Spain; [3]PPGBAN, Department of Zoology, Institute of Biosciences, Federal University of Rio Grande do Sul, Avenida Bento Gonçalves 9500, Porto Alegre Rio Grande do Sul, 91540-000, Brazil; [4]PPGEC, Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, 79090-900, Brazil; [5]Department of Biology and Center for Environmental and Marine Studies, University of Aveiro, Campus Universitário de Santiago, Aveiro 3810-193, Portugal; [6]Department of Zoology and Institute of Biological Sciences, University of Brasília, Campus Darcy Ribeiro, 70.910-900 Distrito Federal, Brazil; [7]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 1352 Boyd Ave., St. Paul, Minnesota, USA; [8]Institute of Virology, University of Bonn Medical Centre, Sigmund-Freud-Str. 25 Bonn 53127, Germany; [9]Corresponding author (email: Christian.Gortazar@uclm.es)

ABSTRACT: Guano samples from 412 Brazilian bats were screened with real-time PCR for the virulence genes (*eae*, *est*, *elt*, *stx*1, *stx*2, *ehx*A, *inv*A, *bfp*A, *agg*R) representing five intestinal pathotypes of *Escherichia coli*. From 82 pooled samples, 22% contained *Escherichia coli* DNA, and *eae*, *est*, *bfp*A, *agg*R were detected.

Bats (Chiroptera) are a taxonomically and ecologically diverse group of animals found in almost all habitats and trophic levels. With 1,152 species of bats currently documented (Reeder and Wilson 2005; Schipper et al. 2008), they are recognized as a natural reservoir for a wide range of viral pathogens, some of which can cause severe disease in humans (e.g., RNA viruses, such as rabies, Ebola, and Nipah viruses; Leroy et al. 2005; Castilho et al. 2010; Kuzmin et al. 2011). In contrast, little is known about bats as carriers of pathogenic bacteria because only a few authors have investigated enteric bacteria, including *Escherichia coli*, in bats (Chaverri 2006; Adesiyun et al. 2009; Oluduro 2012; Muhldorfer 2013).

Escherichia coli is a commensal bacterium found in the intestine of mammals and birds, with a higher prevalence reported in herbivores and omnivores compared with carnivores, marsupials, and bats (Gordon and Cowling 2003). Information concerning the prevalence of *E. coli* in bats is scarce. Previous research has shown that only a low proportion of bats are carrying *E. coli*, which could be due to the lack of a cecum and, thus, microbial fermentation in these animals (Gordon and Cowling 2003). However, *E. coli* has been reported as the cause of urinary tract infection in Vespertilionid bats (Muhldorfer 2013).

To advance epidemiologic knowledge of *E. coli* in bats, we investigated the presence of *E. coli* in guano of Brazilian bats. We captured 412 bats at 27 sampling points in the Serra do Lajeado Environmental Protection Area, Tocantins State, Brazil (10°2′47.79″S, 48°15′45.11″W), using mist-nets. Sampling occurred April–June and October–December in 2012 and 2013 and included three replicates of pristine areas and three replicates of areas subjected to two levels of anthropogenic modification. Mild changes included removal of native trees and planting of trees with economic interest. Areas with severe modifications were characterized by widespread tree removal. Each captured bat was identified and weighed before release. Fecal samples were collected immediately after bats were taken, directly from the animal (no swabs were used). We collected 412 individual fecal samples from 33 species of bats in four families (Table 1), including nectarivores, frugivores, insectivores, and omnivores. Samples

TABLE 1. Bat species (suborder Microchiroptera), feeding habits, and genotypes found in each pool of DNA from samples of guano from bats collected in Tocantins State, Brazil, 2012 and 2013.

Bat family, species	Feeding[a]	No. samples	Pool pattern[b] (No. positive pools)
Phyllostomidae			
Anoura caudifer	N	2	0
Anoura geoffroyi	N	5	*st* (1)
Artibeus concolor	F	2	*agg*R (1), *eae/bfp*A/*st* (1)
Artibeus glaucus	F	2	*agg*R (1)
Artibeus gnomus	F	1	0
Artibeus lituratus	F	8	*bfp*A (1)
Artibeus obscurus	F	3	0
Artibeus planirostris	F	1	0
Artibeus planirostris/ A. fraterculus	F	28	*bfp*A (1), *eae* (2), *eae/bfp*A (1)
Carollia perspicillata	F	286	*eae* (6), *bfp*A (2), *st* (3), *agg*R (1), *eae/bfp*A (2), *eae/bfp*A/*est* (1)
Glossophaga soricina	N	4	*eae* (1)
Lonchophylla dekeyseri	N	4	0
Lonchophylla mordax	N	3	0
Lonchophylla mordax/dekeyseri	N	1	*agg*R (1)
Lonchophylla sp.	N	5	*eae* (1), *eae/bfp*A/*st* (1)
Lonchorhina aurita	I	4	*eae* (1)
Lophostoma carrikeri	I	1	*bfp*A (1)
Lophostoma silvicola	I	2	0
Micronycteris megalotis	I	1	*eae* (1)
Mimon crenulatum	I	4	*eae* (2)
Phyllostomus hastatus	O	3	0
Platyrrhinus helleri	F	8	0
Rhinophylla pumilio	F	2	*eae/bfp*A/*st* (1)
Sturnira lilium	F	3	*eae/bfp*A (1)
Sturnira tildae	F	6	*eae/bfp*A (1)
Tonatia saurophilla	I	1	*eae* (1)
Uroderma bilobatum	F	1	0
Vampirodes caracciolli	F	4	0
Emballonuridae			
Saccopteryx canescens	I	2	*eae/bfp*A (1)
Saccopteryx leptura	I	1	*eae* (1)
Vespertilionidae			
Myotis albescens	I	1	*st* (1)
Myotis nigricans	I	1	0
Myotis riparius	I	3	*st* (1)
Rhogeessa io	I	1	*eae* (1)
Mormoopidae			
Pteronotus parnellii	I	4	*eae* (1)
Unknown		4	0
Total		412	

[a] N = nectarivore, F = frugivore, I = insectivore, O = omnivore.

[b] The pool pattern can be duplicated because different species comprise each pool.

were preserved in 500 μL of RNAlater RNA stabilization reagent (QIAGEN, Lisbon, Portugal), stored at 4 C, and analyzed 6–12 mo after collection. We extracted DNA from all samples individually with a commercial kit, and 20 μL of each were pooled to a final volume of 100 μL in each pool (belonging to different species and sampling points).

The DNA was analyzed by real-time PCR in pools ($n=82$) for nine *E. coli* virulence genes (VGs) characteristic of five pathotypes

TABLE 2. Prevalence (95% confidence intervals [CI]) of single virulence genes and patterns detected by real-time PCR of pools of DNA from samples of guano from bats collected in Tocantins State, Brazil, 2012 and 2013.

Target	Positive pools (n=82)	% (exact 95% CI)
*uid*A	18	4.84 (2.87–7.55)
Eae	16	4.25 (2.43–6.82)
*bfp*A	5	1.25 (0.41–2.89)
est (ST)	2	0.49 (0.06–1.77)
*agg*R	1	0.25 (0.01–1.36)
*ehx*A	0	0
*stx*1	0	0
*stx*2	0	0
*inv*A	0	0
elt (LT)	0	0
*eae/bfp*A	3	0.74 (0.15–2.15)
eae/bfpA/est	1	0.25 (0.01–1.36)

affecting humans (enterohemorrhagic *E. coli* [EHEC], enteroaggregative *E. coli* [EAEC], enteropathogenic *E. coli* [EPEC], enteroinvasive *E. coli* [EIEC], and enterotoxigenic *E. coli* [ETEC]) using previously published primers (Cabal et al. 2013) and newly designed TaqMan probes (available under request). The VGs associated with each pathotype were *stx*1, *stx*2, *eae*, and *ehx*A for EHEC; *agg*R for EAEC, *elt*, and *est* for ETEC; *eae* and *bfp*A for EPEC; and *Inv*A for EIEC. A genus-specific gene (*uid*A) was also included in the PCR assay to estimate the prevalence (WINPEPI updated, version 11.35) of total *E. coli* in each pooled sample. Analysis was conducted using WINIPEPI version 11.35 (Abramson 2011). No culture isolation methods were applied.

Of 82 pooled samples, 18 (22%) were positive for *E. coli* (*uid*A gene), but four VGs characteristic of EPEC, ETEC, and EAEC pathotypes were also detected (Table 2). One sample positive for *bfp*A was sequenced and confirmed as *E. coli* O157:H45.

Our results (22% positive) were in agreement with those from other authors who reported 11–28% of positive samples (Pinus and Muller 1980; Adesiyun et al. 2009; Apun et al. 2011). In addition, we showed that some VGs (*eae*, *bfp*A, *est*, *agg*R) were present in bat feces, suggesting the presence of pathogenic *E. coli*. The pathotypes present in guano may include EAEC, typical EPEC, and ETEC strains. No indications of STEC or EIEC-associated VGs were seen. This supports Apun et al. (2011) who found no EHEC-positive samples in bats. In the literature, there are no animal reservoirs reported for other pathotypes different from STEC, but previously (Cabal et al. 2013), we showed that typical human *E. coli* VGs might be more widespread in animals than commonly believed. In addition, the sequenced DNA product matching an O157:H45 strain in one pooled sample suggested the presence of atypical EPEC in bats. This serotype was described as a causative agent of enterocolitis and sporadic diarrhea in humans (Stephan et al. 2004). Detection of multiple VGs within pooled samples does not imply that a single bacterium is carrying them simultaneously. In addition, it is unknown whether bats were infected with pathogenic *E. coli* or were temporary carriers of these pathogens. It is possible that, because of their feeding habits and the physiology of their digestive tract (Gordon and Cowling 2003), bats may become transiently infected by using farms or urban areas as foraging grounds (Chaverri 2006).

Although plating of the VG-positive samples will be necessary, isolation might be complicated, given the low proportion of VGs. Therefore, the possibility of bats being infected with pathogenic *E. coli* cannot be excluded, and further research is needed to elucidate the role bats may have in the epidemiology of human infections with important pathovars of *E. coli*.

Bats were collected under permits from the Brazilian Ministry of Science and Technology (343, 2012), Sisbio (33542, 2012) and Naturatins (1633, 2012). We thank K. VanderWaal for her contribution to the manuscript.

LITERATURE CITED

Abramson JH. 2011. WINPEPI updated: Computer programs for epidemiologists, and their teaching potential. *Epidemiol Perspect Innov* 8:1. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3041648/pdf/1742-5573-8-1.pdf. Accessed June 2015.

Adesiyun AA, Stewart-Johnson A, Thompson NN. 2009. Isolation of enteric pathogens from bats in Trinidad. *J Wildl Dis* 45:952–961.

Apun K, Kho KL, Chong YL, Hashimatul FH, Abdullah MT, Rahman, MA, Lesley MB, Samuel L. 2011. Detection of *Escherichia coli* O157:H7 in wildlife from disturbed habitats in Sarawak, Malaysia. *Res J Microbiol* 6:132–139.

Cabal A, Gomez-Barrero S, Porrero C, Barcena C, Lopez G, Canton R, Gortazar G, Dominguez L, Alvarez J. 2013. Assessment of virulence factors characteristic of human *Escherichia coli* pathotypes and antimicrobial resistance in O157:H7 and non-O157:H7 isolates from livestock in Spain. *Appl Environ Microbiol* 79:4170–4172.

Castilho JG, Carnieli P Jr, Oliveira RN, Fahl WO, Calvacante R, Santana AA, Rosa WL, Carrieri ML, Kotait I. 2010. A comparative study of rabies virus isolates from hematophagous bats in Brazil. *J Wildl Dis* 46:1335–1339.

Chaverri G. 2006. Aerobic bacterial flora from the digestive tract of the common vampire bat, *Desmodus rotundus* (Chiroptera: Phyllostomidae). *Rev Biol Trop* 54:717–724.

Gordon DM, Cowling A. 2003. The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: Host and geographic effects. *Microbiology* 149:3575–3586.

Kuzmin IV, Bozick B, Guagliardo SA, Kunkel R, Shak JR, Tong S, Rupprecht CE. 2011. Bats, emerging infectious diseases, and the rabies paradigm revisited. *Emerg Health Threats J* 4:7159.

Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Delicat A, Paweska JT, Gonzalez JP, Swanepoel R. 2005. Fruit bats as reservoirs of Ebola virus. *Nature* 438:575–576.

Muhldorfer K. 2013. Bats and bacterial pathogens: A review. *Zoonoses Public Health* 60:93–103.

Oluduro AO. 2012. Antibiotic-resistant commensal *Escherichia coli* in faecal droplets from bats and poultry in Nigeria. *Vet Ital* 48:297–308.

Pinus M, Müller HE. 1980. Enterobacteria of bats (Chiroptera).*Zentralbl Bakteriol A* 247:315–322. [In German.]

Reeder DM, Wilson DE. 2005. *Mammal species of the world: A taxonomic and geographic reference*. 3rd ed. Johns Hopkins University Press, Baltimore, Maryland, 2142 pp.

Schipper J, Chanson JS, Chiozza F, Cox NA, Hoffmann M, Katariya V, Lamoreux J, Rodrigues ASL, Stuart SN, Temple HJ, et al. 2008. The status of the world's land and marine mammals: Diversity, threat, and knowledge. *Science* 322:225–230.

Stephan R, Borel N, Zweifel C, Blanco M, Blanco J. 2004. First isolation and further characterization of enteropathogenic *Escherichia coli* (EPEC) O157: H45 strains from cattle. *BMC Microbiol* 4:10.

Submitted for publication 30 January 2015.
Accepted 23 April 2015.