Brief Communication
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Quantitative Analysis of Fecal Flora in Goat Kids with and without Floppy Kid Syndrome

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Background: Floppy kid syndrome (FKS) affects goat kids in the first month of life and is associated with high morbidity and mortality rates. The condition is characterized by neurological signs that can be ascribed to increased plasma D-lactate concentrations. The source of D-lactate has not been identified conclusively, but D-lactate-producing bacteria in the large intestine are thought to be involved.

Objectives: To determine the number of colony-forming unit (CFUs) of certain groups of bacteria in the feces of kids with and without FKS.

Animals: Nineteen goat kids with clinical signs of FKS, acidemia (pH $\leq$ 7.2), and plasma D-lactate concentration >7 mM and 15 healthy goat kids without acidemia (pH >7.2) and D-lactate concentration <1 mM.

Methods: In this case-control study, the goat kids were examined clinically and blood was collected to measure D-lactate concentration, blood gases, and acid–base parameters. Fecal samples were collected and the total aerobic bacterial count and CFU counts of coliforms, enterococci, staphylococci, streptococci, lactobacilli, and clostridia were determined using the surface plating method.

Results: Goat kids with FKS had a mean plasma D-lactate concentration of 10.9 $\pm$ 3.7 mM compared with 0.3 $\pm$ 0.9 mM in healthy kids, and significantly greater CFU counts for enterococci, streptococci, staphylococci, and lactobacilli than healthy kids.

Conclusions and Clinical Importance: The groups of bacteria present in greater numbers in the feces of goat kids with FKS include several D-lactate-producing species, which makes dysbacteriosis a likely cause of the increased plasma D-lactate concentration in FKS.

Key words: Acidosis; D-lactate; Dysbacteriosis; Goat kids.

Floppy kid syndrome (FKS) is characterized by wobbly gait and knuckling in the forelimbs with progressive listlessness and eventual somnolence or even coma. Affected goat kids do not have diarrhea or septicemia.[1] This syndrome was first described in 1991 and has since been seen in North America and Europe as a disease of 4- to 30-day-old goat kids.[1–3] Mortality rates may increase gradually during the kidding season and be as high as 60%.[2,4] Blood gas analysis identified metabolic acidosis in the absence of diarrhea and increased anion gap caused by increased D-lactate concentrations with an average of 7.43 $\pm$ 6 2.71 mM (reference range, 0.26 $\pm$ 0.24 mM).[1] The mechanism of increased D-lactate production has not been identified. However, because mammals produce only small amounts of D-lactate via the methyl-glyoxal pathway, the most likely source is bacterial fermentation of carbohydrates in the gastrointestinal tract.[5] This mechanism would explain D-lactic acidosis in animals receiving large amounts of rapidly fermented carbohydrates, but this type of feed is not normally part of the ration of young goat kids. It is therefore assumed that excessive proliferation of D-lactate-producing bacteria in the gastrointestinal tract results in the generation of large amounts of D-lactate, which then are reabsorbed.[1,2] If this is correct, the gastrointestinal flora of affected kids and healthy controls should differ. The goal of this study therefore was to compare the fecal flora of kids with FKS to that of healthy kids by determining fecal bacterial counts for defined microbial species.

Abbreviations:

BE	base excess
CFU	colony-forming unit
FKS	floppy kid syndrome
PCO_2	partial pressure of CO_2
TABC	total aerobic bacterial count

Materials and Methods

Animals and Collection of Samples

The group of goat kids with FKS (group F) consisted of 5 males and 14 females from 11 herds in the eastern and southern parts of Switzerland. Breeds included Oberhasli, Saanen, Toggenburg, Valais Blackneck, and Nera Verzasca. They ranged in age from 3 to 16 days, and all were presented for evaluation because of failure to nurse, listlessness, or recumbency. The control group (group C) consisted of 6 male and 9 female kids from 5 herds in the canton of Zurich. Breeds included Oberhasli and Saanen, and the kids ranged in age from 5 to 18 days. All kids underwent a clinical examination before collection of blood from a jugular vein for measurement of PCO_2, pH, plasma bicarbonate concentration, and base excess (BE).[a] For the determination of

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the D-lactate concentration, blood was centrifuged and plasma harvested and stored at −20°C. Analysis was carried out at the Clinic for Ruminants and Ambulatory Field Service Clinic and Herd Health, Ludwig-Maximilians-University Munich using an enzymatic test.[6,b]

Only kids between 3 and 20 days old that had not received any treatment and had a rectal temperature <39.6°C were included in the study. Group F did not contain animals with other diseases, particularly diarrhea, and group C included only healthy kids. Additional requirements for inclusion in group F were venous blood pH ≤7.2 and D-lactate concentration >7 mM. The D-lactate concentration was <1 mM in kids of group C. At least 1 g of feces that was passed spontaneously or after gentle stimulation of the rectum with a thermometer was collected, transported to the laboratory at 4°C, and examined bacteriologically immediately or after storage at −20°C for a maximum of 4 weeks.

Bacteriological Examination

The spread-plate method was used for culture, and bacterial counts were obtained for the following groups of organisms: total aerobic bacteria, coliforms, enterococci, staphylococci and streptococci, lactobacilli, and clostridia. Except for the total aerobic bacterial count, a variety of selective media were used for all groups. The total aerobic bacterial count was determined by counting the colonies on Colombia blood agar plates[c] and the number of coliforms by counting all dark green lactose-positive colonies on Gassner agar plates.[c] Enterococcal colonies were counted on bile esculin azide agar plates[c] and staphylococcal and streptococcal colonies on Colombia CNA agar plates.[c] Plates were incubated aerobically for 24 hours at 37°C. De Man Rogosa Sharpe agar[c] was used to grow lactobacilli under aerobic conditions for 72 hours at 30°C. Clostridial colonies were counted on lactose egg yolk agar plates after strict anaerobic incubation at 37°C.[d,7]

A geometric dilution series was used for surface plating of the fecal samples; this was accomplished by making serial 10-fold dilutions with 0.15 M NaCl solution from an initial fecal suspension to a final dilution step of 10.[9] From each dilution step, 0.1 mL was plated on the different agar plates in duplicate. The number of bacteria per gram of feces was calculated based on the number of CFU, the volume applied to the agar plate and the dilution step using the following formula[8]:

$$c = \frac{\sum a}{n1 \times n2 \times 0.1} \times d$$

where c, weighted arithmetic mean of number of CFU; Σa, sum of CFU of all plates that were used for calculation (lowest and next higher dilution step); $n1$, number of plates of the lowest dilution step that could be evaluated; $n2$, number of plates with the next higher dilution step that could be evaluated; d, fold-step of the lowest dilution that could be evaluated.

Statistical Analysis

For comparison of blood gas and acid–base parameters, D-lactate concentrations and number of CFUs for the various group of organisms between groups F and C, the Shapiro–Wilk test was applied to test for normal distribution.[e] For all group of organisms, numbers of CFUs were not normally distributed, and therefore various transformations were tested to identify the one that generated the best linearity. Square root transformation was used for the total aerobic bacterial count, coliforms and clostridia, and logarithmic transformation was used for enterococci, streptococci and staphylococci, and lactobacilli. Differences in the transformed variables and in normally distributed nontransformed variables between groups were analyzed using a 2-sample t-test. Differences were considered significant at $P < .05$.

This study was approved (05/2011) by the Committee for the Permission of Animal Experimentation of the Canton of Zurich.

Results

All goat kids of group C were healthy. There was some degree of failure to nurse normally in all goat kids of group F. Thirteen of the 19 kids with FKS were obtunded to somnolent and the remaining 5 were comatose. Twelve had an unsteady gait and knuckling in the front feet, and the other 7 were in sternal or lateral recumbency.

The pH, PCO_2, plasma bicarbonate concentration, and BE were significantly lower in group F than in group C (Table 1) and the D-lactate concentration was significantly higher in group F (10.9 ± 3.7 mM) than in group C (0.3 ± 0.9 mM).

Goat kids in group F had significantly higher CFU counts/ml of fecal suspension for enterococci, streptococci and staphylococci, and lactobacilli than kids in group C (Fig 1). The 2 groups did not differ with respect to total aerobic bacterial count and CFU counts for coliforms and clostridia.

Discussion

A variety of predisposing factors and causes of FKD has been proposed.[4,9,10] An effect of breed and specific disease-causing agents such as rotaviruses, enteropathic *Escherichia coli*, *Campylobacter jejuni*, *Cryptosporidium parvum*, *Giardia intestinalis,* and *Clostridium perfringens* type D could not be established.[1] Recent studies have shown that bottle or bucket feeding could reduce morbidity.[2,4] Furthermore, one of these studies indicated that the number of kids with FKS increased gradually as the kidding season progressed, which was coincident with gradually increasing atmospheric temperatures, and at the end of the kidding season, the incidence of FKD had increased to 70%.[2] However, the relationship between the seasonal

Table 1. Blood gas and acid–base variables (mean ± SD) in the venous blood of 19 kids with FKS (group F) and 15 healthy controls (group C).

Variable	Group	Mean (SD)	*P*-Value
pH	F	7.14 (0.09)	<.0001
	C	7.30 (0.05)	
PCO2 (mmHg)	F	33.5 (7.9)	<.0001
	C	54.3 (6.1)	
Bicarbonate (mM)	F	10.7 (4.0)	<.0001
	C	25.4 (2.8)	
BE (mM)	F	−17.6 (5.1)	<.0001
	C	−1.4 (3.1)	
D-lactate (mM)	F	10.9 (3.7)	<.0001
	C	0.3 (0.9)	

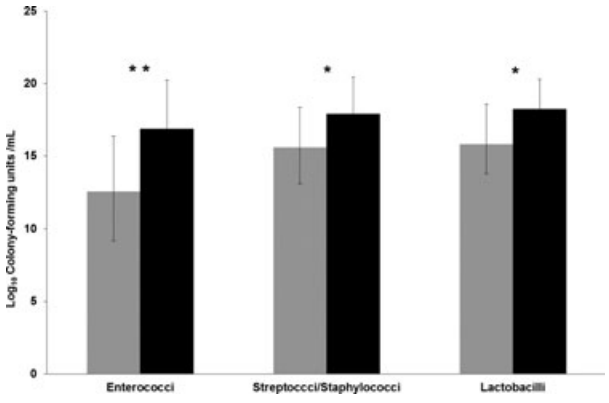


Fig 1. Log_{10} colony-forming unit (CFU) per mL feces for enterococci, streptococci and staphylococci, and lactobacilli in 15 healthy kids (gray) and 19 kids with FKS (black) (*$P < .01$, **$P < .001$).

occurrence of FKS and possible causes of D-lactic acidosis remains unclear.

D-lactic acidosis also has been described in cattle and people.[11] In cattle, high D-lactate concentrations occur in ruminating adult animals with rumen acidosis because of excessive consumption of easily digestible carbohydrates, and in milk-fed calves with ruminal drinking, in which milk enters into the rumen because of esophageal groove dysfunction. In a previous study, the pH of rumnial content did not differ between goat kids with or without FKS.[1] The feces sampled for this study reflect the bacterial flora of the lower large intestine. However, mucosa-associated bacteria differ from those recovered from feces and are rather uniformly distributed throughout the colon.[12] Both diseases are associated with bacterial fermentation and generation of D-lactate and other substances. Large amounts of D-lactate mainly are produced in the large intestine of diarrheic calves because of villus atrophy and malabsorption of carbohydrates in the small intestine and subsequent fermentation of the carbohydrates in the large intestine.[13] An analogous clinical presentation occurs in humans with short bowel syndrome after small intestinal resection and in individuals born with shortening of the small intestine; both conditions are characterized by increased amounts of carbohydrate reaching the large intestine. In a 5-year-old boy with short bowel syndrome, the number of D-lactate producing fecal bacteria increased 2 days before the occurrence of clinical signs.[14] Similarly, the kids of group F had higher numbers of certain fecal bacteria, including enterococci, streptococci, staphylococci, and lactobacilli, than kids of group C. All but the staphylococci belong to the order of *Lactobacillales*, which comprise the lactic acid bacteria and are characterized by their ability to ferment sugar to lactic acid.[15] Most of the *Enterococcus* and *Steptococcus* genera recovered from the feces in group F produce L-lactate and some also produce D-lactate. In contrast, the *Lactobacillaceae* family includes genera that selectively produce D- or L-lactate, and others that produce both.[16] Previous studies have shown that the L-lactate concentration in the blood of kids with FKS is very low, and therefore this compound was not measured in this study.[1,4] A possible reason for the low L-lactate concentration is that this compound is much more rapidly eliminated than D-lactate by mammals. This leads to a preferential accumulation of D-lactate in the blood of affected kids and the typical clinical signs. Thus, similar to short bowel syndrome in people, FKS appears to be accompanied by dysbacteriosis associated with a shift in the intestinal flora toward lactate-producing bacteria. However, the factors leading to dysbacteriosis in kids that are only a few days old remain unclear. Furthermore, to design an effective treatment for FKS and to prevent the shift in intestinal flora, it would be helpful to identify the bacteria responsible for the high D-lactate concentration. Based on such a discovery, it may be possible to find the reason for the dysbacteriosis.

Footnotes

[a] Rapidlab 248, Bayer, Zurich, Switzerland
[b] Hitachi Automatic Analyser 911, Roche, Basel, Switzerland
[c] Oxoid, Pratteln, Switzerland
[d] Biotest, Rupperswil, Switzerland
[e] STATA, StataCorp, College Station, TX

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Conflicts of Interest: Authors disclose no conflict of interest.

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