

INCIDENCE OF *CAMPYLOBACTER* IN PIGS WITH AND WITHOUT DIARRHEA

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SHORT COMMUNICATION

ABSTRACT

Two hundred pigs (1- 21 weeks old), from five piggeries in São Paulo State, Brazil, were divided in two groups of 100 animals each, G1 with diarrhea and G2 without diarrhea. *Campylobacter* was recovered from 43% of G1 and 34% of G2 specimens, and was more frequently recovered from 0-4 week old piglets. *C. coli* was the most common species (44.2% in G1 and 32.4% in G2), followed by *C. jejuni/coli* (16.3% in G1 and 23.5% in G2). *Campylobacter* counts were significantly higher in G1 ($\leq 10^8$ UFC/g) than in G2 ($\leq 10^4$ UFC/g) ($p < 0.01$), which suggests that the bacterium may play a role at least in the aggravation of the diarrheic process.

Key words: *Campylobacter*, diarrhea, pigs

The primary habitat of some species of *Campylobacter* is the intestinal tract of warm-blooded animals. Colitis attributed to *C. jejuni* has been reported in men, dogs, cats, ferrets and hamsters, though colonization by the organism usually causes mild diarrhea without severe colitis (3,5,12). In pigs, the association of diarrhea with *Campylobacter* was first reported by Doyle (6,7), who reproduced the so-called "pig dysentery" in healthy animals through experimental inoculation. Dysentery was also observed when *Campylobacter coli* was inoculated in gnotobiotic piglets by oral route. Sala *et al.* (16) observed diarrhea and bacterial dissemination in the lungs, kidneys and liver of pigs

after experimental inoculation with thermophilic *Campylobacter*. There are many other reports on pig campylobacteriosis, such as Sala *et al.* (17), Boosinger and Powe (4), Smith *et al.* (22) and Sticht-Groh (23).

Since raw and undercooked animal products may be contaminated with microorganisms derived from the stools, representing a potential health hazard, and there is limited information as to whether the presence of *Campylobacter* in substantial numbers in feces can be linked with diarrhea in pig, this study was undertaken to investigate the incidence and number of the microorganism in feces of pigs with and without diarrhea.

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Two hundred pigs (1-21 weeks old), from five piggeries in São Paulo State, Brazil, were divided into two groups, 100 animals each, G1 with diarrhea and G2 without diarrhea. Fecal samples from animals of G1 and G2 groups were collected in sterile flasks, shipped under refrigeration and stored at $\pm 20^{\circ}\text{C}$ after the initial laboratory handling. For isolation of microorganisms, samples were directly smeared onto thioglycolate agar plates with 20% defibrinated bovine blood and Butzler selective supplement (SR 85-Oxoid) (TSA). Following this procedure, 1 g of feces was homogenized in 9 ml of sterile saline (0.85 %). The homogenate was centrifuged at 2500 rpm/5 min and the supernatant was filtered through an acetate membrane (0.65 μM \varnothing Millipore). Five drops of each filtrate were also streaked onto plates of TSA without Butzler selective supplement (TA). Plates of TSA and TA were incubated under microaerobic atmosphere for 72 h at 43°C and 37°C , respectively. Colonies were examined for morphology and motility using phase contrast microscopy (1000 x). The final identification was based on biochemical tests: catalase, hydrogen sulfide production with and without cysteine, oxidase, growth at 25°C , at 43°C , in 0.8% glycine and in 3.5% sodium chloride, reduction of sodium selenite, sodium hippurate hydrolysis and tolerance to 2, 3,5-triphenyl tetrazolium (TTC) (8,9,11,18,20, 21).

Enumeration of *Campylobacter* was done plating 0.1 ml of each filtrate, submitted to serial dilutions in sterile saline, ranging from 10^{-1} to 10^{-10} , onto TA plates, which were incubated under microaerobic atmosphere for 72 h at 43°C . After incubation, plates showing 30-300 colony-forming units (CFU) were selected for counting.

The X^2 test was used to compare G1 and G2 in relation to the incidence of *Campylobacter* according to age of the animals. Student's t test was used to evaluate the significance of the differences in the enumeration of *Campylobacter* in groups G1 and G2 (15).

The incidence and number of *Campylobacter* in fecal specimens of pigs with and without diarrhea are presented in Table 1. *Campylobacter* was recovered from 43% of G1 and from 34% of G2 specimens. These data are similar to those of Piazza and Lasta (14) and Smith *et al.* (22), but not to those of Kashiwazaki *et al.* (10). We found no statistically significant difference between the incidence of *Campylobacter* in G1 and G2, although the

microorganism was more frequently recovered from the 0-4 week old piglets. Despite the presence of *Campylobacter* in all age groups, it is difficult to consider that this microorganisms is the causative agent of diarrhea in G1, in view the broad spectrum of biological factors which affect the diarrheic process, including the association of various enteropathogenic agents. There was a significant difference ($p < 0.01$) between the density of *Campylobacter* in fecal specimens of G1 ($\leq 10^8$ UFC/g) and G2 ($\leq 10^4$ UFC/g), which suggests that *Campylobacter* may at least play a role in the aggravation of the diarrheic process, possibly due to intestinal colonization.

Table 2 shows that there was a higher incidence of *C. coli* in G1 (44.2%) than in G2 (32.4%), though this species was the predominant in both groups. However, other thermophilic species were more frequently recovered from G2 than G1. *C. jejuni* biotype 2 showed a higher incidence than *C. jejuni* biotype 1 in both G1 and G2. With regards to *Campylobacter lari* (nalidixic acid resistant *Campylobacter*), in five out of 11 strains the phenotypic profile of sodium hippurate hydrolysis was comparable to that of *C. jejuni*, and in six the phenotype of TTC tolerance was comparable to that of *C. coli*. In this context, previous studies (13,16) suggested that, based on the biochemical profile, *C. lari* may be considered a distinct species, and that *C. lari*, *C. coli* and *C. jejuni* present common phenotypic characteristics.

In this study, *C. fetus* subsp *fetus* was also isolated, which may have an epidemiological significance, in view the etiological correlation with enzootic abortion in sheep, sporadic abortion in bovines, diarrhea in calves and enteritis in dogs and cats (1,2,19).

Table 1. Incidence of *Campylobacter* in fecal specimens of pigs with (G1) and without diarrhea (G2)

Age (weeks)	G1	G2
	Positive Cultures (%)	Positive Cultures (%)
0 + 4	23/47 (48.9)	10/24 (41.7)
4 + 8	15/42 (35.7)	14/41 (34.2)
8 + 21	5/11 (45.5)	10/35 (28.6)
Total	43/100	34/100

0 + 4 $x^2 = 0.338$	4 + 8 $x^2 = 0.022$	8 + 21 $x^2 = 1.085$	Total: $x^2 = 1.710$
$p > 0.50$	$p > 0.50$	$p > 0.30$	$p > 0.10$
G1 = G2	G1 = G2	G1 = G2	G1 = G2

Table 2. Species of *Campylobacter* isolated from fecal specimens of pigs with (G1) and without diarrhea (G2)

<i>Campylobacter</i>	G1		G2	
	N°	(%)	N°	(%)
<i>C. coli</i>	19	(44.2)	11	(32.4)
<i>C. jejuni</i> biotype 1	3	(6.9)	4	(11.8)
<i>C. jejuni</i> biotype 2	5	(11.6)	5	(14.7)
<i>C. jejuni /coli</i>	7	(16.3)	8	(23.5)
<i>C. lari</i>	6	(13.9)	5	(14.7)
<i>C. fetus</i> subsp. <i>fetus</i>	1	(2.4)	0	
Atypical strains	2	(4.7)	1	(2.9)
Total	43	(100)	34	(100)

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RESUMO

Incidência de *Campylobacter* em suínos com e sem diarreia

Um total de 200 suínos (1 - 21 semanas de idade), originários de cinco criações localizadas no Estado de São Paulo, Brasil foi dividido em dois grupos de 100 animais caracterizando-se o grupo G1 de animais com diarreia e G2, sem diarreia. *Campylobacter* foi isolado em 43% das amostras provenientes de G1 e 34% de G2. O microrganismo foi mais frequentemente encontrado em leitões na faixa de 0 a 4 semanas de idade. *Campylobacter coli* foi a espécie mais comumente observada em G1 (44,2%) e em G2 (32,4%), seguido por *Campylobacter jejuni/coli* com 16,3% em G1 e 23,5% em G2. As contagens de *Campylobacter* foram significativamente maiores ($p < 0,01$) em G1 ($\leq 10^8$ UFC/g) do que em G2 ($\leq 10^4$ UFC/g), fato que sugere que o microrganismo pode, pelo menos, atuar no agravamento do processo diarreico.

Palavras-chaves: *Campylobacter*, diarreia, suínos.

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