Austral J Vet Sci **54, 55-63 (2022)**

ORIGINAL ARTICLE

Captive collared peccary carries ESBL-producing diarrheagenic *Escherichia coli* pathotypes

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ABSTRACT. Collared peccaries (*Pecari tajacu*) roam the forests of natural areas in America. Wild collared peccary appears to be a carrier for bacteria associated with infections in humans and animals, however, the presence of diarrheagenic *E. coli* (DEC) pathotypes has not been studied in the captive collared peccary. This study aimed to study the prevalence of DEC, the susceptibility to antibiotics, and the frequency of β-lactamase genes (ESBL) in captive collared peccary faeces. DEC strains were identified in 44.4% (N=56) of the *E. coli*-carrying peccaries under study. The following DEC strains were identified: ETEC (35.7%), EAEC (28.6%), STEC (21.4%) and EPEC (14.3%). Most of the identified DEC strains belonged to clade I (58.9%). The genetic marker *rfbO157* was not found in any STEC strain. Of the DEC strains, 67.9% (N=38) were considered multidrug resistant and were not susceptible to ampicillin (75%) nor to carbenicillin (51.8%). The combination of the genes blaTEM + blaCTX and blaTEM + blaSHV (6 strains respectively) was the most frequent among the DEC strains. It is concluded that captive collared peccaries are carriers of DEC strains that carry β-lactamase blaTEM, blaCTX and blaSHV genes and are not susceptible to ampicillin. Given the current efforts of the Wildlife Management Units (WMU) to reintroduce the collared peccary into natural areas, these captive collared peccaries act as carriers of diarrheagenic *E. coli* strains and therefore a potential source of gastrointestinal disease in humans and animals.

Key words: β-lactamase, carrier, diarrheagenic pathotypes, *Pecari tajacu*, wildlife management Unit.

INTRODUCTION

The collared peccary *Pecari tajacu* is a gregarious species distributed from the southeast of the United States to Argentina. It is considered a predator and a seed disperser in tropical forests, deserts, and deforested areas (Marinho *et al.*, 2019). Currently, the International Union for the Conservation of Nature (IUCN2019-1) and the Official Mexican Standard NOM-059-SEMARNAT-2010 (PROFEPA 2010) classify peccaries as a species of least concern (LC).

Collared peccaries roam the jungles and forests of Mexico. They are thus vulnerable to hunting and habitat destruction. Wildlife Management Units (WMU) have developed several strategies for the sustainable management of the species for the purpose of conservation, recovery, reproduction, and reintroduction (Sisk *et al.*, 2007). Previous studies have shown that wild collared peccaries can carry bacteria and viruses associated with infections in humans and domestic animals, such as *Clostridium perfringens* type A and multiple serotypes of *Salmonella* spp. (Shender *et al.*, 2009), shiga toxin-producing *Escherichia coli* (Jay-Russell *et al.*, 2014), *Mycobacterium hyopneumoniae*, *Pasteurella multocida*, circovirus type 2 (PCV2) and herpesvirus type 1 (SuHV) (de Castro *et al.*, 2014). The prevalence of *Leptospira* spp. (78%) in collared peccaries was recently reported, the most frequent serovars are *bratislava*, *grippotyphosa*, *icterohaemorrhagiae* and *pomona* (Montenegro *et al.*, 2018). Collared peccaries have also been found to harbour classical swine fever virus (5%), porcine circovirus type 2 (7%) and vesicular stomatitis (33%). They also share pathogens with domestic and wild pigs (*Sus scrofa*) (Montenegro *et al.*, 2018; Molina-Barrios *et al.*, 2018).

Although some efforts have been made to understand the role of captive collared peccaries as pathogen carriers (de Carvalho *et al.*, 2011), their role as carriers of diarrheagenic *Escherichia coli* (DEC) pathotypes is still unknown. The DEC group includes enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC), DEC pathotypes are associated with gastrointestinal infections in humans and animals (Croxen *et al.*, 2013; Shabana *et al.*, 2013; Tamayo *et al.*, 2021). Enterohaemorrhagic *E. coli* (EHEC) is a subgroup of STEC strains associated with diarrhoea and haemorrhagic colitis, occasionally progressing to haemolytic uremic syndrome (HUS), which can have serious consequences in humans, including death (Oh *et al.*, 2016). *Escherichia coli* can contain several antibiotic resistance genes, including those that encode extended spectrum β-lactamases (ESBL) (Faridah

Received: 20.10.2021.

Accepted: 12.01.2022.

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et al., 2020). Therefore, understanding the mechanisms of *E. coli* dissemination is a necessary step in finding a solution to the growing worldwide incidence of multidrug resistant (MDR) and extensively resistant (XDR) bacterial strains (Taghadosi *et al.*, 2019).

This study aimed to identify the role of captive collared peccaries as a carrier of ESBL-producing diarrheagenic *Escherichia coli* pathotypes. This would allow designing sanitary measures to mitigate the potential of collared peccaries to spread pathogens of public health importance as a consequence of their management and a possible reintroduction to natural areas. The objective of this research was to study the prevalence of diarrheagenic *Escherichia coli* pathotypes that colonise the intestine of collared peccaries (*Pecari tajacu*) in captivity. In addition, this study aimed to find out if the DEC strains identified in captive collared peccaries are associated with antibiotic resistance and, if so, the genes involved. To do this, the susceptibility of DEC strains to commonly used antibiotics and the frequency of genes that encode extended spectrum β-lactamases (ESBL) were determined.

MATERIAL AND METHODS

PECCARY SAMPLING

Between the autumn of 2017 and the summer of 2019, 140 clinically healthy collared peccaries with no record of antibiotic therapy were selected among a group kept in 11 intensive wildlife management units (WMU) in the state of Chiapas, Mexico. The specimens of these WMU were obtained by purchase, donation, under protection or loan, and their origin may be wild or captivity. Animal studies were approved by the Ethics Committee of the University of Sciences and Arts of the state of Chiapas (approval #049/02-2018).

In the WMU, an average of 25 peccaries were kept in roofless cages averaging 8 x 24 meters in size, with floors made of dirt, logs or twigs, and walls commonly made of stone and steel mesh. Their daily diet consisted mainly of seasonal agricultural waste and food waste from homes, restaurants and markets: ears of corn, plant waste such as rhizomes (ginger, sugar cane, bamboo, among others), tubers (potato, cassava, sweet potato, carrot, jicama, radishes, among others), bulbs (onion, leek, garlic, among others) and fruits (cucumber, tomato, papaya, watermelon, among others); water was supplied *ad libitum* in concrete or wood drinking troughs.

ISOLATION OF *E. COLI* AND IDENTIFICATION OF DEC STRAINS

The peccaries were transferred to a wooden corridor (average measurements: 1 m high, 1.20 m long and 60 cm wide) with a liftgate at each end to carry out the sampling. Faeces samples were collected by introducing a swab into the rectum of the animal, and then into agar gel medium or Stuart transport medium (Copan Diagnostics, Inc.) for transfer to the laboratory. The swabs were inoculated onto Eosin Agar and Methylene Blue (BD-BBI™) plates and vatted at 37 °C for 24 hours. Up to 10 lactose fermenting colonies were selected and analysed by standard biochemical tests. Plates of MacConkey Agar with sorbitol were used simultaneously to promote the growth of EHEC serotype O157: H7. The genetic identification of the *E. coli* strains was carried out by amplifying the *uid*A gene (Tsai *et al.*, 1993).

IDENTIFICATION OF DIARRHEAGENIC PATHOTYPES OF *E. COLI* BY POLYMERASE CHAIN REACTION (PCR)

In the PCR, the *E. coli* strain ATCC® 25922 TM was used as a negative control. The following strains were used as positive control: STEC EDL933 (O157: H7), EAEC 042 (044: H18), ETEC H10407 (O78: H11), EPEC E2348-69 (O127: H6) and EIEC E11 (O124NM). The strains were provided by Dr. Teresa Estrada García from CINVESTAV, Mexico, and were kept in the bacteriological collection of the University of Sciences and Arts of Chiapas (Universidad de Ciencias y Artes) (Gutiérrez-Jiménez *et al.*, 2015). To obtain the DNA, the selected colonies were suspended in 1 mL of deionized water and the preparation was boiled for 10 min. The suspension was centrifuged at 10,000 rpm for 5 min and the supernatant containing DNA was removed and stored at -80 °C. Specific primers were used to amplify the Enteroaggregative *E. coli* (EAEC) *aap*, *agg*R, and *AAprobe* genes using the technique described by Cerna (Cerna *et al.*, 2003).

For Shiga toxin-producing *E. coli* (STEC) (*stx*1 and *stx*2 genes), Enteropathogenic *E. coli* (EPEC) (*bfp*A and *eae*A genes), Enterotoxigenic *E. coli* (ETEC) (*lt* and *st* genes), and Enteroinvasive *E. coli* (EIEC) (*ial* gene), the targets and conditions used were those previously described (López-Saucedo *et al.*, 2003). Shiga toxin-producing *E. coli* O157 was studied by amplifying the *rfb* gene (specific O-polysaccharide) (Paton & Paton, 1998).

The *E. coli* strains were classified by quadruplex PCR into seven phylogroups: A, B1, B2, C, D, E, F, and the crypto Clado I, based on the presence or absence of the genes *chuA*, *yjaA*, *arpA* and *trpA*, as well as the TSPE4 C2 DNA fragment (Clermont *et al.*, 2013). The amplification of the extended spectrum β-lactamase genes *blaTEM*, *blaSHV*, *blaCTXM*, *blaOXA* and *blaCMY* was carried out using the primers and conditions previously reported (Ahmed *et al.*, 2007). Table 1 shows the sequences of the primers and the PCR conditions used in this study.

ANALYSIS OF ANTIMICROBIAL SUSCEPTIBILITY

The following antimicrobial susceptibility discs (BD BBL™ Sensi-Disc™) were used with first-line antibiotics commonly used of different antimicrobial categories: β-lactam: ampicillin (AMP; 10 µg), carbenicillin (CAR;

Table1. Primers used in this study.

Primer pair	Sequence (5'-3')	Target	Encoded protein	Size (bp)	Reference
uidAF	AAAACGGCAAGAAAAAGCAG	*uid*A	β-glucuronidase	147	(Tsai *et al.*, 1993)
uidAR	ACGCGTGGTTAACAGTCTTGCG				
aapF	CTTGGGTATCAGCCTGAATG	aap	Aggregative adherence fimbria	310	(Cerna *et al.*, 2003)
aapR	AACCCATTCGGTTAGAGCAC				
aggF	CTAATTGTACAATCGATGTA	*agg*R	Transcriptional activator	457	(Cerna *et al.*, 2003)
aggR	AGAGTCCATCTCTTTGATAAG				
AA probeF	CTGGCGAAAGACTGTATCAT	AA probe	Anti-aggregation protein transporter	629	(Cerna *et al.*, 2003)
AA probeR	CAATGTATAGAAATCCGCTGTT				
ltFf	GGCGACAGATTATACCGTGC	lt	Heat-labile enterotoxin	450	(López-Saucedo *et al.*, 2003)
ltRr	CGGTCTCTATATTCCCTGTT				
stFf	ATT TTTCTTTCTGTATTGTCTT	st	Heat-stable enterotoxin	190	(López-Saucedo *et al.*, 2003)
stRr	CACCCGGTACAAGCAGGATT				
bfpAf	AATGGTGCTTGCGCTTGCTGC	*bfp*A	Bundle-forming pili	324	(López-Saucedo *et al.*, 2003)
bfpAr	GCCGCTTTATCCAACCTGGTA				
eaeAf	GACCCGGCACAAGCATAAGC	*eae*A	Structural gene for intimin	384	(López-Saucedo *et al.*, 2003)
eaeAr	CCACCTGCAGCA ACA AGA GG				
Ialf	GGT ATG ATG ATG ATG AGT CCA	ial	Invasion-associated locus	650	(López-Saucedo *et al.*, 2003)
Ialr	GGA GGC CAA CAA TTA TTT CC				
Stx1F	CTGGATTTAATGTCGCATAGTG	*stx*1	Shiga toxin 1	150	(López-Saucedo *et al.*, 2003)
Stx1R	AGAACGCCCACTGAGATCATC				
Stx2F	GGCACTGTCTGAAACTGCTCC	*stx*2	Shiga toxin 2	255	(López-Saucedo *et al.*, 2003)
Stx2R	TCGCCAGTTATCTGACATTCTG				
O157F	CGGACATCCATGTGATATGG	rfbO157	Polisacárido específico O	259	(Paton & Paton, 1998)
O157R	TTGCCTATGTACAGCTAATCC				
chuA.1b	ATGGTACCGGACGAACCAAC	*chu*A	Membrane hemin receptor	288	(Clermont *et al.*, 2013)
chuA.2	TGCCGCCAGTACCAAAGACA				
yja.1b	CAAACGTGAAGTGTCAGGAG	*yja*A	Stress response protein	211	(Clermont *et al.*, 2013)
yja.2b	AATGCGTTCCTCAACCTGTG				
ArpAgpE.f	GATTCCATCTTGTCAAAATATGCC	*arp*A	Regulator of acetyl CoA synthetase	301	(Clermont *et al.*, 2013)
ArpAgpE.r	GAAAAGAAAAAGAATTCCCAAGAG				
trpAgpC.1	AGTTTTATGCCCAGTGCGAG	*trp*A	Operon leader peptide	219	(Clermont *et al.*, 2013)
trpAgpC.2	TCTGCGCCGGTCACGCCC				
trpBA.f	CGGCGATAAAGACATCTTCAC	*trp*A (control interno)	Operon leader peptide	489	(Clermont *et al.*, 2013)
trpBA.r	GCAACGCGGCCTGGCGGAAG				
TSPE4C2 f	CACTATTCGTAAGGTCATCC	TspE4.C2	Anonymous DNA fragment	152	(Clermont *et al.*, 2013)
TSPE4C2 r	AGTTTATCGCTGCGGGTCGC				
blaTEMf	ATAAAATTCTTGAAGACGAAA	*bla*TEM	beta-lactamase TEM	1080	(Ahmed *et al.*, 2007)
blaTEMr	GACAGTTACCAATGCTTAATC				
blaSHVf	TTATCTCCCTGTTAGCCACC	*bla*SHV	beta-lactamase SHV	795	(Ahmed *et al.*, 2007)
blaSHVr	GATTTGCTGATTTCGCTCGG				
blaCTXM f	CGCTTTGCGATGTGCAG	*bla*CTXM	beta-lactamase CTX M	550	(Ahmed *et al.*, 2007)
blaCTXM r	ACCGCGATATCGTTGGT				
blaOXAf	TCAACTTTCAAGATCGCA	*bla*OXA	beta-lactamase OXA	591	(Ahmed *et al.*, 2007)
blaOXAr	GTGTGTTTAGAATGGTGA				
blaCMYf	GACAGCCTCTTTCTCCACA	*bla*CMY	beta-lactamase CMY	1000	(Ahmed *et al.*, 2007)
blaCMYr	TGGAACGAAGGCTACGTA				

100 µg) and oxacillin (OXA; 1 µg); aminoglycosides: amikacin (AMK; 30 µg), netilmicin (NET; 30 µg) and gentamicin (GEN; 10 µg); cephalosporins: cephalothin (CEF; 30 µg) and cefotaxime (CTX; 30 µg); quinolones: ciprofloxacin (CIP; 5 µg) and norfloxacin (NOR; 10 µg); phenicols: chloramphenicol (CHL; 30 µg); folate inhibitors: trimethoprim-sulfamethoxazole (SXT; 25 µg); furans: nitrofurantoin (NIT; 300 µg); Tetracyclines: Tetracycline (TET; 30 µg). β-lactam-resistant strains were subsequently analysed using the disc diffusion method with amoxicillin-clavulanic acid discs (AMC; 20/10 µg). The antibiotics and the disk diffusion method were used following the recommendations of the Clinical and Laboratory Standards Institute (Wayne, 2020). The *E. coli* strain ATCC® 25922 TM was used as a negative control and STEC EDL933 (O157: H7) as the positive control. *Escherichia coli* strains (including intermediate and resistant phenotypes) not susceptible to at least three antibiotics and from different antimicrobial categories were classified as multidrug resistant strains (MDR), while strains not susceptible to at least one antibiotic and belonging to each of the tested antimicrobial categories were classified as Extensively Drug-Resistant (XDR) (Magiorakos *et al.*, 2012).

STATISTICAL ANALYSIS

The prevalence of *E. coli* pathotypes, identified phylogenetic groups, resistance to antibiotics and the frequency of ESBL genes were analysed using descriptive statistics. The proportion of molecular markers among DEC strains was determined by binomial test. The two-tailed Fisher's exact test was used to test for associations between the categorical variables (when the expected frequencies were under 5), with a significance level of $P<0.05$. The statistical analysis was carried out using the IBM SPSS statistical package (Chicago SPSS Inc).

RESULTS

IDENTIFICATION OF DIARRHEAGENIC *E. COLI*

One hundred and twenty-six (90%) strains of *E. coli* were isolated from faecal samples of 140 captive collared peccaries. DEC strains were identified in 44.4% (N = 56) of the peccaries carrying *E. coli*. Among the DEC strains, ETEC (35.7%) was the category with the highest prevalence, followed by EAEC (28.6%), STEC (21.4%) and EPEC (14.3%). The PCR analysis showed a statistically significant prevalence for the ETEC and EAEC categories. No EHEC strain was detected on MacConkey/sorbitol agar plates, these strains did not carry the *eae* and *hlyA* genes, and the genetic marker *rfbO157* was not amplified by PCR in any of the STEC strains (table 2).

PCR was used to determine the phylogenetic group of DEC strains isolated in captive collared peccary. Among the identified strains of *E. coli* (N= 56), the highest number of DEC strains were grouped in Clade I (58.9%), followed by the phylogroup B2 (10.7%) and A (8.9%). Some DEC strains could not be assigned to any known phylogroup (21.4%).

Table 2. DEC strains and their virulence genes isolated in *Pecari tajacu* from Chiapas, Mexico.

Faeces samples with DEC pathotype: % (N)	Virulence gene: % (N)	P
ETEC: 35.7 (20)	*lt*, *st*: 30.3 (17)	0.001
	st: 3.6 (2)	
	lt: 1.8 (1)	
EAEC: 28.6 (16)	*aap*, AA probe: 25 (14)	0.01
	AA probe: 3.6 (2)	
STEC: 21.4 (12)	*stx*1: 12.5 (7)	0.08
	*stx*1 *stx*2: 8.9 (5)	
EPEC: 14.3 (8)	*bfpA*, *eaeA*: 10.7 (6)	0.15
	eaeA: 3.6 (2)	
Total: 100 (56)		

ANTIMICROBIAL SUSCEPTIBILITY OF DEC STRAINS

Three-thirds of the DEC strains were not susceptible to the antibiotic ampicillin (75%), while half of the strains were not susceptible to carbenicillin (51.8%). Resistance to ampicillin was also found in EAEC (81.3%), ETEC (80%), EPEC (62.5%) and STEC (50%) (table 3). Among the DEC strains not susceptible to ampicillin, 88.1% (N= 37) were susceptible to the antibiotic amoxicillin-clavulanic acid. Sixty-seven-point nine per cent (67.9%; N = 38) of the DEC strains were not susceptible to at least one antibiotic in three different categories of antimicrobials; thereby, these strains were considered Multidrug Resistant (MDR). No DEC strain was classified as Extensively Drug-Resistant (XDR). In general, most of the strains were susceptible mainly to chloramphenicol (94.6%), gentamicin (92.9%), amoxicillin-clavulanic acid (91.1%), sulfamethoxazole trimethoprim (89.3%) and tetracycline (87.5%).

The PCR was used to determine the frequency of genes encoding β-lactamase in the 42 DEC strains not susceptible to ampicillin. The combination of the genes blaTEM + blaCTX and blaTEM + blaSHV (6 strains respectively) was the most frequent among the DEC strains, followed by the combination of the blaTEM and blaCTX genes (4 strains respectively). The blaTEM (N=3) and blaCTX (N=3) genes were amplified more frequently among ETEC strains not susceptible to ampicillin. In the EAEC strains, both blaTEM + blaCTX genes were amplified (N=3). In STEC strains susceptible to ampicillin, both blaTEM + blaCTX and blaTEM + blaSHV genes were amplified (2 strains, respectively) (table 4).

Table 3. Antimicrobial non-susceptibility of DEC strains (56).

Antimicrobial	Diarrheagenic *Escherichia coli* pathotypes % non-susceptibility (n)				
	DEC	ETEC	EAEC	STEC	EPEC
	(N=56)	(N=20)	(N=16)	(N=12)	(N=8)
AMP	75 (42)	80 (16)	81.3 (13)	50 (6)	62.5 (5)
AMC	8.9 (5)	10 (2)	0	16.7 (2)	12.5 (1)
CAR	51.8 (29)	60 (12)	37.5 (6)	50 (6)	62.5 (5)
OXA	10.7 (6)	15 (3)	6.3 (1)	16.7 (2)	0
AMK	12.5 (7)	5 (1)	12.5 (2)	25 (3)	12.5 (1)
GEN	5.4 (3)	5 (1)	6.3 (1)	8.3 (1)	0
NET	33.9 (19)	45 (9)	31.3 (5)	25 (3)	25 (2)
CEF	10.7 (6)	5 (1)	12.5 (2)	16.7 (2)	25 (2)
CTX	32.1 (18)	30 (6)	31.3 (5)	25 (3)	50 (4)
CIP	37.5 (21)	40 (8)	50 (8)	33.3 (4)	12.5 (1)
NOR	30.4 (17)	35 (7)	37.5 (6)	25 (3)	12.5 (1)
CHL	3.6 (2)	10 (2)	0	8.3 (1)	12.5 (1)
SXT	5.4 (3)	5 (1)	0	8.3 (1)	12.5 (1)
NIT	12.5 (7)	5 (1)	12.5 (2)	25 (3)	12.5 (1)
TET	8.9 (5)	5 (1)	6.3 (1)	25 (3)	0

AMP; Ampicillin, AMC; Amoxicillin-clavulanic acid, CAR; Carbenicillin, OXA; Oxacillin, AMK; Amikacin, GEN; Gentamicin, NET; Netilmicin, CEF; Cephalotin, CTX; Cefotaxime, CIP; Ciprofloxacin, NOR; Norfloxacin, CHL; Chlorampphenicol, SXT; Trimethoprim-sulfamethoxazole, NIT; Nitrofurantoin, TET; Tetracycline.

Table 4. Genes of extended spectrum β-lactamase producing DEC strains isolated in collared peccary.

DEC groups (n)	Non susceptible profile	β-lactamase gene	
	AMP	Gene	Number of isolates
DEC (56)	42	blaTEM	4
		blaCTX	4
		blaTEM+blaCTX	6
		blaTEM+blaSHV	6
ETEC (20)	16	blaTEM	3
		blaCTX	3
		blaTEM+blaSHV	2
EAEC (16)	13	blaTEM+blaCTX	3
		blaTEM	1
		blaTEM+blaSHV	1
STEC (12)	6	blaTEM+blaCTX	2
		blaTEM+blaSHV	2
EPEC (8)	5	blaCTX	1
		blaTEM+blaCTX	1
		blaTEM+blaSHV	1

DISCUSSION

In several countries of the Americas, sustainable strategies for the conservation, reproduction, and reintroduction of collared peccary have been proposed through Wildlife Management Units (WMU) (Sisk *et al.*, 2007). The present study showed that 44.4% of the *E. coli* strains isolated in collared peccary faeces kept in WMU are carriers of genes that encode virulence factors of diarrheagenic pathotypes, mainly ETEC (*lt* and *st*) and EAEC (*aap* and AA probe), followed by STEC non-O157 (*stx*1 and *stx*2) and EPEC (*bfp*A and *ea*eA). The DEC virulence is mainly based on the ability to produce isoforms of the encoded protein related to infections in humans (Taghadosi *et al.*, 2018; Angulo *et al.*, 2021; Alfinete *et al.*, 2022). These results are consistent with previous studies demonstrating that the wild collared peccary is a carrier of bacteria associated with humans and domestic animals. In the southern areas of the United States, isolates of *Clostridium perfringens* type A and multiple Salmonella serotypes were reported in wild *Pecari tajacu* (Shender *et al.*, 2009), in addition to other possible Salmonella and STEC non-O157 serotypes (Jay-Russell *et al.*, 2014). Other bacteria have been reported in the collared peccary, such as *Mycoplasma hyopneumoniae*, *P. multocida* (de Castro *et al.*, 2014), *Brucella* and different serovars of *Leptospira* (Mendoza *et al.*, 2007; Montenegro *et al.*, 2018). However, to date there are no reports on the prevalence of diarrheagenic pathotypes of *E. coli* in captive collared peccary.

The present study demonstrated a higher prevalence of virulence genes of DEC pathotypes in captive collared peccaries compared with the prevalence of *stx1*, *eaeA* and *hlyA* showed in a study conducted on wild collared peccaries (Jay-Russell *et al.*, 2014). This suggests that captive peccaries are more exposed to these pathogens. Wild peccaries may have suitable environmental conditions such as the type of habitat and availability of food resources (Hernández-Pérez, 2019). The population density of wild peccaries is also much lower. In the WMU containing collared peccary, food and vegetables are frequently introduced and it cannot be ruled out that plant material can spread diarrheagenic *E. coli* strains; there is evidence that these products can be a reservoir of multidrug resistant bacteria harbouring antibiotic resistance and virulence genes (Richter *et al.*, 2021).

The presence of livestock also plays an important role in the dissemination of this bacteria to the environment and could be the main link in the contamination route to collared peccaries. There is evidence of domestic animals acting as carriers of strains of DEC mainly cattle (Gutema *et al.*, 2021), sheep, and goats (Shabana & Al-Enazi, 2020), birds (Kimura *et al.*, 2021) and pigs (Misumi *et al.*, 2021). In Mexico, DEC strains have been reported in humans and animals (Rivas-Ruiz *et al.*, 2020; Tamayo-Legorreta *et al.*, 2020). In southeastern Mexico, where the WMU of the collared peccaries under study are located, this type of DEC strains have been reported in captive green iguanas (Bautista-Trujillo *et al.*, 2020).

A PCR-based phylogenetic group assay was performed as proposed by Clermont *et al.* (2013). The highest frequencies of the ETEC, EAEC, STEC stx1 and STEC stx2 pathotypes, as well as EPEC, were found in Clade I, with lower frequencies in phylogroups B2 and A. Some strains could not be assigned to a known clade. A previous study on the characterisation of *E. coli* isolated in wastewater found the highest frequency of EPEC in phylogroup B2, followed by B1, C and A, while STEC strains were grouped in phylogroup B1 (Cho *et al.*, 2018). Clonal lineage strains in diarrheic poultry, in Tunisia, which could constitute a risk of their transfer to healthy animals and humans, enteropathogenic (EPEC) and extraintestinal (ExPEC) infections mainly belonging to the phylogroups B2 (Jouini *et al.*, 2021). The concentration of environmental strains into different groups could be explained by the phylogenetic variability of *E. coli*, based on their genetic content and diverse lineage. A consequence of this is the difficulty to associate pathogenic strains and commensals to a specific phylogroup, which is why the use of molecular techniques with greater clonal discrimination power for DEC strains such as MLST and PFGE is suggested (Su *et al.*, 2021).

In the present study, most (75%) of the DEC strains isolated in captive collared peccary were not susceptible to ampicillin, while 51.8% were not susceptible to carbenicillin. Overall, more than half of the DEC strains were not susceptible to at least one antibiotic from three different categories tested (MDR), mainly beta-lactams, aminoglycosides, cephalosporins, fluoroquinolones and nitrofurantoin. Resistance to beta-lactams such as ampicillin was observed in DEC isolated from canaries (Kimura *et al.* 2021), pigs (Misumi *et al.*, 2021) and bovines, cow meat and humans (Gutema *et al.*, 2021). In Mexico, multidrug resistant *E. coli* infections are a common occurrence, and the incidence of DEC strains resistant mainly to ampicillin and tetracycline has been reported in bovine faeces (Navarro *et al.*, 2018), beef and pork (Martínez-Vázquez *et al.*, 2018), as well as in humans (Castro *et al.*, 2019). There is evidence that the constant oral-faecal transmission of antibiotic-resistant *E. coli* between animals, humans, and the environment, favours the horizontal transfer of resistance genes between different microorganisms sharing the same niche (Abdel-Rhman *et al.*, 2021; Puvača & Frutos, 2021). This could be the hypothesis of a possible route of dissemination of multidrug resistant DEC strains among captive collared peccaries; however, further research is required.

Other antibiotic resistance genes have been reported in *E. coli* from livestock such as those from the polymyxins family (Ilbeigi *et al.*, 2021), however, efficacy is poorly understood in the veterinary practice in Mexico (Martínez *et al.*, 2020). In the present study, most of the DEC strains isolated in the captive collared peccary were not susceptible to beta-lactams. The presence of genes coding

for β-lactamase was evidenced in DEC strains isolated in captive collared peccary, mainly blaTEM, blaCTX and blaSHV. These findings are consistent with those reported by other authors who found the blaTEM, blaCTX and blaSHV genes in multidrug-resistant diarrheagenic *E. coli* strains in humans and animals (Quino *et al.*, 2020; Shafiq *et al.*, 2021). The results confirm that captive collared peccaries can act as carriers of multiresistant *Escherichia coli* containing genes associated with ESBL. Further research is required to make a genetic characterization of *bla* profiles and carbenicillin-hydrolyzing ß-lactamases.

This study reported the presence of DEC in captive collared peccaries. The ETEC, EAEC, STEC stx1, STECstx1 stx2 and EPEC pathotypes, carrying ESBL genes, were identified in captive collared peccary faeces, with resistance characteristics against beta-lactam antibiotics. The information from this work contribute to develop sanitary strategies for the management of collared peccary in captivity. Further research on the risk factors associated with the prevalence of DEC in captive peccary would allow to better understand how bacteria spread among captive animals and to reduce their capacity to act as carriers of DEC strains.

COMPETING INTERESTS STATEMENT

The authors declare that this study was carried out in the absence of commercial or financial relationships that could be interpreted as a potential conflict of interest and all persons gave their informed consent prior to their inclusion in the study.

AUTHOR CONTRIBUTIONS

CQ-B, CT-C and GUB-T conceived and design of the study. CQ-B, CI-M, MO-Ll, MR-S and GUB-T obtained faecal samples from peccaries, as well laboratory work. CAC-G and JG-J determine virulence genes and phylogenetic analyses. GB-T, CAC-G, SM-G and JG-J performed the statistical analysis and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

Financial support was partially granted to JG-J by the Program or Strengthening Educational Quality of the University of Science and Arts of the State of Chiapas (grant number C/PFCE-2016-07MSU0002G-13-43), also financial support was partially granted to QB-B by the support of the National Council of Science and Technology (Conacyt).

ACKNOWLEDGEMENTS

The authors would like to thank the personnel of the Wildlife Management Units of the State of Chiapas, Mexico, for the assistance provided. Permission to handle the animals under study was given by the personnel of the Wildlife Management Units.

Financial support was partially granted to JG-J by the Program or Strengthening Educational Quality of the University of Science and Arts of the State of Chiapas (grant number C/PFCE-2016-07MSU0002G-13-43). Also, financial support was partially granted to QB-B by the National Council of Science and Technology (Conacyt).

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