Acta vet. scand. 1996, *37*, 163-169.

Escherichia Coli and Virus isolated from "Sticky Kits"

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Jørgensen, M., F. Scheutz and B. Standbygaard: *Eschericia Coli* and virus isolated from sticky kits. Acta vet. scand. 1996, 37, 163-169. – A total of 121 Escherichia coli strains isolated from 3-week-old mink kits were serotyped and examined for virulence factors. 56 strains were isolated from healthy kits while 65 were from "sticky kits". Among these, 34 different serotypes were detected. No difference in serotypes or the presence of virulence factors could be detected between healthy and diseased kits.
By electron microscopy of faecal samples corona-, rota-, and calicivirus were demonstrated among healthy as well as diseased kits.

mink; diarrhoea; E. coli; coronavirus; rotavirus; calicivirus.

Introduction

"Sticky kits" have been registered as a multifactorial disorder among mink for several years, particularly in Denmark. The disease occurs in mink kits 1-5 weeks old. As the name indicates, the kits are covered by a greasy, sticky, scabby exudate on the skin surface. The exudation starts dorsally behind the head and spreads to the rest of the body. Typically, distinct scabs are present on the claws. The abdomen is distended, the anal region is oedematous and hyperemic and when pressing the abdomen of the animal foaming yellowish-greenish faeces flows from the anus. Loss of hair is a common feature that may develop into total alopecia *(Henriksen* 1987). These are the classical symptoms, but frequently a foaming diarrhoea without accompanying skin affections is seen. At post mortem examination, the mesenterial vessels are dilated and the intestinal wall oedematous, frequently with hyperemia and diffuse bleeding to the lumen. Stasis in liver and spleen is common together with petecchial bleedings in cardiac muscle, kidneys, lungs and liver, indicating septicaemia or hypoxia *(Henriksen* 1994). Morbidity is usually 1-50%, while the mortality rarely exceeds 1%.

At birth the intestinal tract of mink is sterile, but during the first days after birth an intestinal flora begins to establish. During the first 3-4 weeks, while the kits live exclusively on maternal milk, this intestinal flora consists mainly of staphylococci and faecal streptococci. However, when the kits begin to eat solid food, *Escherichia coli* becomes dominant *(Clausen* 1988, *Jørgensen* 1990).

During the eighties, hemolytic *staphylococci* were the dominant findings in the intestinal tract of "sticky kits", and less frequently *E. coli* was found. Lately, however, this has changed into a dominance of *E. coli* (60%) irrespectively of the age of the kits. Clinical observations seem to indicate that infections with *E. coli* cause higher mortality than hemolytic staphylo-

cocci *(Jørgensen* 1990). To the authors knowledge no systematic investigations of *E. coli* from mink have hitherto been carried out, nor is it known whether *E. coli* from mink could appear as human pathogens. In the present paper we investigated *E. coli* strains, isolated from mink kits in 1993, with respect to serotypes (O-, H- and K-antigens), haemolysin, heat-stable toxin (ST), heat-labile toxin (LT), verotoxin (VT) and other virulence factors. In addition, faecal samples were investigated for the presence of virus.

Materials and methods

Experimental animals and collection of faecal samples

The experimental animals were 14 "sticky kits" and 12 healthy kits randomly chosen from 11 farms. Among the diseased kits 11 were younger than 3 weeks and 8 of the healthy kits were younger than 3 weeks. The oldest kit was hardly 4 weeks old.

Faecal samples were collected on sterile cotton wool swabs directly from the anus after a light pressure on the abdomen.

Bacteriological examination

Faecal samples for qualitative bacteriological examination were suspended in 5 ml sterile saline (0.9% w/vol) followed by inoculation onto blood agar added esculin (bloodagarbase no. 2, code CM271 + 0.05% aesculin, Oxoid) and onto Drigalski agar (Danish Veterinary Laboratory, Århus, Denmark). Agar plates were incubated aerobically at 37°C for 24 h. Up to 5 *E. coli* colonies displaying different morphology were collected from each Drigalski agar and transferred to deep nutrient agar stabs (SSI). After aerobic incubation at 37°C for 24 h, strains were stored at room temperature until further investigation. A total number of 121 *E. coli* strains were collected.

Serotyping

The somatic (O), capsular (K) and flagellar (H) antigens were determined by standard methods *(Ørskov & Ørskov* 1984). Antisera to 171 O, 74 K and 53 H antigens were used. Capsular polysaccharides K1, K5, K12 and K13 were identified by specific phages *(Ørskov & Ørskov* 1984).

Haemolysin

For detection of haemolysin, bacteria were grown aerobically at 37°C for 24 h on blood agar plates containing 5% defibrinated unwashed sheep erythrocytes and on blood plates containing erythrocytes washed 3 times in phosphate-buffered saline, pH 7.2 *(Beutin et al.* 1989). Strains producing a clear haemolytic zone larger than the overlying colony on agar plates containing washed erythrocytes as well as on plates containing unwashed erythrocytes were considered to produce α haemolysin. Strains producing haemolytic zones which were clearly different between unwashed (weak haemolysis) and washed (strong haemolysis) erythrocytes were considered to produce enterohaemolysin. Strains producing haemolytic zones which were not clearly different after overnight incubation were described as haemolytic (Hly^+).

DNA probes

All the different O:K:H serotypes from both healthy and "sticky kits" were examined for the presence of the *E. coli attaching & effacing(eae)* (pCVD434) *(Jerse & Kaper* 1991), EnteroAggregative *E. coli* (EAggEC) (pCVD432) *(Baudry et al.* 1990) and EnteroInvasive *Eschericia coli* (EIEC) (pPS2.5) *(Small & Falkow* 1985) gene sequences by colony blot hybridisation under stringent conditions using DNA probes labelled with Digoxigenin as described by the manufacturer (Boehringer Mannheim).

Toxins

All the different O:K:H serotypes from both healthy and sticky kits were tested for the production of heat-stable toxin (ST) by EIA (Oxoid kit TD 700; Kebolab, Sweden & Oxoid, Basingstoke, UK), for heat-labile toxin (LT) by the Y1 adrenal cell assay *(Sack & Sack* 1975), and for vero cytotoxin (VT) in the vero cell assay (VCA) *(Konowalchuch et al.* 1977).

Virological examination

Faecal samples for virological examination were taken from 13 diseased and 10 healthy kits. The faecal samples were diluted 1:10 in Ringer chloride solution and centrifuged at 3400 g for 15 min at 5°C. One drop of supernatant was used for electron microscopy, while the remaining supernatant was centrifuged at 30000 g for 1 h at 5°C. The pellet was resuspended in saline solution (0.9% w/vol), pH 7.2 and used for electron microscopy. One drop of virus suspension was transferred to a formvar carbon coated grid for 5 min, washed with 2 drops of water and stained with uranyl acetate (UA) or sodium silicowolframate (NaSiW). Specimens were analysed using a Zeiss 10 electron microscope at 60 kV.

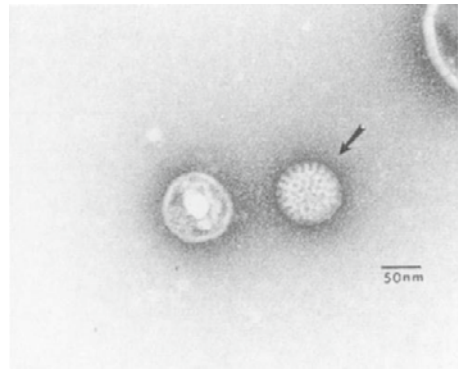

Figure 1. Electron microscopy of mink rotavirus detected in the faeces of mink. Negative stained with uranyl acetate. Magnification 200.000X. Foto: B Strandbygård.

Results

E. coli was isolated from 33 of 41 faecal samples from kits (80%). A total of 121 *E. coli* strains were collected from 14 diseased and 12 healthy kits for further examination. Faeces was collected from each kit only once.

O:K:H serotypes

The results of the serotypings are shown in Table 1. A total of 34 different serotypes were demonstrated. In 16 of the 26 kits only one serotype was detected, while in remaining kits, 2 or more, up to 5 serotypes were recovered. Five different serotypes were found in diseased as well as in healthy animals. Two isolates cross-reacted with 2 O-groups, O5,O86 and O8,O25 respectively.

Virulence factors

None of the strains tested produced ST, LT or VT.

Two healthy kits had *E. coli* that produced haemolysin, one of which was α-haemolytic. Eae^+ *E. coli* strains were identified from 10 and 6 different serotypes from healthy and sticky kits, respectively, while none of the strains hybridized with the *EAggEC* or *EIEC* DNA probes.

Virological examination

The results of the virological examination are shown in Table 2. Rota-(Fig.1), corona- and calicivirus were detected in one or more samples, while either parvo- or enterovirus were detected in one sample. There were no significant differences between healthy and diseased animals ($p<0.77$) with respect to the number of virus positive samples (Epi-Info programme).

Table 1. The results of serotyping of 121 *E. coli* strains isolated from faeces of mink kits. O: O-antigen; OR: O rough; O-antigen could not be determined due to auto-agglutination; K^+: presence of polysaccharid K-antigen that could not be determined; K^-: K-antigen could not be demonstrated; NM: nonmotile; ON, KN and HN: non-typeable with respect to O, K or H antigen, respectively.

Healthy kits		Diseased kits	
Serotypes	Virulence factors	Serotypes	Virulence factors
O5,086:K^+:H11	*eae*$^+$	O8:K^+:H19	
O7:K^+:H7	α Hly	O8,O25:H49	
O9:K^+:NM		O9:K^+:NM	
O15:NM	*eae*$^+$	O25:K^+:H9	
O18abc:NM		O39:K^-:H49	
O33:KN:H6	*eae*$^+$	O45:K^-:NM	
O51:H49	*eae*$^+$	O51:K^-:H49	*eae*$^+$
O61:KN:H34		O61:KN:H34	
O68:K^+:H49			
O76:H51	*eae*$^+$	O76:H51	*eae*$^+$
O125ab:K^-:H6	*eae*$^+$	O113:KN:H21	
O130:KN:H11			
O157:K^-:H39	*eae*$^+$	O157:K^-:H39	*eae*$^+$
O166:K^-:H49		O157:K^-:H42	
O168:KN:NM	*eae*$^+$	O169:K^-:H18	
O170:KN:H49			
OR:K^-:H8	Hly$^+$	OR:K^-:H6	*eae*$^+$
OR:K^-:H28		OR:KN:H6	*eae*$^+$
OR:KN:NM	*eae*$^+$	OR:KN:H19	
ON:K84:H2		OR:KN:NM	*eae*$^+$
ON:K^-:NM	*eae*$^+$		

Discussion

The pathogenesis of enteric infections includes absorption of agent, colonisation and diarrhoea. To exert a negative effect on the host animal *E. coli* has to bypass several barriers. For example in the oesophagus in pigs *E. coli* is restricted by lactic acid produced by *Lactobacillus species* adherent to the oesophagal epithelium *(Pedersen & Tannock* 1989). Next hindrance is the low pH in the stomach. In the intestines bacteria compete mutually for colonization and sometimes for adhesion at the epithelium. Chemical compounds as colicin, hydrogen peroxide, lactic acid, free oxygen radicals and others produced by different types of bacteria, impede the growth of *E. coli* and

Table 2. Detection of virus in faecal samples from diseased and healthy mink kits.

	No. of positive samples				
Kits	Rota	Corona	Calici	Parvo or entero	Total no. of samples
Diseased	2	1	3	1	13
Healthy	2	0	2	0	10

other types of bacteria. The constant production of mucus from the intestinal epithelial cells and secretory antibodies contribute a barrier as well. Furthermore there is the passive transport caudally because of peristaltic movements.

Investigations in adult mink and kits have shown that no bacterial adhesion takes place anywhere in the gastrointestinal tract. Even after challenge on healthy, sucking kits with *Staphylococcus intermedius* and *E. coli* isolated from "sticky kits", no adherence to the epithelium anywhere in the gastro-intestinal tract was observed *(Jørgensen & Pedersen* 1992). In spite of this, one examination of the intestinal flora in kits younger than 3 weeks showed bacterial adhesion to the intestinal epithelium or mucuslayer. From the results of cultivation it was most likely that the bacteria of *Lactococcus species (Pedersen & Jørgensen* 1992, *Pedersen et al.* 1994). These bacteria are thought to have the quality of a probiotic, but unfortunately we did not succeed in retrieving the bacteria. The oral part of the intestinal tract in mink is almost sterile, and compared to other species of animals there is a sparse flora in the aboral part of the intestinal tract. The probable explanation for this is the short intestinal passage time (3-4 h).

Microbial pathogenicity is a complex phenomenon involving many mechanisms. One of these is adherence to host epithelium. There is a clear correlation between ability of adherence of pathogenic *E. coli* strains and their ability to cause disease *(Krogfelt* 1991). Adhesion per se does not cause disease but makes it possible for other virulence factors to take effect. Adhesion is often mediated by fimbriae or adhesins.

Human Enteropathogenic *Escherichia coli* (EPEC) with the gene sequence *eae*, which adhere to the intestinal epithelium and efface microvilli have been shown to produce attaching and effacing (A/E) lesions *(Rothbaum et al.* 1982).

In rabbits *E. coli,* O15:H- produce A/E lesions similar to those of human EPEC. This strain is therefore used in rabbits in an experimental model for human EPEC *(Okerman* 1987). As seen in Table 1, this serotype was found in this study.

Some *E. coli* produce exotoxins such as the classical LT, ST and VT. Other toxins produced by *E. coli* are Entero Aggregative Heat Stable Toxin (EAST)(Savarino et al., 1993) produced by Entero Aggregative *Escherichia coli* (EAggEC), CytoLethal Distending Toxin (CLDT) *(Scott & Kaper* 1994) and Cytotoxic Necrotizing Factor (CNF) *(Oswald et al.* 1991). In this investigation it has not been possible to examine for these 3 toxins.

The *E. coli* strains found in this study probably belong to the non-enterotoxic *E. coli.* It is most likely that none of the strains belong to known human pathogenic strains. The high diversity in serotypes does not indicate any common virulence factors. In spite of this it occurs likely, that unknown virulence factors are present in these *E. coli* strains. The pathological changes in the intestines with oedema and hyperaemia in the intestinal epithelium indicate the presence of toxins *(Henriksen* 1994). It is also possible that the methods used *in vitro* do not sufficiently imitate the conditions *in vivo.* Thus it has recently been shown, that a *Pseudomonas* strain which does not express LPS in vitro actually expresses LPS when colonising the lungs *(Cohen* 1994).

When 13 strains are *eae*+ and 2 produce haemolysin, they might contain virulence factors possibly contributing to the pathogenesis. It has been documented, that expression of the cromosomal gene *eae* is regulated at the transcription level by a trans-acting, positive regulator encoded on a plasmid *(Gomezduarte & Kaper* 1995). These strains from mink have not been examined for plasmids, and therefore it cannot be excluded that there are differences in the

plasmid profiles in strains from healthy and diseased mink.

The importance of the findings of corona-, calici- and rotavirus is unknown but interesting. In a scientific work by *Svansson* (1991) it appears that especially corona- and calicivirus are widely spread among Danish mink, but presumably they do not have any primary importance for diarrhoea in mink. Svansson too found reo-, calici and coronavirus in the faeces from healthy and diseased mink kits. A single study showed greater incidences of rotavirus in faeces from "sticky kits" than from healthy kits *(Henriksen* 1987). A preliminary study in 1994 using Rota test strip (On-Site Biotech, Uppsala, Sweden) showed rotavirus in faeces from grown mink with "3 days weakness" and no virus in faeces from healthy mink.

Conclusion

The *E. coli* and virus found in this study are not found to be obligate pathogenic with the methods used, as *E. coli* with virulence factors and virus are found in healthy and diseased kits respectively. It is more likely to consider them as potential pathogens, causing disease in weakened kits, e.g. because of dietetic diarrhoea or dehydration. Some times it seems as if "sticky kits" is a contagious disease. This can be explained by the presence of these disease germs. If there is one or more of the former mentioned germs present they can provide *E. coli* and virus respectively.

The results from this investigation leave some questions for further investigations:

- a wider explanation of *E. coli* and their virulence factors in mink,
- the importance of the virus found is still unclear. Challenge on seronegative animals would be relevant in order to make a deeper investigation of the pathogenesis.

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Sammendrag

Escherichia coli og virus isoleret fra fedtede minkhvalpe.

I alt 121 *Escherichia coli* stammer isoleret fra fæces fra 3 uger gamle minkhvalpe blev serotypet og undersøgt for virulensfaktorer. 56 stammer blev isoleret fra raske hvalpe, mens 65 stammer blev isoleret fra fæces fra »fedtede hvalpe«. Blandt disse blev fundet 34 forskellige serotyper. Der blev ikke iagttaget forskelle i serotyper og tilstedeværelse af virulensfaktorer mellem raske og syge hvalpe. Ved elektronmikroskopering af fæcesprøver blev der iagttaget corona-, calici-, og rotavirus såvel i raske som syge hvalpe.

(Accepted February 23, 1996).

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