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INFECTIOUS DISEASE

Enteric Colonization by *Staphylococcus delphini* in Four Ferret Kits with Diarrhoea

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Summary

Four, 1-to 4-week-old ferret kits were submitted to the Diagnostic Center for Population and Animal Health at Michigan State University for post-mortem examination. Grossly, multiple bowel loops in all ferret kits were distended by mucoid faecal material. Microscopically, there was no evidence of inflammation or notable alteration to the normal mucosal morphology. Gram-positive coccoid bacteria colonized variable segments of the small intestine. These bacteria were identified as *Staphylococcus delphini* by phenotypic and molecular analyses. Enzyme-linked immunosorbent assay for detection of *Staphylococcus* enterotoxins was positive and polymerase chain reaction detected the gene for *Staphylococcus* enterotoxin E in the isolates. The hypersecretory diarrhoea in these ferret kits may have been associated with colonization of the small intestine by *S. delphini*, cultures of which were shown *in vitro* to be potentially capable of producing enterotoxin E. The condition described in these ferrets is similar to ‘sticky’ kit syndrome in mink.

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‘Sticky’ kit syndrome is an enteric disease that affects mink kits between 10 and 14 days of age. Clinical signs include hypersecretion of cervical apocrine glands and mucoid diarrhoea, which causes severe dehydration that can rapidly proceed to death. Large outbreaks of sticky kit syndrome at mink farms are not uncommon, causing death in up to 30–40% of affected kits (Englund *et al.*, 2002). During one such outbreak, post-mortem examinations conducted at the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH), in Lansing, MI, revealed that the intestinal mucosa of affected kits was colonized by gram-positive coccoid bacteria. Bacterial culture and typing indicated that they were a strain of *Staphylococcus delphini* that was capable of producing enterotoxins A and E (Sledge *et al.*, 2010).

Staphylococcus delphini is coagulase-positive and is part of the *Staphylococcus intermedius* group. Mustelids

are reported to be natural hosts of *S. delphini* group A, which has been isolated from their skin, urinary tract, nasal swabs and faeces (Guardabassi *et al.*, 2012), and from urinary tract lesions (Guardabassi *et al.*, 2012) and enteric disease in mink (Sledge *et al.*, 2010). The purpose of this report is to describe enteric colonization by *S. delphini* in four ferret kits with diarrhoea, which is similar to ‘sticky’ kit syndrome previously described in mink.

Four ferret kits, comprising a <1-week-old female (kit 1), a 2-week-old female (kit 2), a 3-week-old male (kit 3) and a 4-week-old male (kit 4), all from the same facility, had a 1-day history of pasty, yellow (kits 1, 2 and 4) to green (kit 3) diarrhoea. They were submitted dead to the Michigan State University DCPAH. On gross examination, the coats of kits 1, 2 and 4 appeared normal except for mild faecal staining in the perineal region, while kit 3 had a rough and greasy hair coat. The stomachs of all kits contained milk. The small and large intestines of kits 1, 2 and 4 contained moderate amounts of yellow–orange,

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mucoïd to liquid faecal material (Fig. 1). The jejunal and ileal contents of the third kit were yellow—white and mucoïd, while the colonic contents were bright green and of mucoïd to liquid consistency. Brain, heart, lung, liver, spleen, thymus, kidney, pancreas and small and large intestine were collected from the four kits and were fixed in 10% neutral buffered formalin for at least 24 h. Representative sections were processed routinely and embedded in paraffin wax. Sections (5 µm) were stained with haematoxylin and eosin (HE). Selected sections of the small intestine were stained by the Gram's method (Hucker-Twort stain). Immunohistochemistry (IHC) was performed for coronavirus as previously described (Garner *et al.*, 2008).

Microscopical examination of small intestine revealed numerous 1–2 µm diameter, gram-positive coccoid bacteria along the apical and lateral surfaces of the intestinal villi (Figs. 2 and 3). In some segments, the cocci were also present in large numbers in the lumen of intestinal crypts. Rarely, sections of small intestine from kit 3 were characterized by mild degeneration of the mucosal epithelium associated with minimal neutrophilic inflammation. The small intestine was negative by IHC for ferret enteric coronavirus in all the kits. For bacterial culture, representative sections of fresh frozen small intestine were pooled based on the age of the affected kits. For each pooled sample, intestinal contents were cultured as described by Sledge *et al.* (2010). Similarly, pooled samples of faeces were collected for parasitological evaluation and sections of small intestine were tested for the presence of ferret enteric coronavirus and group C rotavirus using polymerase chain reaction (PCR) assays (Wise *et al.*, 2006, 2009). Small

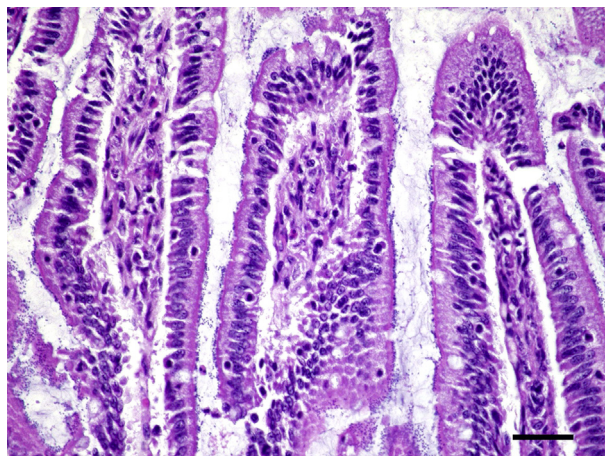


Fig. 2. Ferret kit intestine. Large numbers of coccoid bacteria are adherent to the villi of the small intestine. HE. Bar, 100 µm.

intestinal samples were negative by PCR for ferret enteric coronavirus and group C rotavirus and by qualitative faecal examination for intestinal parasites in all the kits.

Pooled small intestinal culture from the two younger kits grew gram-positive cocci that were identified as *Staphylococcus* spp. by colony morphology, production of catalase, haemolysis on enriched sheep blood agar, the presence of coagulase enzyme and positive Gram stain. The bacteria did not grow on P agar and were Voges—Proskauer test negative, which characterized them as member of the *S. intermedius* group. Few *Escherichia coli* and few *Enterococcus* spp. were also cultured from the small intestine. There was no growth of *Salmonella* in any culture.

Samples of the bacterial colonies phenotypically consistent with *Staphylococcus* spp. were submitted for extraction of DNA and subsequent PCR assays for



Fig. 1. Necropsy image of the intestines of ferret kit 2. Approximately 60–70% of the small intestine is distended by soft, pasty, yellow faecal material. Bar, 1 cm.

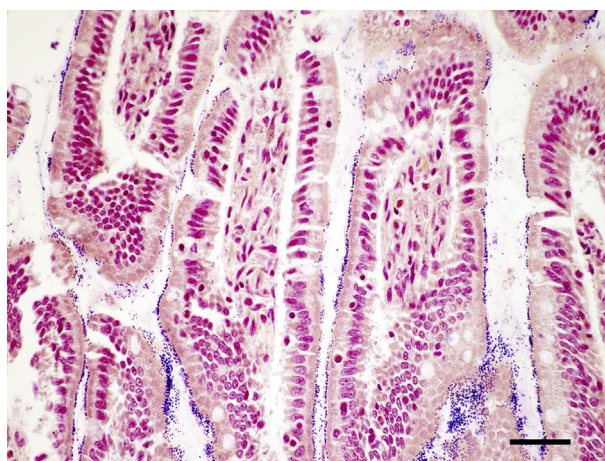


Fig. 3. Ferret kit intestine. The coccoid bacteria adherent to the villi are gram positive. Gram stain. Bar, 100 µm.

characterization of the bacteria. These assays targeted the 16S ribosomal DNA (16S rDNA), the superoxide dismutase (*SodA*) gene and the heat shock protein 60 (*Hsp60*) gene (Sledge *et al.*, 2010). Phylogenetic analysis using the nucleic acid sequence of the three selected genes identified the bacteria as *S. delphini* (Fig. 4). The amplified nucleic acid sequences of the 16S rDNA and *Hsp60* genes were identical among the bacterial isolates obtained in the current study and the *S. delphini* isolated previously from mink with sticky kit syndrome (unpublished sequence) (Sledge *et al.*, 2010). Further, the 1,052 base pair sequence derived from 16S rDNA in the current study was 100% identical to multiple corresponding sequences from *S. delphini* in the GenBank database and the 485 base pair sequence of the *Hsp60* gene had 95–99% similarity with corresponding sequences for *S. delphini* in GenBank. The 348 base pair segment of the *SodA* gene of the *S. delphini* from sticky kit disease in mink was identical to that of the bacterial isolates from the current study except for a single A to G transition that did not result in a change of the predicted amino acid sequence.

To determine if the *S. delphini* isolated in the current study contained toxin genes, overnight cultures of bacteria in tryptic soy broth were centrifuged individually at 1,730 g for 10 min at 10°C. The clarified culture supernatants were injected through low protein binding 0.22 µm syringe filters. The filtered culture supernatants were then screened for enterotoxin production using a commercial enzyme-linked immuno-

sorbent assay (ELISA) kit that detects the presence of *Staphylococcus* enterotoxins (SETs) A, B, C, D and E following procedures recommended by the manufacturer (RIDASCREEN® SET Total, R-Biopharm AG, Darmstadt, Germany). The clarified bacterial culture supernatants were positive for enterotoxin(s) by ELISA. The presence of staphylococcal enterotoxin genes A to E was assessed by PCR assays, using PCR primers designed to amplify fragments of the *Staphylococcus aureus* enterotoxin genes (*sea*, *seb*, *sec*, *sed* and *see*) (Fischer *et al.*, 2009). Nucleic acid sequence analysis of the amplicons confirmed the detection of an enterotoxin gene fragment that was 92% similar to a corresponding fragment of the enterotoxin E gene of *S. aureus*.

In this study, small intestinal colonization with *S. delphini* was seen in four ferret kits with diarrhoea. Minimal degeneration of intestinal epithelium with rare associated neutrophils, likely representing a mild reaction to bacterial toxins, was present in one ferret kit. In previous reports, bacterial colonization of the intestine by *S. delphini* with associated diarrhoea and excessive cervical apocrine gland secretion was described in mink kits ranging from 10 to 14 days of age (Sledge *et al.*, 2010). The four ferret kits described in this report showed similar clinical signs to those ‘sticky’ mink kits: the ferret kits had pasty, yellow diarrhoea and one kit had also a sticky coat. However, ferret kits were affected at a wider age range (1–4 weeks) than mink kits. Many of the features of the diarrhoeal disease described in these ferret kits, including the intestinal colonization by gram-positive cocci, the isolation of *S. delphini* and the partial nucleic acid sequences of three genes of the isolates, are consistent with the findings previously described in the mink kits with sticky kit syndrome (Sledge *et al.*, 2010). The similar clinical and histological features and the comparable bacterial isolates suggest that ferret kits can experience a ‘sticky kit syndrome’ similar to the syndrome described in mink.

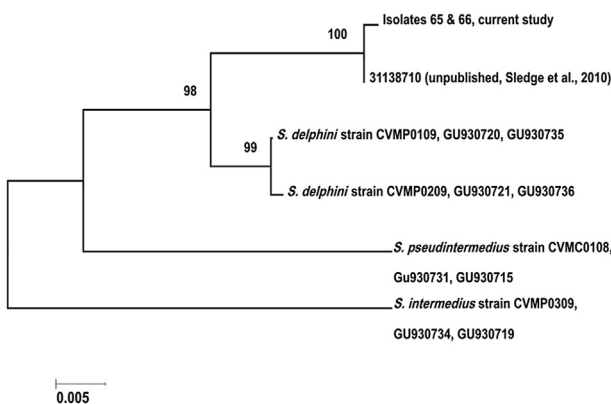


Fig. 4. Phylogenetic tree based on concatenated sequence of *Hsp60* gene (448 base pairs) and *SodA* gene (313 base pairs). The concatenated sequence for the *HSP60* and *SodA* genes was trimmed to match the length of sequences for both genes that were available in GenBank. The tree was constructed using the neighbour-joining method (Saitou and Nei, 1987) with 1,000 bootstrap replicates (Felsenstein, 1987). The percentage of replicate trees in which the associated taxa clustered together is shown above the branches. The phylogenetic analyses were conducted in MEGA4 (Tamura *et al.*, 2007).

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Conflict of Interest Statement

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article. The authors received no financial support for the research, authorship and/or publication of this article.

References

- Englund L, Chriél M, Dietz HH, Hedlund KO (2002) Astrovirus epidemiologically linked to pre-weaning diarrhoea in mink. *Veterinary Microbiology*, **85**, 1–11.
- Felsenstein J (1987) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.
- Fischer A, Francois P, Holtfreter S, Broeker B, Schrenzel J (2009) Development and evaluation of a rapid strategy to determine enterotoxin gene content in *Staphylococcus aureus*. *Journal of Microbiological Methods*, **77**, 184–190.
- Garner MM, Ramsell K, Morera N, Juan-Sallés C, Jiménez J, Ardiaca M *et al.* (2008) Clinicopathologic features of a systemic coronavirus-associated disease resembling feline infectious peritonitis in the domestic ferret (*Mustela putorius*). *Veterinary Pathology*, **45**, 236–246.
- Guardabassi L, Schmidt KR, Petersen TS, Espinosa-Gongora C, Moodley A, Agerso Y *et al.* (2012) Mustelidae are natural hosts of *Staphylococcus delphini* group A. *Veterinary Microbiology*, **159**, 351–353.
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.
- Sledge DG, Danieu PK, Bolin CA, Bolin SR, Lim A, Anderson BC *et al.* (2010) Outbreak of neonatal diarrhoea in farmed mink kits (*Mustella vison*) associated with enterotoxigenic *Staphylococcus delphini*. *Veterinary Pathology*, **47**, 751–757.
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596–1599.
- Wise AG, Kiupel M, Maes RK (2006) Molecular characterization of a novel coronavirus associated with epizootic catarrhal enteritis (ECE) in ferrets. *Virology*, **349**, 164–174.
- Wise AG, Smedley RC, Kiupel M, Maes RK (2009) Detection of group C rotavirus in juvenile ferrets (*Mustela putorius furo*) with diarrhoea by reverse transcription polymerase chain reaction: sequencing and analysis of the complete coding region of the VP6 gene. *Veterinary Pathology*, **46**, 985–991.

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