ISOLATION OF *ESCHERICHIA COLI* O157 FROM ZOO ANIMALS

Isolatie van Escherichia coli O157 uit Zoodieren

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ABSTRACT

During a nine month survey in the Royal Zoological Society of Antwerp, *E. coli* O157 was isolated from six out of 300 faecal samples collected from 258 mammals, 33 birds and nine reptiles. Enterohaemorrhagic *E.coli* O157:H7 (EHEC) strains were isolated from a horse (*Equus caballus*) and two primates: a ring-tailed lemur (*Lemur catta*) and a goeldi's monkey (*Callimico goeldii*). Atypical *E. coli* O157 strains, which fermented sorbitol and were β-glucuronidase positive, were isolated from two silvered leaf monkeys (*Presbytis cristatus*) and a ring-tailed lemur (*Lemur catta*). These strains were classified as enteropathogenic (EPEC), as they only possessed the *eae*A gene as a virulence marker. With five isolations out of 48 samples, the primates can be considered a potential source of infection by *E. coli* O157.

SAMENVATTING

Tijdens een 9 maanden durend onderzoek in de Koninklijke Maatschappij voor Dierkunde van Antwerpen werden 6 *E. coli* O157 stammen geïsoleerd uit 300 meststalen van 258 zoogdieren, 33 vogels en 9 reptielen. Enterohemorrhagische *E. coli* O157: H7 (EHEC) stammen werden geïsoleerd bij een paard (*Equus caballus*) en 2 primaten: een ringstaartmaki (*Lemur catta*) en een goeldi's tamarin (*Callimico goeldii*). Atypische *E.coli* O157 stammen, die sorbitol vergisten en β-glucuronidase vormen, werden geïsoleerd uit 2 mutslangoeren (*Presbytis cristatus*) en een ringstaartmaki (*Lemur catta*). Deze stammen werden geclassificeerd als enteropathogeen (EPEC), daar zij enkel het *eae*A gen als virulentiefactor bezitten. Met 5 isolaties uit 48 meststalen kunnen de primaten beschouwd worden als een mogelijke infectiebron van *E. coli* O157.

Key words: *E. coli* O157 - Zoo animals - Primates - EHEC - EPEC

INTRODUCTION

Escherichia coli is part of the normal intestinal microflora of animals and man. Most strains are harmless, but a limited number of serotypes are responsible for diarrhoea or more serious forms of illness. These strains are categorised as enteropathogenic, enterotoxigenic, enteroinvasive, enteroaggregative or enterohaemorrhagic according to their pathogenicity. Virulence is expressed in terms of their ability to adhere to or invade the mucosal surface of the intestine, and to produce haemolysins and toxins (Levine, 1987; Pohl, 1993). Enterohaemorrhagic *E.coli* (EHEC) are the most pathogenic strains among the verocytotoxin or Shiga toxin-producing *E. coli* (VTEC/STEC). They have been increasingly recognized as a cause of haemorrhagic colitis (HC) and the life-threatening haemolytic uremic syndrome (HUS) in man, particularly in children and the elderly (Karmali, 1989). Serotype O157:H7 and its non-motile variant O157:H- (O157:[H7]) are the predominant cause of human infection (Boyce *et al.*, 1995). The pathogenicity of these important zoonotic pathogens is determined by the verotoxins VT1 and VT2, enterohaemolysin (Ehly) and the intimin adherence factor, an outer membrane protein encoded by the *eae*A gene (Donnenberg *et al.*, 1993; Nataro and Kaper, 1998).

Since the first reported hamburger-related outbreak (Riley *et al.*, 1983), minced beef has been considered the most common vehicle of infection, though the organism has also been isolated from pork, lamb and poultry (Doyle and Schoeni, 1987; Chapman *et al.*, 1993a). Other reported sources of infection include fresh apple cider, unpasteurised milk and drinking water supplies (Swerdlow *et al.*, 1992; Besser *et al.* 1993; Chapman *et al.* 1993b). Several studies have shown that the gastrointestinal tract of domestic ruminants is a natural reservoir of EHEC O157 (Chapman *et al.*, 1989;

Hancock *et al.*, 1994). These pathogens can induce a mild transient diarrhoea in calves and an asymptomatic transient carrier status in older animals (Brown *et al.*, 1997; Dean - Nystrom *et al.*, 1997). Apart from the main reservoir, EHEC O157:[H7] may be present in the faecal flora of horses, pigs, dogs, cats, chickens and gulls (Griffin and Tauxe, 1991; Hancock *et al.*, 1998).

Epidemiological investigation of EHEC O157 strains in animal populations has focused mainly on the bovine reservoir, so the prevalence in other animals is not well known. The aim of this study was to determine the prevalence of *E. coli* O157 in faecal samples collected from animals in the Royal Zoological Society of Antwerp (RZSA).

MATERIALS AND METHODS

Between January and October 1999, 300 faecal samples were collected from animals kept in the RZSA. Whenever possible, individual samples were collected, but from animals living in groups composite samples were examined. One hundred and five different animal species, representing 69 species of mammals, 29 of birds and 7 of reptiles, were examined. The most investigated groups of animals were ruminants (101 samples), primates (48 samples), horses (42 samples), carnivores (22 samples), birds of prey (16 samples), camels (15 samples) and marsupials (9 samples). Forty-nine horses and ruminants examined during the winter were re-sampled during the summer. A horse stabled at the entrance of the zoo belonged to a theatre company and had no contact with the animal collection.

Samples were homogenised in Maximum Recovery Diluent (LabM) and 0.5 ml was added to 10 ml buffered peptone water supplemented with 8 mg/l vancomycin and 10 mg/l cefsulodin (BPW-VC) as described by Chapman *et al.* (1993a). The addition of cefexime was omitted since this product was not available in Belgium at the time of examination. After incubation at 37°C for 6 h, the broth cultures were subjected to immunomagnetic separation (IMS) using Dynabeads anti-*E. coli* O157 (Dynal AS), followed by culture of the magnetic beads on cefixime tellurite sorbitol MacConkey agar (CT-SMAC; LabM) according to the manufacturer's recommendations. Sorbitol non-fermenting colonies and colonies with indistinct fermentation or reduced growth, were subcultured on MacConkey agar (LabM). Coliform colonies were tested for O157 antigen using a latex agglutination kit (Oxoid). Agglutinating strains were confirmed as *E. coli* with reactive strips (Api; BioMérieux) and examined for their motility, β-glucuronidase activity (PGUA; Rosco Diatabs) and fermentation of cellobiose, sorbitol and rhamnose in sugar broth. Haemolysis was examined on washed sheep blood agar as described by Beutin *et al.* (1989). The isolates were sent to the National Reference Laboratory for EHEC/VTEC (Dr. Piérard, VUB, Brussels) for serotyping and determination of the genotype by polymerase chain reaction (PCR) for VT1, VT2, Ehly and the *eae*A gene (Piérard *et al.*, 1997; Paton and Paton, 1998).

RESULTS

E. coli O157 was isolated from six out of 300 individual or pooled faecal samples collected in the RZSA. Phenotypic and genotypic characteristics of the isolated strains are summarised in Table 1. Typical enterohaemorrhagic *E. coli* O157 strains (Table 1: no. 3, 5 and 6) were isolated from a ring-tailed lemur (*Lemur catta*), a goeldi's monkey (*Callimico goeldii*), and a horse (*Equus caballus*) that belonged to a theatre company. These strains presented the different virulence characteristics as they produced verotoxins, possessed the *eae*A gene and were enterohaemolytic. In addition, three sorbitol-fermenting VT and Ehly negative O157 strains (Table 1) were isolated from 2 silvered leaf monkeys (*Presbytis cristatus*) and a ring-tailed lemur (*Lemur catta*). They were positive for the *eae*A gene and were thus classified as enteropathogenic *E. coli* (EPEC). These strains initially showed reduced growth on CT-SMAC agar, were motile but not H type 7 and produced β-glucuronidase. All six strains fermented rhamnose, none fermented cellobiose.

DISCUSSION

Ruminants are considered to be the reservoir of EHEC O157 but in the present study no isolations were made from the 101 faecal samples collected on two occasions from ruminants. A study in Belgium using the sensitive IMS technique demonstrated that 6.3% of the cattle examined were healthy carriers of EHEC O157 (De Zutter *et al.*, 1999). Infection in cattle is transient and a seasonal peak exists during the warmer months of the year. Therefore, repeated examination of sufficient samples over a longer period of time may result in more positive isolations. On the other hand, the isolation of five *E. coli* O157 strains from 48 primates examined might indicate that this animal group may act as a reservoir.

The epidemiology of *E. coli* O157 remains unclear. The infected horse of the theatre company had no contact with the animal collection. The other positive animals, all primates, had been living in the zoo for several years. These primates were housed in the same building, but were kept in separated cages. Contaminated food is generally considered to be the source of infection. It is possible that the vegetables and fruits fed to the primates were contaminated, although reports on the presence of EHEC in vegetables are rare (Samadpour *et al.*, 1990). In farming situations, grass-

Table 1. Characteristics of the isolated *E. coli* O157 strains in the Royal Zoological Society of Antwerp.
Tabel 1. Eigenschappen van de E. coli O157 stammen geïsoleerd in de Koninklijke Maatschappij voor Dierkunde van Antwerpen.

No.	Species	Month of sample	Sorbitol fermentation	PGUA[a]	H antigen	Ehly[b]	Verotoxin VT1	Verotoxin VT2	eaeA gene	Patho[d] group
1	Silvered Leaf Monkey (Presbytis cristatus)	Febr. '99	+	+	MNH7[c]	-	-	-	+	EPEC
2	Silvered Leaf Monkey (Presbytis cristatus)	Febr. '99	+	+	MNH7	-	-	-	+	EPEC
3	Ring-tailed Lemur (Lemur catta)	Febr. '99	+	+	MNH7	-	-	-	+	EPEC
4	Ring-tailed Lemur (Lemur catta)	June '99	-	-	7	+	-	+	+	EHEC
5	Horse (Equus caballus)	July '99	-	-	7	+	-	+	+	EHEC
6	Goeldi's Monkey (Callimico goeldii)	Oct. '99	-	-	7	+	+	+	+	EHEC

a: β-Glucuronidase activity
b: Enterohaemolysin production
c: Motile but not H type 7
d: EPEC: enteropathogenic *E. coli* / EHEC: enterohaemorrhagic *E. coli*

land and slurry management may be important in the spread of this organism (Hancock *et al.*, 1994). Free roaming animals (e. g. gulls) feeding on contaminated sites can spread the infection over large areas and may act as a reservoir from which interspecies transmission to domestic animals can occur (Rice *et al.*, 1995; Wallace *et al.*, 1997).

Results of surveys in the Emperor Valley Zoo in Trinidad and in the Kansas City Zoological Gardens (Jacobson *et al.*, 1998; Adesiyun, 1999) remained negative. However, these surveys were performed without the use of the IMS method. An *E. coli* O157: H7 strain, negative for verotoxins and positive for *eae*A, was isolated from an orang-utan living in Stuttgart's Zoological Gardens with acute watery diarrhoea (Beutin *et al.*, 1996).

All strains isolated in the present study possessed the eaeA gene. In enterohaemorrhagic and enteropathogenic *E. coli*, the intimin-coding gene determines the adherence to intestinal epithelial cells and effacement of microvilli (Donnenberg *et al.*, 1993). Verocytotoxin producing strains were also enterohaemolytic. This is a useful marker for screening EHEC strains, as there is a close relationship between the presence of both characteristics (Beutin *et al.*, 1989). Although isolates no. 4, 5 and 6 (Table 1) were considered to be fully pathogenic EHEC O157 strains, none of the animals with positive faecal culture had gastrointestinal disorders. In order to avoid overlooking tellurite-sensitive strains, colonies showing reduced growth resulting in indistinct fermentation were also subcultured. This led to the isolation of three atypical sorbitol-fermenting O157 strains. On subculture these strains grew equally well on SMAC agar with or without the addition of the CT mixture, so reduced growth could not be due to tellurite susceptibility. The three sorbitol-fermenting *E.coli* O157 strains no. 1, 2 and 3 (Table 1) that were nontoxigenic but possessed the eaeA gene were classified as enteropathogenic. EPEC O157 strains cause diarrhoea in infants and *eae*-positive non-VTEC have been implicated in neonatal calf diarrhea (Schmidt *et al.*, 1993; China *et al.*, 1998).

The isolation of 3 EPEC O157 strains and 2 EHEC 0157:H7 strains from primates indicates that this group of zoo animals may be a potential reservoir. However, the shedding of these pathogens seemed to be transitory and no clinical symptoms were observed. The health risk can be reduced if prevention measures such as food hygiene, personal sanitation and regular cleaning are applied. The existence of subclinical carriers of *E. coli* O157 may have important epidemiologic implications for the transfer of animals between zoos. Consequently, newly acquired animals should be quarantined and examined for EHEC/VTEC, as is already done for other enteric pathogens.

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