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1 **Changes in faecal bacteria and metabolic parameters in foals during the first**
2 **six weeks of life**

3 Juliane Kuhl^{1,4*}, Nora Winterhoff², Manuela Wulf¹, Florian J. Schweigert³,
4 Ilse Schwendenwein⁴, Rupert M. Bruckmaier⁵, Jörg E. Aurich⁴,
5 Peter Kutzer², Christine Aurich^{1,4}

6
7 ¹*Graf Lehndorff Institute for Equine Science, Neustadt (Dosse), Germany*

8 ²*Berlin - Brandenburg State Laboratory, Frankfurt (Oder), Germany*

9 ³*Institute for Nutritional Science, University of Potsdam, Potsdam, Germany*

10 ⁴*University of Veterinary Science, Vienna, Austria*

11 ⁵*Institute for Physiology, Vetsuisse Faculty, University of Berne, Berne, Switzerland*

12
13 **Corresponding author at Graf Lehndorff Institute for Equine Science*

14 *16845 Neustadt (Dosse), Germany, e-mail juliane.kuhl@vetmeduni.ac.at,*

15 *tel. +43-125077-6405, fax +43-125077-5490*

16

17

18 **Abstract**

19

20 Many foals develop diarrhoea within the first two weeks of life which has been
21 suggested to coincide with postpartum oestrus in their dams. To analyse the
22 pathogenesis of this diarrhoea we have determined faecal bacteria in foals and their
23 dams (n=30 each), and serum IGF-1 and γ -globulins for 6 weeks after birth. In
24 addition, effects of β -carotene supplementation to mares (group 1: 1000 mg/day,
25 n=15, group 2: control, n=15) on diarrhoea in foals were studied. Diarrhoea occurred
26 in 92 and 79% of foals in groups 1 and 2, respectively, but was not correlated with
27 oestrus in mares. Beta-carotene supplementation was without effect on foal
28 diarrhoea. In mares, bacterial flora remained stable. The percentage of foals with
29 cultures positive for *E. coli* was low at birth but increased within one day, the
30 percentage positive for *Enterococcus sp.* was low for 10 days and for *Streptococcus*
31 *sp.* and *Staphylococcus sp.* was low for 2 to 4 weeks. By 4 weeks of age, bacterial
32 flora in foals resembled an adult pattern. Concentration of serum IGF-1 was low at
33 birth (group 1: 149 ± 11 , group 2: 166 ± 17 ng/ml), increased after day 1 (day 7 group 1:
34 384 ± 30 , group 2: 372 ± 36) but at no time differed between groups. Serum γ -globulin
35 concentration in foals was low before colostrum intake and highest on day 1 ($p<0.001$
36 over time). In conclusion, neonatal diarrhoea in foals does not coincide with
37 postpartum oestrus in their dams but with changes in intestinal bacteria and is not
38 influenced by β -carotene supplementation given to mares.

39

40 **Keywords:** foal; diarrhoea; faecal bacteria; IGF-I; carotene supplementation

41

42 1. Introduction

43

44 Many foals develop transient diarrhoea in the first two weeks of life. This condition
45 has been suggested to coincide with first oestrus after parturition in their dams and is
46 often named foal heat diarrhoea. This diarrhoea is non-infectious and self-limiting but
47 little is known about the causative factors. Changes in milk composition (Johnston et
48 al. 1970), postnatal maturation of the gastrointestinal tract and establishment of
49 intestinal bacterial flora have been suggested (Masri et al. 1986). Foals are born with
50 virtually no immunoglobulins and passive transfer of maternal immunoglobulins via
51 colostrum is required to provide protection against infections in the neonatal period
52 (McGuire et al. 1977, LeBlanc et al. 1992). In addition to immunoglobulins, colostrum
53 contains a variety of growth factors (Hess-Dudan et al. 1994, Berg et al. 2007, Stel-
54 wagen et al. 2009). In cattle and pigs, positive effects of colostrum on development of
55 the intestinal mucosa have been demonstrated (Xu 1996, Bühler et al. 1998).

56

57 Beta-carotene is present in equine colostrum at high concentrations (Schweigert and
58 Gottwald 1999) and transferred to the foal via colostrum (Kuhl et al., 2010). In calves,
59 β -carotene status may affect the occurrence of diarrhoea (Kume and Toharmat
60 2001). Clinical reports but no controlled studies indicate positive effects of β -carotene
61 supplementation to mares on diarrhoea in their foals (Enbergs and Klemm 1987).

62

63 In the current study, changes in faecal bacteria and parasites of newborn foals and
64 their dams were analysed from foaling until 6 weeks after birth. We hypothesized that
65 foal heat diarrhoea is not linked to postpartum oestrus but is associated with changes
66 in intestinal bacterial flora. Factors potentially influencing intestinal maturation,
67 function and immunity such as IGF-1 concentration and serum γ -globulins were
68 analysed as well. In addition, effects of β -carotene supplementation to mares on
69 faecal bacterial flora and occurrence of neonatal diarrhoea in their foals were studied.

70 2. Material and Methods

71

72 2.1. Animals and sampling procedures

73

74 A total of 30 mares and their foals of the Brandenburg State Stud (Neustadt/Dosse,
75 Germany) were included into the study. Foals were born between March and May.

76 Details on the animals are given in table 1.

77

78 Mares were kept in group stables and had access to an outdoor paddock for several
79 hours every day. Approximately two weeks before the expected date of foaling,
80 mares were transferred to individual boxes and were returned to their group together
81 with the foal 5 days after parturition. Mares were fed concentrates and hay twice daily
82 and had free access to fresh water at all times. Foals were fed a pelleted foal starter
83 in an area of the group stable not accessible to the mares and had also access to the
84 mares' hay. When the foals had reached an age of 3 to 4 weeks, the group was
85 turned out on pasture. Mares were dewormed with ivermectin (Eraquell, Virbac, Bad
86 Oldesloe, Germany) on the day of foaling and on day 28 thereafter if not yet pregnant
87 again. All foals received ivermectin on day 28 of life. Three foals were excluded from
88 the study for reasons not related to the experiment (one foal treated for meconium
89 impaction in a veterinary clinic from days 2 to 7 of life, one foal receiving systemic
90 antibiotic treatment because of a superficial wound with local swelling on the first day
91 of life, one foal receiving antibiotic treatment because of profound diarrhoea with
92 signs of clinical illness between days 22 and 32 of life).

93

94 2.2. Experimental design

95

96 Mares were tested daily for oestrous behaviour with a teaser stallion, beginning on
97 day 6 postpartum. In addition, signs of oestrus were checked by rectal palpation and

98 ultrasonography of the ovaries and uterus at one to two-day intervals. Oestrus was
99 defined using standard criteria (Behrens et al. 1993). Foals were checked daily for
100 signs of diarrhoea and/or illness. The presence of watery faeces, smearing of faeces
101 and loss of hair in the perianal area were used as indicators for diarrhoea. To
102 characterize diarrhoea, a score from 0=absent, 1=mild, 2=moderate to 3=high grade
103 diarrhoea was used.

104

105 For bacteriological analysis, faecal samples were taken from the rectum of the
106 horses using a sterile swab (WDT, Garbsen, Germany) on day -14, 0 (=day of
107 foaling), 14, 28, 42 in mares and on day 0 (=day of birth), 4, 6, 8, 10, 12, 14, 28, 30,
108 32, 34, 36 in foals, respectively. The sampling regime takes into account the
109 expected time of first and second postpartum oestrus in the mares. To observe
110 infestation with intestinal parasites, faecal samples from the mares were taken on
111 days -14, 0, 14, 28 and 42. At the time of collection of faecal swabs from foals, it was
112 always attempted to take also faecal material for parasitological analysis but
113 sufficient amounts of faeces for parasitology (50 g) could not be obtained at all times.
114 The number of samples evaluated is indicated in the results section. Bacteriologic
115 samples were placed in transport medium (Amies medium without charcoal, WDT),
116 chilled at 5°C and transported to the laboratory within 24 hours. Samples for
117 parasitological analysis were shipped without transport medium.

118

119 Blood samples for plasma and serum analysis were taken via jugular venipuncture
120 on day -14, -7, 0, 7, 14, 21, 28, 34, 42 in mares and on day 0 (= within 1 hour after
121 birth and before first nursing), 1 (=18-36 hours after birth), 3, 7, 21, 28, 34, 42 in
122 foals. Plasma was centrifuged at 1600 g for 10 min, serum was centrifuged at 2200 g
123 for 15 min after a waiting period of 2 hours at room temperature. Aliquots of all
124 samples were frozen at -20°C until further processing.

125

126 The mares were randomly allocated to two groups. Mares of group 1 (n=15) received
127 β -carotene orally (1000 mg per day; Blattiviko beta 8000, Blattin Mineralfutterwerk
128 Seitschen, Göda, Germany) from 14 days before the expected date of foaling to 42
129 days after parturition. Mares of group 2 (n=15) remained untreated as controls. The
130 study was approved by the Committee on Animal Experimentation of the Bran-
131 denburg State Office for Consumer Protection, Agriculture and Rural Development.

132

133 2.3. Experimental procedures

134

135 Detection and identification of bacteria in faecal swabs

136 Faecal swabs were plated onto a set of standard media including Columbia agar
137 supplemented with 5% sheep blood (CA), Gassner agar (GA), Yersinia selective agar
138 (YA; all Oxoid, Wesel, Germany), and anaerobic agar supplemented with 5% sheep
139 blood and 0.01% neomycin (AA) (Heipha, Eppelheim, Germany). Incubation was
140 performed as follows: CA and GA aerobically at 36°C overnight, YA aerobically at
141 29°C for 48 hours, and AA anaerobically at 36°C overnight. For the detection of
142 salmonellae, the swab was inoculated into 10 ml of Preuss's potassium tetrathionate
143 enrichment broth (Merck, Darmstadt, Germany). After overnight incubation at 36°C,
144 aliquots of 10 μ l were plated onto Xylose Lysine Desoxycholate agar and Brilliance
145 Salmonella agar (Oxoid). Inoculated agar plates were incubated again at 36°C
146 overnight. Identification of bacterial isolates was performed by standard
147 bacteriological procedures described elsewhere (Songer and Post 2005).

148 Bacterial quantities on solid media (except post-enrichment) were classified as no
149 growth (score 0), sparse growth (<30 colony forming units, cfu; score 1), moderate
150 growth (30-100 cfu; score 2) and pronounced growth (>100 cfu; score 3).

151

152 Parasitology

153 Flotation using a saturated sodium chloride solution (specific gravity of 1.18-1.20),
154 sedimentation and the Baermann technique were carried out for each faecal sample.
155 Parasitic development stages were identified by morphologic characteristics using a
156 reticule equipped light microscope (magnification 100-400x).

157

158 Insulin-like growth factor 1

159 Serum IGF-1 in foals was measured by RIA using a non-extraction method
160 (Daxenberger et al. 1998). The antiserum was raised in rabbits against bovine IGF-1
161 and showed a cross-reactivity with IGF-2 <0.002%. Intra and interassay coefficients
162 of variation were 5.1 and 13.4%, respectively.

163

164 Gamma-globulins and total protein

165 Serum electrophoresis was performed by an automated electrophoresis system and
166 densitometric scanning (Menarini Diagnostics, Vienna, Austria). A proportion of the
167 20 µl serum sample was blotted on a cellulose acetate strip with a plastic support
168 (Mylor). The strip was transferred into the migration chamber filled with buffer
169 solution (1.09% Tris-hydroxymethyl-aminomethan and 0.5% 55-diaethylbarbiturate-
170 sodium), the migration time was 15 min at 140 V. After migration, the strip was
171 blotted and transferred into a staining solution (Ponceau-red-5, 1.5% trichloroacetic
172 acid) for 180 sec and then transferred into a destaining solution (5% citric acid) for
173 120 sec and into a clearing solution (32% N-methyl-pyrrolidon, acetic acid, methanol)
174 for 120 sec. Drying took 620 sec. Densitometric scanning was performed in green
175 light and the readings were performed with Efolab software (Menarini Diagnostics).

176

177 Statistical analysis

178 Statistical comparisons were made with the SPSS statistics package (version 17.0
179 SPSS, Chicago, Illinois, USA). Because part of the data are based on scoring, non-
180 parametric tests were used throughout. For comparisons between times within

181 groups test for related samples were used, taking into account the repeated
182 measures in the same animals (Friedman-test: > two time points, Wilcoxon-test: two
183 time points). Comparisons between groups for individual time points were made by
184 Mann-Whitney-U-test. A p-value <0.05 was considered significant. All data given are
185 means \pm standard error of mean (S.E.M.).

186 **3. Results**

187

188 Clinical findings

189 All but one mare showed signs of oestrus within two weeks after foaling with a mean
190 interval of 9.2 ± 0.4 days until first observation of oestrus in group 1 and 8.6 ± 0.5 days
191 in group 2. Occurrence of first postpartum oestrus did not differ between groups
192 (figure 1). All foals were viable at birth and no problems with regard to first standing
193 and suckling and colostrum intake were observed. Diarrhoea occurred in 92 and 79
194 % of foals in groups 1 (foals born to β -carotene supplemented mares) and 2 (foals
195 born to control mares), respectively, at least once during the study period (n.s.).
196 Diarrhoea was seen for 4.1 ± 1.0 and 2.6 ± 0.8 days in groups 1 and 2, respectively,
197 and was detected for the first time on day 11.0 ± 1.8 and 17.9 ± 3.0 in foals of group 1
198 and 2 (n.s.). Within the first two weeks of life 62% and 43% of foals in groups 1 and
199 2, respectively, showed diarrhoea at least on one day (n.s.). With the exception of the
200 first 5 days and days 24 to 42 of life, the occurrence of diarrhoea was spread over
201 the whole observation period and not correlated to signs of first postpartum oestrus in
202 the mares (figure 1).

203

204 Bacteriology

205 A variety of bacterial species considered as markers of gastrointestinal flora was
206 isolated from faecal swabs. Bacteria species isolated at least in two individuals per
207 group and at least on two days were *E. coli*, *Enterococcus* sp., β -haemolytic
208 *Streptococci* and coagulase-negative *Staphylococci* in both mares and foals,

209 *Acinetobacter* sp. in mares only and *Proteus* sp. and *Klebsiella* sp. in foals only. In
210 foals *Pantoea* sp., *Acinetobacter* sp., *Clostridium perfringens* and *Citrobacter* sp.
211 were seen less frequently, i.e. in less than two individuals per group or not on two
212 days. Only occasionally *Pseudomonas* sp., *Staphylococcus intermedius*, *Bacillus* sp.,
213 *Acinetobacter* sp., *Corynebacterium* sp. and *Enterobacter* sp. were detected.
214 Coagulase-positive *Staphylococci*, *Kluyvera* sp., *Arthrobacter* sp. and apathogenic
215 *Yersina enterocolitica* occurred in one sample each. In mares, single isolates of
216 *Proteus* sp., *Pseudomonas* sp., *Pantoea* sp. and *Bacillus* sp. could be detected.
217 *Klebsiella* sp. and *Citrobacter* sp. were found in one sample each.

218

219 In mares, bacterial flora remained largely stable throughout the period studied. *E. coli*
220 was present in all swabs of group 1 and 2 mares throughout the study except for two
221 swabs of group 2 on the day of foaling. *Enterococcus* sp. was isolated over the whole
222 observation period from between 57 to 93% of samples with no significant
223 differences between groups. The occurrence of β -haemolytic *Streptococci* decreased
224 at the time of parturition in both groups compared to all later time points ($p=0.05$).
225 Coagulase-negative *Staphylococci* did not change significantly over time in group 1.
226 In group 2, they were detected in fewer samples on day 0 and 42 compared to day
227 -14 ($p<0.05$) but did not differ when day -14 was compared to days 14 and 28 and on
228 day 0 compared to days 14 and 42 (figure 2a and c). Average intensity of bacterial
229 growth is summarized in table 2.

230

231 In foals, occurrence of bacterial species and intensity of growth changed over time.
232 *E. coli* occurred in 2 out of 13 and 5 out of 14 samples in groups 1 and 2, respective-
233 ly, directly after birth (n.s.). On day four 12/13 and 13/14 faecal swabs in groups 1
234 and 2, respectively, (n.s.) contained *E. coli* which could be isolated in all samples
235 from the age of 10 days onwards (Figure 2b). *Enterococcus* sp. were present in 4/13
236 and 6/14 samples of group 1 and 2, respectively, on the day of birth and in 5/13 and

237 2/14 swabs of group 1 and 2 respectively on day 8. After the first week of life, the
238 percentage of samples positive for *Enterococcus* sp. increased significantly ($p<0.05$)
239 in group 2 but only tended to increase in group 1 ($p=0.08$). On day 32, *Enterococcus*
240 sp. occurred in more faecal swabs from foals of mares treated with β -carotene than
241 from foals of non-treated mares ($p<0.05$; figure 2b). Beta-haemolytic *Streptococci*
242 were first isolated on day 4 of life and until day 10 detected in only 1 to 2 samples in
243 both groups. On day 30, β -haemolytic *Streptococci* were detected in more samples,
244 i.e. 9/12 in group 1 and 8/10 in group 2, compared to day 8 ($p<0.05$). No samples
245 could be obtained from one foal in group 1 and 4 foals in group 2. Coagulase-negati-
246 ve *Staphylococci* were isolated in 10/13 and 12/14 swabs in groups 1 and 2, respecti-
247 vely, at the day of birth and in none (group 2) or only one (group 1) of the samples
248 until day 10 where 3/11 and 2/14 samples in group 1 and 2, respectively, were positi-
249 ve for coagulase-negative *Staphylococci*. Thereafter, they were present in up to 50%
250 and 36 % of samples in groups 1 and 2, respectively. *Clostridium perfringens* was
251 present in one sample of 3 foals ($n=2$ group 1, $n=1$ group 2), in two consecutive
252 samples of one foal (group 1) and in 4 consecutive samples of two foals (group 2).
253 *Clostridium perfringens* could be detected during the first two weeks of life only and
254 not at later times. Its occurrence was not associated with diarrhoea in these foals.
255 Intensity of bacterial growth in faecal swabs of foals is summarized in table 3.

256

257 Parasitology

258 Eggs of small strongyles occurred in 12 of 15 faecal samples in mares of both groups
259 on day -14 and in 13 (group 1) and 12 (group 2) of 15 samples on the day of
260 parturition. Eggs of large strongyles occurred in 7 (group 1) and 2 (group 2) of 15
261 faecal samples on day -14 (n.s.) and in 8 (group 1) and 5 (group 2) of 15 samples on
262 the day of parturition (n.s.). No eggs of endoparasites could be detected thereafter. In
263 the foals, one faecal sample was positive for eggs of small strongyles on day 8. In

264 the remaining samples (n=32) parasitic stages were non-detectable throughout the
265 study period.

266

267 IGF-1

268 Concentrations of IGF-1 in serum of foals were low on the day of birth (149 ± 11 and
269 166 ± 17 ng/ml in groups 1 and 2, respectively) and decreased slightly but significantly
270 ($p < 0.05$) until day 1 (133 ± 13 and 154 ± 12 ng/ml in groups 1 and 2, respectively).
271 Thereafter, IGF-1 concentrations increased continuously during the first week of life
272 (day 7: 384 ± 30 and 372 ± 36 ng/ml in groups 1 and 2, respectively, $p < 0.001$ versus
273 day 0). No further changes were seen between days 7 and 42. At no time serum IGF-
274 1 concentrations differed between foals born to β -carotene-treated mares and control
275 mares (figure 3a).

276

277 Gamma-globulins

278 Concentration of γ -globulins in serum was lowest on the day of birth before colostrum
279 intake (group 1: 315 ± 56 , group 2: 317 ± 51 mg/dl), and highest on day 1 (group 1:
280 958 ± 140 , group 2: 943 ± 161 mg/dl, $p < 0.01$ versus day 0). Thereafter, γ -globulin
281 concentration decreased continuously during the first 4 weeks of life (day 28: 492 ± 46
282 and 509 ± 62 mg/dl in groups 1 and 2, respectively, $p < 0.05$ versus day 1). Serum γ -
283 globulin concentrations at no time differed between foals born to β -carotene-treated
284 and control mares (figure 3b).

285

286 **4. Discussion**

287

288 The majority of foals showed signs of diarrhoea for some time during the first 6
289 weeks of life. Diarrhoea was transient and none of the foals received antibiotic or
290 other treatment. Diarrhoea in the foals occurred throughout the experimental period
291 but was most pronounced during the second and third week of life. There was no

292 temporary relation between diarrhoea in foals and first or second postpartum oestrus
293 in their dams. During the first two weeks of life, diarrhoea occurred in approximately
294 50% of foals only. While older studies have reported a prevalence of foal heat
295 diarrhoea between 75 and 80% (Rumbaugh 1983, Masri et al. 1986), the total
296 prevalence of diarrhoea in newborn foals between days 5 and 15 of life in our study
297 is in the same range as reported recently by Sgorbini et al. (2008). Onset and
298 duration of diarrhoea in foals of our study was in accordance with occurrence of
299 diarrhoea in orphan foals raised on a milk replacer diet (Cymbaluk et al. 1993). Foal
300 diarrhoea is thus neither an effect of first postpartum oestrus in their dams nor does it
301 occur in all foals. Because no close temporal relation between foal heat and
302 diarrhoea in foals exists, the term foal heat diarrhoea is confusing and its adequacy
303 should be questioned.

304

305 Beta-carotene supplementation to periparturient mares was without effect on foal
306 diarrhoea. This contradicts the report by Enbergs and Klemm (1987), however in that
307 study, data were not submitted to critical statistical evaluation. In calves, β -carotene
308 status at 6 days of age affects the occurrence of diarrhoea (Kume and Torhamat
309 2001), however, the aetiology of diarrhoea in calves and foals is not comparable.
310 Diarrhoea in neonatal dairy calves is caused either by inadequate feeding manage-
311 ment or by infectious agents (reviewed by Bartels et al. 2010).

312

313 Frequency and intensity of bacterial growth in faeces of foals underwent changes
314 especially within the first two weeks of life, i.e. at a time when neonatal diarrhoea
315 occurred in most foals. Faecal bacterial flora represents a combination of shed
316 mucosal bacteria and non-adherent luminal bacteria. It can thus be assumed that the
317 changes in faecal bacterial flora observed in our study are due to changes in the
318 intestinal bacterial flora. Changes in intestinal flora occur in all species and have
319 been analysed in depth in human infants (Palmer et al., 2007). In infants, a first

320 rearrangement of the faecal bacterial population occurred around 5 days of age while
321 the transition to an adult-like pattern is found later and usually after the introduction of
322 solid foods. In contrast to humans, foals start to consume small amounts of
323 concentrates already around 10 days of age. To our knowledge, the horse is the only
324 domestic animal species where a non-infectious diarrhoea occurs regularly in most
325 neonates. The temporal coincidence with changes in bacterial flora is no proof yet of
326 a causal relationship and our data do not allow excluding postnatal maturational
327 changes of the intestinal epithelium as a cause of foal diarrhoea.

328

329 The percentage of foals with faecal cultures positive for *E. coli* was low on the day of
330 birth but had increased to near 100% within one day. In contrast, the percentage of
331 foals with faecal cultures positive for *Enterococcus sp.* remained low for approxima-
332 tely 10 days before increasing to the same range as in mares. The percentage of
333 faecal cultures positive for *Streptococcus sp.* and *Staphylococcus sp.* increased even
334 later, i.e. between two and 4 weeks of life. Consistent with other reports which
335 consider *Clostridium perfringens* part of the normal intestinal flora in neonatal foals
336 (Yuyama et al. 2004, Tillotson et al. 2002), its presence was not associated with
337 diarrhoea in the foals of our study. By 4 weeks of age, the bacterial flora in foals
338 largely resembled the spectrum isolated from faeces of their dams, indicating that the
339 intestinal bacterial flora had changed from a neonatal to a postnatal pattern. The time
340 window of these changes was closely correlated with the time range for foal
341 diarrhoea. During the first 10 days of life, in foals also coprophagy can be seen
342 (Kazunori et al.1985, Thompson et al. 1988, Cymbaluk et al. 1993). Microorganisms
343 from the faeces of the mares present on the floor of the stable might therefore
344 colonise the intestinal tract of the foals at this time. Interestingly, diarrhoea occurred
345 in approximately 50% of foals only during the first two weeks of life, although
346 changes in bacterial flora did not differ between foals with and without signs of
347 diarrhoea. Some foals are thus able to develop a postnatal intestinal flora without

348 diarrhoea. This might be an advantage with regard to growth and weight gain in
349 these foals.

350

351 Results of a study using the same deworming regimen of the dams as in our
352 experiment indicated that the regimen was efficient to reduce shedding of
353 *Strongyloides* eggs and that foal diarrhoea was not caused by infestation with
354 *Strongyloides westeri* (Ludwig et al. 1982). As no mare was shedding eggs of
355 endoparasites postpartum, worm burden can largely be excluded as a cause of
356 diarrhoea in our foals.

357

358 Colostrum uptake does not only provide the neonate with immunoglobulins but, at
359 least in piglets, enhances also postnatal maturation of the intestinal epithelium (Xu
360 1996, Jensen et al. 2001, Blum and Baumrucker 2008). In pigs, intestinal maturation
361 is not fully complete before 3 weeks of age (Jarvis et al. 1977). It can be assumed
362 that also in foals, maturation of the intestinal tract at large occurs during a
363 comparable time period and may in part be linked to foal heat diarrhoea.

364

365 In our study, serum IGF-1 reached highest levels within the first week and remained
366 constant thereafter whereas Hess-Dudan et al. (1994) reported an increase over a
367 period of 3 weeks until a plateau phase was reached. Concentrations of IGF-1 in
368 colostrum of mares are high but decrease dramatically within less than 24 hours
369 (Hess-Dudan et al. 1994, Berg et al. 2007). The postnatal increase in IGF-1
370 concentrations in foals therefore is not due to absorption of colostral IGF-1 but to
371 IGF-1 synthesis by the foal. Although, as in calves (Baumrucker et al. 1994) and
372 piglets (Xu 1996), colostral IGF-1 might have local effects on postnatal development
373 of the intestinal epithelium in foals, these effects will take place before the occurrence
374 of neonatal diarrhoea.

375

376 Gamma-globulin concentrations in foals were low before first suckling and, as
377 expected, increased rapidly after ingestion of colostrum (Jeffcott 1974, Hess-Dudan
378 et al. 1994). The concentration of γ -globulins was highest one day after foaling and
379 decreased thereafter. Another increase was seen after week 4 of life and is
380 apparently due to immunoglobulin synthesis by the foals (Holznagel et al. 2003).
381 There was no period of markedly reduced γ -globulin concentrations in foals and thus
382 no gap in immunological protection. Neither the occurrence nor the characteristics of
383 neonatal diarrhoea differed between foals with high and with lower plasma γ -globulin
384 concentration, indicating that neonatal diarrhoea in foals is not affected by passive
385 transfer of immunity via colostrum and not caused by reduced γ -globulin transfer in
386 our study. Gamma-globulins were already detectable before first intake of colostrum.
387 This is in agreement with a recent study in cattle, indicating that more than 50% of
388 calves had detectable serum IgG concentrations before first colostrum uptake
389 (Chigerwe et al. 2008). However, further investigations are needed to determine
390 causes for the comparably high concentration of γ -globulins prior to first nursing in
391 our foals. Most probably this is caused by the fact that in our study, total γ -globulins
392 in serum were measured which consist not only of immunoglobulins but also of
393 additional proteins with no known immune function.

394

395 In conclusion, neonatal diarrhoea in foals is not linked to postpartum oestrus in their
396 mares but is observed at a time when changes in faecal bacterial flora from a
397 neonatal to a postnatal pattern occur. These changes in bacterial flora in many foals
398 are accomplished without clinical signs of diarrhoea. Beta-carotene supplementation
399 to mares was without effect on neonatal diarrhoea in foals.

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401

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404

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496 Table 1: Age of the mares and parity (number of foals produced so far)

	Age (years)	Parity (number of foals)	Number of mares
	Range	Range	rebred within the
	(means \pm SEM)	(means \pm SEM)	study period
Group 1 (n=15)	5 – 17 (8.0 \pm 1.0)	1 – 13 (4.0 \pm 0.9)	10/15
Group 2 (n=15)	4 – 19 (8.5 \pm 1.1)	1 – 13 (4.7 \pm 1.0)	14/15
Total (n=30)	4 – 19 (8.3 \pm 0.7)	1 – 13 (4.4 \pm 0.6)	24/30

497

498

499 Table 2: Intensity of bacterial growth in mares (n=15 group 1, n=15 group 2; score
500 from 0 to 3; data are means \pm SEM)

Isolated bacteria	Group	Day -14	Day 0	Day 14	Day 28	Day 42
<i>E.coli</i>	1	2.4 \pm 0.1*	2.3 \pm 0.2	2.6 \pm 0.2	2.5 \pm 0.1	2.8 \pm 0.1
	2	2.9 \pm 0.1*	2.3 \pm 0.3	2.4 \pm 0.2	2.7 \pm 0.1	2.9 \pm 0.1
<i>Enterococcus sp.</i>	1	2.1 \pm 0.2*	2.1 \pm 0.3	2.3 \pm 0.3	2.3 \pm 0.3	1.6 \pm 0.4
	2	2.5 \pm 0.2*	2.1 \pm 0.3	1.5 \pm 0.3	1.9 \pm 0.3	1.9 \pm 0.3
<i>β-haemolytic</i>	1	1.5 \pm 0.3 a,b	0.6 \pm 0.3 a	1.8 \pm 0.2 b	2.1 \pm 0.2 b	2.5 \pm 0.2b
<i>Streptococci</i>	2	1.8 \pm 0.3 a,b	0.9 \pm 0.3 a	1.5 \pm 0.3 b	1.5 \pm 0.3 b	1.9 \pm 0.3 b
<i>Coagulase-neg.</i>	1	0.4 \pm 0.3 a	1.5 \pm 0.3 b	1.1 \pm 0.3 a,b	1.1 \pm 0.4 a,b	0.9 \pm 0.4 a,b
<i>Staphylococci</i>	2	0.1 \pm 0.1 a	1.3 \pm 0.3 b	0.5 \pm 0.2 a,b	0.6 \pm 0.3 a,b	1.7 \pm 0.4 b
<i>Proteus sp.</i>	1	0	0.1 \pm 0.1	0	0	0
	2	0.1 \pm 0.1	0.1 \pm 0.1	0	0	0
<i>Pantoea sp.</i>	1	0	0.1 \pm 0.1	0.4 \pm 0.3	0.1 \pm 0.1	0
	2	0	0	0	0.1 \pm 0.1	0
<i>Acinetobacter</i> <i>sp.</i>	1	0.2 \pm 0.2	0.8 \pm 0.4	0.6 \pm 0.3	0.4 \pm 0.3	0.4 \pm 0.3
	2	0	1.2 \pm 0.4	0.4 \pm 0.3	0.2 \pm 0.2	0.5 \pm 0.3

501 * significant differences between groups ($p < 0.05$); a,b: values with different

502 superscript letters in the same line differ significantly ($p < 0.05$)

Table 3: Intensity of bacterial growth in foals from the day of birth to day 36 of life (n=13 group 1, n=14 group 2; score from 0 to 3; data are means \pm SEM)

Isolated bacteria	Group	Day after birth											
		0	4	6	8	10	12	14	28	30	32	34	36
<i>E.coli</i>	1 a	0.2 \pm 0.2	2.5 \pm 0.2	2.5 \pm 0.2	2.7 \pm 0.1*	2.6 \pm 0.2	2.8 \pm 0.1	2.7 \pm 0.1	2.9 \pm 0.1	2.9 \pm 0.1	2.8 \pm 0.1	2.8 \pm 0.1	2.9 \pm 0.1
	2 a	0.8 \pm 0.3	2.2 \pm 0.2	2.4 \pm 0.1	2.2 \pm 0.1*	2.4 \pm 0.1	2.4 \pm 0.2	2.6 \pm 0.1	2.9 \pm 0.1	2.6 \pm 0.2	2.7 \pm 0.1	2.6 \pm 0.1	2.7 \pm 0.1
<i>Enterococcus</i>	1 a	0.6 \pm 0.3	1.4 \pm 0.4	0.7 \pm 0.4	1.0 \pm 0.4	2.3 \pm 0.4	2.1 \pm 0.4	1.9 \pm 0.4	2.3 \pm 0.3	1.9 \pm 0.4	2.8 \pm 0.1*	2.0 \pm 0.4	2.4 \pm 0.4
	2 a	0.8 \pm 0.3	0.4 \pm 0.2	0.4 \pm 0.2	0.3 \pm 0.2	1.4 \pm 0.3	2.1 \pm 0.3	1.3 \pm 0.3	2.3 \pm 0.4	1.9 \pm 0.4	1.5 \pm 0.4*	2.3 \pm 0.3	2.2 \pm 0.3
β -haem. <i>Streptococci</i>	1 a	0	0.2 \pm 0.2	0.1 \pm 0.1	0.2 \pm 0.2	0.7 \pm 0.4	0.4 \pm 0.2	0.2 \pm 0.2	1.2 \pm 0.3	1.9 \pm 0.4	1.6 \pm 0.4	1.6 \pm 0.3	1.6 \pm 0.4
	2 a	0	0.1 \pm 0.1	0.3 \pm 0.2	0.4 \pm 0.2	0.4 \pm 0.2	0.4 \pm 0.2	0.7 \pm 0.3	1.3 \pm 0.4	2.1 \pm 0.4	1.5 \pm 0.3	1.6 \pm 0.3	1.7 \pm 0.4
<i>Coagulase-neg. Staph.</i>	1 a	1.4 \pm 0.3	0	0.2 \pm 0.2	0.2 \pm 0.2	0.8 \pm 0.4	0.5 \pm 0.3	0.9 \pm 0.4	1.5 \pm 0.5	1.4 \pm 0.4	0.7 \pm 0.4	0.7 \pm 0.4	0.3 \pm 0.3
	2 a	1.9 \pm 0.3	0	0	0	0.4 \pm 0.3	0.8 \pm 0.4	0.9 \pm 0.4	0.7 \pm 0.4	0.6 \pm 0.4	0.9 \pm 0.4	0.8 \pm 0.4	1.0 \pm 0.4
<i>Proteus</i>	1 a	0.1 \pm 0.1	0.9 \pm 0.1	1.1 \pm 0.2	0.8 \pm 0.2	0.4 \pm 0.2	0.3 \pm 0.1	0.5 \pm 0.2	0	0	0	0	0
	2 a	0.1 \pm 0.1	1.0 \pm 0.0	0.9 \pm 0.1	0.6 \pm 0.1	0.4 \pm 0.1	0	0.1 \pm 0.1	0	0	0.1 \pm 0.1	0	0
<i>Clostridium perfringens</i>	1	0	0.2 \pm 0.1	0.4 \pm 0.3	0.4 \pm 0.3	0.5 \pm 0.3	0.3 \pm 0.3	0	0	0	0	0	0
	2	0	0	0.2 \pm 0.2	0.1 \pm 0.1	0.1 \pm 0.1	0	0	0	0	0	0	0
<i>Klebsiella</i>	1 a	0	0.7 \pm 0.3	0.5 \pm 0.2	0	0	0	0	0	0	0	0	0
	2 a	0	0.3 \pm 0.2	1.1 \pm 0.3	0.4 \pm 0.3	0	0	0.4 \pm 0.3	0	0	0	0	0
<i>Pseudomonas</i>	1	0	0	0	0	0	0.3 \pm 0.3	0	0.2 \pm 0.2	0	0.5 \pm 0.3	0.2 \pm 0.2	0
	2	0.1 \pm 0.1	0	0	0	0	0	0	0.3 \pm 0.3	0.3 \pm 0.3	0	0	0
<i>Pantoea</i>	1 a	0.7 \pm 0.3	0.2 \pm 0.2	0	0	0	0	0	0	0	0	0	0
	2 a	0.9 \pm 0.3	0	0	0	0	0	0	0.5 \pm 0.3	0	0	0	0
<i>Acinetobacter</i>	1	0.4 \pm 0.3	0.2 \pm 0.2	0	0	0.3 \pm 0.3	0	0.2 \pm 0.2	0.3 \pm 0.3	0	0.3 \pm 0.3	0	0.2 \pm 0.2
	2	0.5 \pm 0.3	0	0	0	0.1 \pm 0.1	0	0.4 \pm 0.3	0.6 \pm 0.4	0.3 \pm 0.3	0.2 \pm 0.2	0	0
<i>Citrobacter</i>	1	0	0.5 \pm 0.3	0.5 \pm 0.3	0.2 \pm 0.2	0.3 \pm 0.3	0	0	0	0	0	0.2 \pm 0.2	0
	2	0	0.3 \pm 0.2	0.1 \pm 0.1	0	0.2 \pm 0.2	0	0	0	0	0	0	0

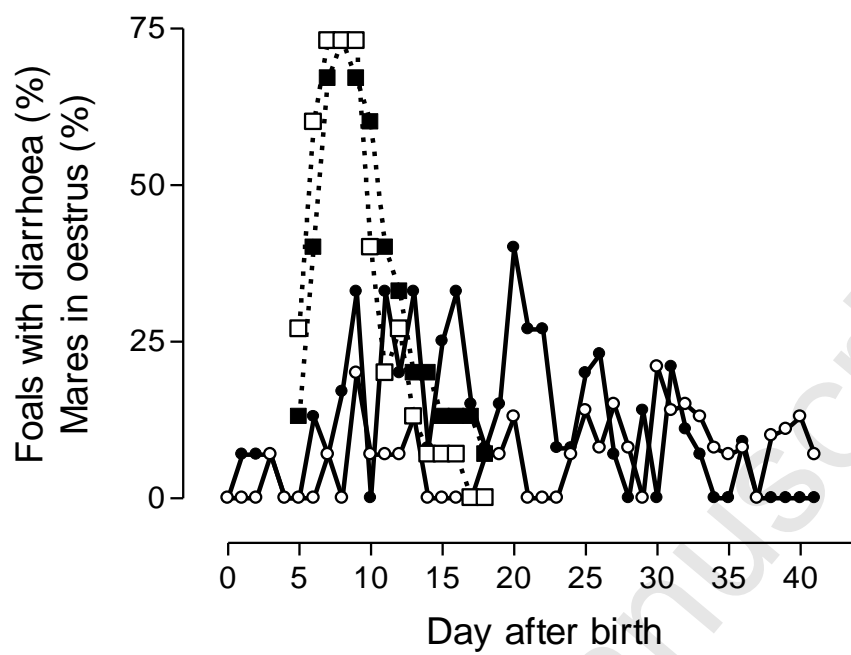
*significant differences between groups (p<0.05); a: significant changes within group over time (p<0.01; Friedman-test)

Figure legends

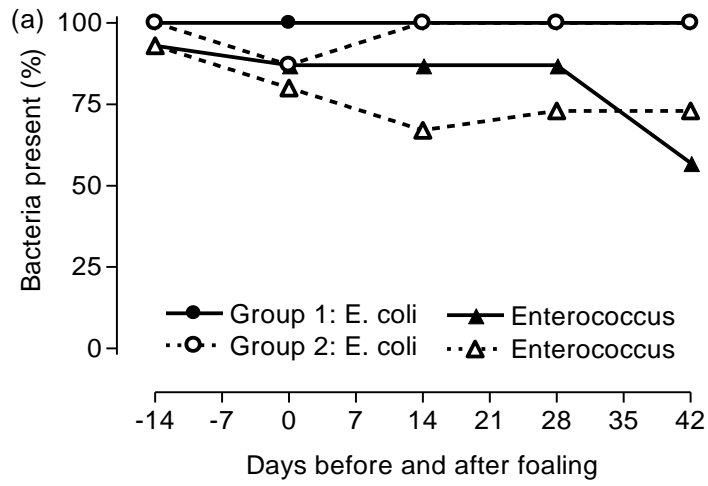
Figure 1: Percentage of mares in first postpartum oestrous (■ β -carotene treated, n=15; □ control, n=15) and foals showing signs of diarrhoea during 42 days after foaling and birth, respectively (● foals born to β -carotene-treated mares, n=13; ○ foals born to control mares, n=14)

Figure 2: Percentage of (a, c) mares (n=15 per group) and (b, d) foals (n=13 group 1, n=14 group 2) with *E. coli*, *Enterococcus* spp., β -haemolytic *Streptococcus* spp. and coagulase-negative *Staphylococcus* spp. in faecal swabs throughout the experimental period. Significant changes over time in mares of both groups for β -haemolytic *Streptococcus* spp. ($p < 0.05$) and group 2 mares for coagulase-negative *Staphylococcus* spp. ($p < 0.01$); in foals for *E. coli*, *Enterococcus* spp., β -haemolytic *Streptococcus* spp. and coagulase-negative *Staphylococcus* spp. ($p < 0.001$); * $p < 0.05$ between groups.

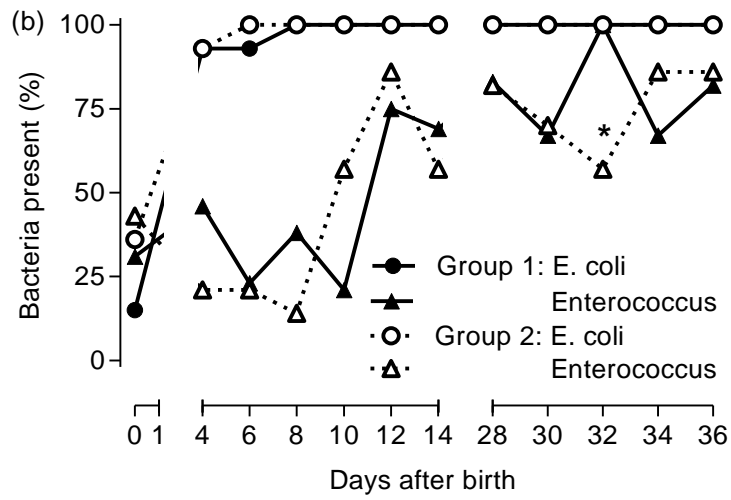
Figure 3: (a) Serum IGF-1 and (b) γ -globulin concentrations in foals born to β -carotene-treated (●; n=13) and control mares (○; n=14). Significant changes over time for both IGF-1 ($p < 0.001$) and γ -globulins ($p < 0.001$).



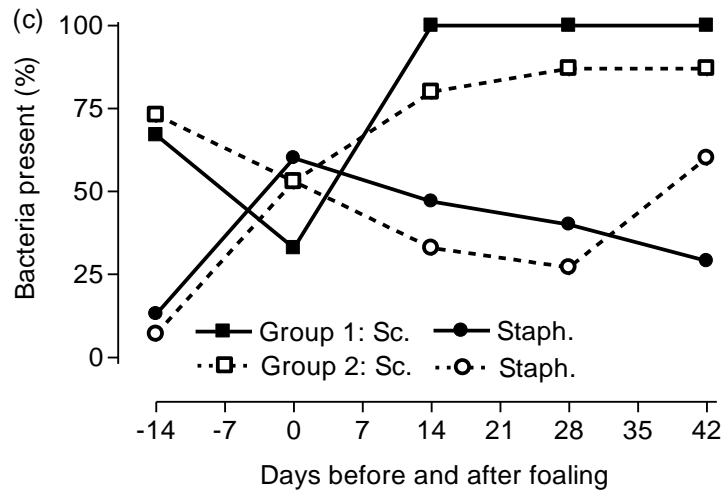
Kuhl et al., Figure 1



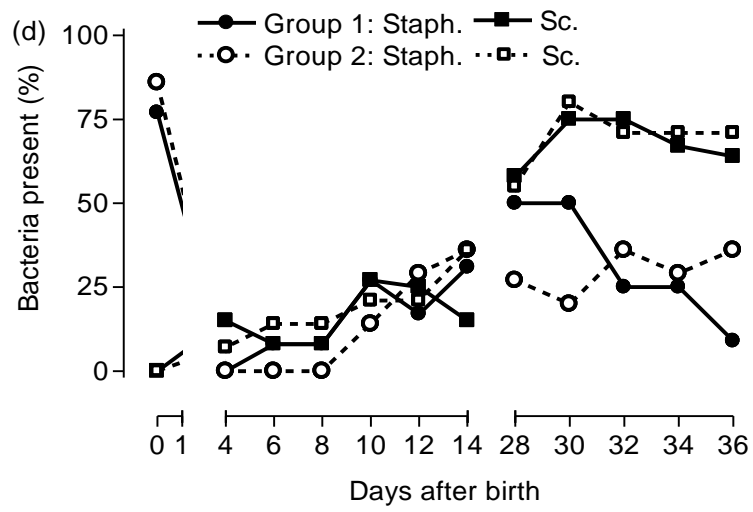
Kuhl et al., Figure 2a



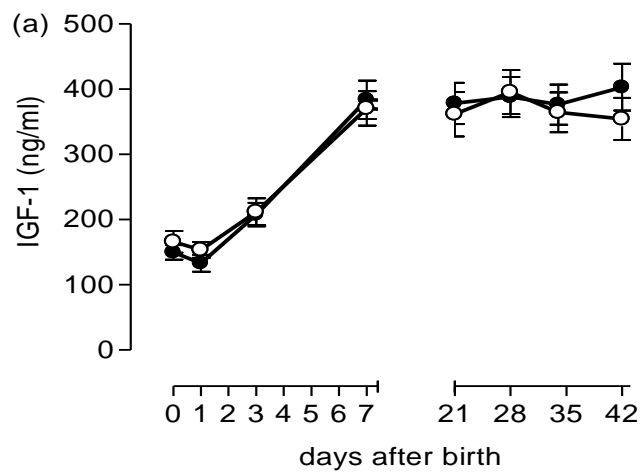
Kuhl et al., Figure 2b



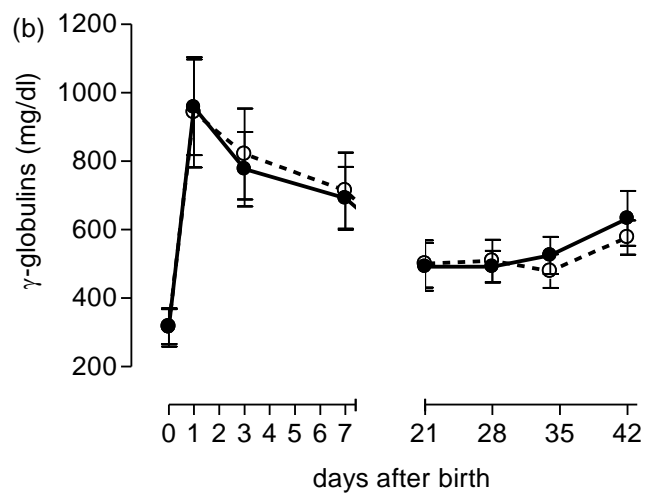
Kuhl et al., Figure 2c



Kuhl et al., Figure 2d



Kuhl et al., Figure 3a



Kuhl et al., Figure 3b