212 - ENTEROPATHOGENIC ESCHERICHIA COLI (EPEC) ISOLATED FROM PSITTACINE BIRDS.

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1.INTRODUCTION

The normal flora of psittacine birds is composed mostly or exclusively of Gram-positive bacteria (Hoefer, 1997). Many authors have suggested that all Gram-negative bacteria are abnormal inhabitants of the psittacine gut and should be considered as pathogens (Mattes et al., 2005; Marietto-Gonçalves et al., 2007; Knöbl et al., 2009).

Enteropathogenic Escherichia coli (EPEC) are important pathogens of infantile diarrhea in developing countries and have been isolated from man and domestic animals (Goffaux et al., 2000; Nakazato et al., 2004; Gladys, 2005). The pathogenic effects result from intimate adhesion to the intestinal mucosa, called attaching-effacing (AE) lesion. The ability to induce AE lesion is mediated by the expression of some 40 bacterial genes organized in a “pathogenicity island”, known as the Locus Enterocyte Effacement (LEE) (Wales and Woodward, 2004).

Although not essential for the development of AE lesions, fimbriae called bundle-forming pili (BFP) encoded by the Escherichia attaching factor plasmid (EAF) promote the localized adherence of bacteria to the epithelial cells, facilitating the occurrence of the lesion. Those EPEC strains which harbor the EAF plasmid are designated typical EPEC, and those which lack the plasmid are called atypical EPEC (Carvalho et al., 2003).

Humans are the mainly reservoir of typical EPEC, while atypical EPEC have been associated with domestic animals (cattle, sheep, goats, dogs, cats, pigs and chicken) (Gladys, 2005). Recently, some atypical EPEC serotypes were described in wildlife animals (Carvalho et al., 2003; Hernandez et al., 2007). The impact of anthropogenic pathogens for wildlife animals and the potential zoonotic risk implicated in the animal-human contact are unclear. The purpose of this survey was to investigate the presence of typical and atypical EPEC in psittacine birds.

2. MATERIALS AND METHODS

2.1 E. coli isolates

This study was conducted with 80 swabs fresh fecal samples, collected from psittacine birds in São Paulo State, Brazil, during two consecutive years (2006 to 2008).

Standard bacteriological methods were employed for E. coli isolation and identification (Bangert et al., 1988). All isolates were stored at -70oC in brain heart infusion broth (BHI) (Difco Laboratories, Detroit, MI, USA) to which 15% glycerol was added after incubation.

2.2 PCR

The primer sequences were used to detect genes encoding, amplicon sizes and the relevant literature are given in Table 1.

The DNA extraction was performed as described by Boom et al (1990). The standard PCR amplification mixture consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.001% (wt/vol) gelatin, 200 M each of the four deoxynucleoside triphosphates, sets of primers, and 0.5 U of Taq DNA polymerase in a final volume of 25 l. Amplified products were separated in 1.5% agarose gel and examined after ethidium bromide staining. A 100-bp DNA ladder was used as a molecular size marker.

Table 1- The primers used for detection of EPEC by PCR, amplicon size, and references.

Gene	Oligonucleotide primer pairs (5`-3`)	Amplicon (bp)	Reference
*eae*A	ACG TTG CAG CAT GGG TAA CTC GAT CGG CAA CAG TTT C AC CTG	815	Gannon et al., 1993
EAF	CAG GGT AAA AGA AAG ATG ATA A TAT GGG GAC CAT GTA TTA TCA	397	Franke et al., 1994

2.3 Serogroup determination

Positive PCR strains were serotyped by the method described by Guinée et al. (1981) with all available O antisera (O1 to O185). Antisera were obtained and absorbed with corresponding cross-reaction antigens to remove nonspecific agglutinins.

3. RESULTS

Twenty four strains (30%) were isolated from 80 samples, but only two isolates were positive for the eae gene. These isolates belonged to serogroups O128 and O76 and were negative when tested for the presence of genes encoding for EAF.

4. DISCUSSION

In this study were analyzed amazon parrots without signs of diarrhea. E. coli strains were isolated from 24/80 (30%) fecal samples collected from wildlife parrots.

It is very difficult to differentiate pathogenic from nonpathogenic strains, because E. coli are frequently secondary invaders in birds, associated with stress, malnutrition, poor hygiene and hypovitaminosis A. The potentially pathogenic E. coli strains can be screened by different tests, like phenotypic assays as Congo red binding (Styles and Flammer, 1991), serotyping (Schremmer et al, 1999), and genotypic assays (Knöbl et al., 2001; Pakpinyo et al., 2002). Polymerase Chain Reaction (PCR) is the most fast and sensitive molecular method to determine the virulence proprieties of E. coli. In this study, PCR detected two strains eae+ (8,3%). Nakazato et al. (2004) showed the same positive percentile (8,3%) from 36 dogs (with no diarrhea) in Campinas and São Paulo cities (Brazil).

Hernandez et al. (2007) report an occurrence of atypical EPEC in Antarctic fur seals (Arctocephalus gazelle). The authors discussed the concern about harmful pathogens introduced into Antarctica by human activities.

Schremmer et al. (1999) examined E. coli isolated from psittaciformes and emphasized the presence of seven strains belonged serovars O63:H10, O110:H6, O131:H-, O153:H10 and ONT:H6, that were positive for the eae gene, four of which were also positive for the bfpA gene. The authors concluded that EPEC should be considered as potential pathogens in psittaciform birds, and may be a reservoir of human EPEC infections. Likewise, in our study two isolates of serogroups O128 and O76 were positive for eaeA gene, and thus were considered atypical enteropathogenic E. coli (aEPEC), a diarrheagenic pathotype characterized by the absence of the EAF region.

In conclusion, our results show that atypical EPEC serotypes were present among psittacine birds in Brazil. Future epidemiological studies are needed to clarify the bacterial pathogenesis in wild birds and the risks implicated in this zoonosis.

ACKNOWLEDGEMENTS.

FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo – grant 2005/57500-9).

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ENTEROPATHOGENIC ESCHERICHIA COLI (EPEC) ISOLATED FROM PSITTACINE BIRDS.

ABSTRACT

Fecal swabs were collected from 80 psittacine birds, analyzed for the presence of Escherichia coli and investigated for the genes encoding the attaching and effacing (eae) and enteropathogenic E. coli EAF plasmid (EAF). The results showed that two of 24 E. coli isolates, belonged to serogroups O76 and O128, were eae positive. All strains were negative for gene encoding for EAF. Our findings showed that some E. coli isolated from psittacine birds present the same virulence factors as human enteropathogenic E. coli and suggest that amazon parrots constitute a reservoir of atypical EPEC.

KEYWORDS: Psittacine birds, APEC, Zoonosis.

ESCHERICHIA COLI ENTEROPATÓGENAS (EPEC) AISLADAS DE AVES PSITÁCIDAS
RESUMEN

Hisopos fecales fueron recolectadas de 80 aves psitácidas, analizó la presencia de Escherichia coli y de investigación por los genes que codifican la adhésion estricta (eae) y E. coli enteropatógenos EAF plásmido (EAF). Los resultados mostraron que dos de los 24 E. coli aisladas pertencían a los serogrupos O76 y O128, se eae positivas. Todas las cepas fueron negativas para el gen que codifica para el EAF. Nuestros resultados mostraron que algunos de E. coli aisladas de aves psitácidas presentan los miesmos factores de virulencia, como humanos enterophatogenic E. coli y sugieren que los loros amazónicos constituyen una reserva de EPEC atípica.

PALABRAS CLAVE: aves psitácidas, EPEC, Zoonosis.

ESCHERICHIA COLI ENTÉROPATHOGÈNES (EPEC) OISEAUX ISOLE DE PSITTACIDÉS
RESUME

Écouvillons fécaux ont été recueillis à partir de 80 psittacidés, a analysé pour la presénce d'Escherichia coli et d enquêtes pour le gènes codant pour les attachant et effaçant (eae) et de E. coli entéropathogènes EAF plasmide (EAF). Les rèsultants ont montré que deux des 24 E.coli isolés appartenaient aux sérogroupes O128 at O76, eae ont été positifs. Toutes les souches étaient négatifs pour le gène codant pou l'EAF. Nos résultats ont montré que certains E. coli isolée de psittacidés de présenter les mêsmes facteurs de virulence comme l'homme enterophatogenic E. coli et de suggérer que les perroquets amazone constituent un réservoir de atypique EPEC.

MOTS-CLÉS: Psittacidés, APEC, Zoonose.

ESCHERICHIA COLI ENTEROPATOGÊNICA (EPEC) ISOLADAS DE PSITACÍDEOS.
RESUMO

Esfregaços fecais foram coletados de 80 Psittaciformes, analisados para a presença de Escherichia coli e investigados para os genes que codificam aderência intima à mucosa (eae) e para o plasmídio EAF de E. coli enteropatogênica (EAF). Os resultados mostraram que dois dos 24 isolados de E. coli, pertenciam aos sorogrupos O76 e O128, foram eae positivos. Todas as amostras foram negativas para o gene que codifica o EAF. Nossos resultados mostraram que cepas de E. coli isoladas de psitacídeos apresentam os mesmos fatores de virulência de E. coli enteropatogênicas para humanos e sugerem a participação dos psitacídeos como reservatório de EPEC atípica.

PALAVRAS-CHAVE: Psittaciformes, APEC, Zoonose.

PUBLICAÇÃO NO FIEP BULLETIN ON-LINE: http://www.fiepbulletin.net/80/a1/212