Pharmacokinetics of amoxicillin administered in drinking water to recently weaned 3- to 4-week-old pigs with diarrhea experimentally induced by *Escherichia coli* O149:F4

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**Objective**—To measure effects of *Escherichia coli* O149:F4–induced diarrhea on water consumption and pharmacokinetics of amoxicillin after administration in drinking water.

**Animals**—24 recently weaned 24- to 28-day-old crossbred pigs.

**Procedure**—10 pigs were inoculated with *E. coli* O149:F4; all 10 pigs subsequently developed diarrhea. Pigs were medicated by administration of amoxicillin in the drinking water (0.75 mg/mL) for a 4-hour period on 2 consecutive days. Fourteen age-matched noninfected healthy pigs (control group) were medicated in a similar manner. Blood samples were obtained from both groups daily, and plasma concentrations of amoxicillin were analyzed by use of high-performance liquid chromatography.

**Results**—Diarrhea reduced the area under the plasma concentration-versus-time curve (AUC) and maximum plasma concentration 

![](https://example.com/image.png)

**Conclusions and Clinical Relevance**—*E. coli*–induced diarrhea reduced the AUC of amoxicillin and time that pigs with diarrhea caused by *E. coli* O149:F4 has often been associated with diarrhea in 3- to 4-week-old weaned pigs, and treatment is commonly accomplished by administration of medication in the drinking water. *Escherichia coli* is efficiently eliminated by amoxicillin, and the antimicrobial is soluble in water and, hence, suitable for administration in drinking water. 

The dose achieved by administration in drinking water is determined by the water consumption of each pig. Consequently, the largest doses are achieved during peak drinking periods. Approximately 75% of daily water consumption in healthy baby pigs is associated with feeding periods, and the lowest water intake is observed during periods of darkness. *Escherichia coli*–induced diarrhea does not affect water consumption of baby pigs, relative to water consumption before infection.

Enteric disease attributable to infection with *E. coli* may influence plasma concentrations of amoxicillin in various ways. In pigs, secretory diarrhea induced by *E. coli* O149:F4 causes a significant reduction in plasma concentrations of amoxicillin after oral administration of a single dose of amoxicillin, possibly because *E. coli* shares the same saturable intestinal sodium-dependent transport mechanism used by amoxicillin. In contrast, investigators in another study reported that IV injection of *E. coli* endotoxin causes a significant increase in plasma concentrations of amoxicillin, presumably as a result of a decrease in renal clearance of amoxicillin. Furthermore, the addition of ß-lactam antimicrobials to cell cultures of *E. coli* reportedly causes an in vitro release of *E. coli* endotoxin.

Medication in the drinking water for the treatment of pigs with diarrhea caused by *E. coli* O149:F4 is preferable to IM injections because the use of medicated drinking water is less time-consuming for farm personnel and less stressful for the pigs. However, administration of amoxicillin in the drinking water to diarrheic pigs may be less efficient when pharmacokinetic dynamics are unfavorable. The objectives of the

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>ABBREVIATION</th>
<th>DESCRIPTION</th>
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<tbody>
<tr>
<td>AUC</td>
<td>Area under the plasma concentration-versus-time curve</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximum plasma concentration</td>
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<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Time to reach C&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td>K&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Elimination rate constant</td>
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<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>Elimination half-life</td>
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<td>SEE</td>
<td>Standard error of the estimate</td>
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study reported here were to measure the effect of E.coli O149:F4-induced diarrhea on water consumption and plasma concentrations of amoxicillin in recently weaned pigs during 2 consecutive days and to calculate pharmacokinetic variables and the amount of time that plasma concentrations were greater than a certain value in diarrheic pigs, compared with results for healthy pigs.

Materials and Methods

Animals and housing—Twenty-four healthy 24- to 28-day-old Danish crossbreed pigs were obtained from a commercial company for use in the study. The pigs (16 castrated males and 8 females) were from 3 litters and were weaned from 21 to 25 days of age during various weeks in the summer. None of the pigs had a history of intestinal diseases or treatment with amoxicillin. Pigs were delivered to the animal faculty of the Department of Veterinary Pathobiology of The Royal Veterinary and Agricultural University 3 days after weaning. Day of arrival was designated as day 0. Each pig weighed between 5.0 and 9.2 kg at the time of arrival. Each pig was housed separately in a pen (minimum floor area of each pen 0.5 m²/pig) but had visual contact with the other pigs. Each pen had a solid concrete floor, and sawdust was used as bedding. Room temperature was maintained at 22° to 24°C, but each pen had a heated resting area that was maintained at 30° to 32°C. Ventilation was maintained at a rate of 7 total room air exchanges/h. Antimicrobial-free weaning food was offered 4 times/d (6:30 AM, 10 AM, 3:30 PM, and 7:30 PM); total amount of feed was restricted to 200 g/pig/d. The study was approved by the Danish Animal Experiments Inspectorate.

Experimental design—Phenotypes of the pigs with regard to susceptibility toward adherence of E.coli O149:F4 subtype ac were examined by use of an intestinal adhesion test. On arrival at our facility, the pigs were randomly assigned to 2 groups (10 pigs in a diarrhea group and 14 pigs in a control group). The 2 groups were housed in the same unit and maintained under identical environmental and feeding conditions.

Induction of diarrhea—The 10 pigs in the diarrhea group were inoculated with E.coli O149:F4 subtype ac, whereas the 14 pigs in the control group were not inoculated. The inoculum was a strain of E.coli O149:F4 subtype ac isolated from the intestinal contents of a pig with postweaning diarrhea. The strain harbored the genes for heat-stable enterotoxin type b, heat-labile enterotoxin, and heat-stable enterotoxin type EA1 and for fimbria F4 subtype ac and caused hemolysis when grown on blood agar. The inoculum culture was grown at 37°C for 6 to 7 hours in 1 L of veal infusion broth with constant shaking. An aliquot (600 mL) of this culture was centrifuged (17,700 × g for 30 minutes), after which the supernatant was discarded and the pellet resuspended in 300 mL of PBS solution. Each pig in the diarrhea group was administered 25 mL of inoculum by use of gastric intubation within 5 hours after arrival and every morning and afternoon thereafter for a maximum of 3 days or until diarrhea developed. Daily dose per pig ranged from 10⁹ to 10⁷ CFUs (median daily dose per pig, 10⁸ CFUs). After each inoculation, the gastric tube was flushed with 30 mL of 10% NaHCO₃ to increase the gastric pH and extend bacterial survival. Presumably, the typical gastric pH was fully restored before amoxicillin treatment was initiated a minimum of 12 hours after NaHCO₃ was administered; therefore, the control pigs were not administered NaHCO₃.

Amoxicillin administration—Amoxicillin trihydrate was provided in the drinking water (0.75 mg/mL) of diarrheic and control pigs. Pigs were expected to consume an amount of water that would result in a dose of 20 mg of amoxicillin/kg. However, the actual dose was determined by the amount of medicated water consumed, and this resulted in an actual mean dose of 26 to 32 mg/kg. Water was withheld from all pigs for 3 hours, and the medicated water was then provided for 4 hours (9:30 AM to 1:30 PM). This procedure was repeated the following day. Water was provided in drinking troughs, and water consumption was recorded for 4 consecutive periods (9:30 AM to 1:30 PM [medication period], 1:30 PM to 3:30 PM, 3:30 PM to 7:30 PM, and 7:30 PM to 9:30 AM) on each of the 2 consecutive days. Administration of medicated water to pigs with diarrhea was initiated on the day that diarrhea was first observed, whereas administration of medicated water to control pigs was initiated on the day after arrival.

Collection of blood samples—Blood samples (5 mL) were collected and used for analysis of plasma concentrations of amoxicillin. Blood samples were collected from a jugular vein into tubes that contained heparin as an anticoagulant. Collection of samples from pigs in the diarrhea group was initiated on the day diarrhea was first observed, whereas collection of samples in the control group was initiated on the day after arrival. Blood samples were collected before (time 0) and 2, 4, 6, 8, 10, and 24 hours after administration of amoxicillin (ie, medicated water) for 2 consecutive days. Blood samples were maintained at 5°C until centrifugation (1,200 × g for 10 minutes) to separate the plasma; all blood samples were centrifuged within 3 hours after collection. Plasma was harvested and stored at –80°C until analysis; all plasma samples were analyzed within 2 months after they were obtained.

Microbiological analysis—Fecal samples were collected daily from the rectum of each pig. Swab specimens collected on the day of arrival were cultured to detect common pathogens of pigs, such as E.coli, rotavirus, and coccidia. Swab specimens collected on subsequent days were cultured to detect the inoculation strain of E.coli. For detection of E.coli, swab specimens were streaked on blood agar plates and cultured overnight at 37°C. Two colonies from each plate were chosen for serotyping by use of a panel of rabbit O-antisera, which is used for routine typing of common pathogenic E.coli isolates of pigs. For fecal swab specimens from pigs with diarrhea, 5 colonies were tested by use of the serotyping method. Isolates that yielded positive results were further examined to detect virulence factor genes by use of a 5′ nuclease PCR assay and to detect fimbria F4 subtype ac. An ELISA was used to detect rotavirus. A flotation technique was used to detect coccidia; detection was followed by determination of oocyte counts by use of light microscopy.

Clinical examination—Clinical examination included assessment of fecal consistency, measurement of rectal temperature, visual examination to detect cyanosis, and evaluation of possible behavioral disturbances. Fecal consistency was visually assessed on the day of arrival before inoculation and each morning and evening thereafter until the development of diarrhea. Pigs were considered to have diarrhea when they had semiconfluent or watery feces. Pigs were considered to not have diarrhea when they had fully formed feces. Fecal consistency was evaluated as the percentage of fecal dry matter by weighing feces before and after it was dried for 18 to 24 hours at 100°C.

Rectal temperature was measured on the day of arrival and each subsequent morning. The pigs were observed for signs of behavioral disturbances and cyanosis on the day of arrival and each subsequent morning and afternoon. Behavioral disturbances were defined as slow reactions, an unsteady and slow gait during walking, and inattentiveness...
when encouraged to move. Typical behavior was defined as immediate reactions, a fast and steady gait during walking, and attentiveness when encouraged to move. Cyanosis was defined as blue discoloration of the ears or limbs.

Both groups of pigs were observed clinically until the last blood sample was collected. To prevent contamination of control pigs, investigators changed boot covers, gloves, and overalls between groups.

Analysis of amoxicillin concentrations—Plasma concentrations of amoxicillin were determined by use of a high-performance liquid chromatography method, as described elsewhere. Briefly, the system was equipped with a fluorescence detector, which monitored excitation and emission at wavelengths of 355 and 435 nm, respectively. Separation was achieved on a reverse-phase C18 column fitted with a guard column, which was maintained at 30°C. The mobile phase consisted of a mixture of methanol:water (40:60). Flow rate was set at 1.0 mL/min. Amoxicillin was eluted at 5.0 minutes. Assay results were calculated from ratios of peak areas between the analyte (amoxicillin) and an internal standard (ampicillin; 0.02 µg/mL) by use of calibration curves generated by use of standards (range, 0.05 to 12.5 µg/mL) in porcine plasma. Limit of quantification for amoxicillin was 0.02 µg/mL, and the between-day coefficient of variation was 4% at an amoxicillin concentration of 0.05 µg/mL.

Pharmacokinetic analysis—Data were fitted to an open 1-compartment model. The AUC for amoxicillin was calculated by use of the trapezoidal rule from time zero to the last experimental time point (i.e., AUC0–24 hours). The AUC for amoxicillin was calculated by use of regression analysis of the linear portion of the descending slope of the logarithmically transformed plasma concentration curve. Value for t1/2 was calculated as 0.693/ke.

Statistical analysis—Analysis of water consumption, plasma concentrations, and rectal temperatures was performed by use of a repeated-measures ANOVA on ranks, and results were reported as estimated means ± SEE. Pharmacokinetic variables were analyzed by use of a Mann-Whitney rank sum test, and results were reported as median and range. Percentages of fecal dry matter were analyzed by use of a 1-way ANOVA, and results were reported as estimated means ± SEE. Values of P < 0.05 were considered significant.

Results

All pigs except 1 were observed eating at 3 or 4 of the 4 feeding periods each day. The exception was 1 pig in the control group that ate at only 2 of the 4 feeding periods on day 1.

All 10 pigs inoculated with Escherichia coli O149:4F subtype ac developed diarrhea; thus, they were included in the diarrhea group for day 1. However, 6 of the pigs had recovered and did not have diarrhea on day 2. Thus, they did not meet the criteria for being in the diarrhea group for day 2. For this reason, only results from the remaining 4 pigs with diarrhea were included in the data for day 2.

Mean water consumption per hour during the 4 consecutive periods and total daily water consumption of the 2 days were calculated (Figure 1). Total daily water consumption did not differ significantly between groups; however, it increased significantly (by 30%) on day 2, compared with consumption on day 1. Diarrhea resulted in increased water consumption during the periods from 1:30 PM to 3:30 PM on days 1 (P < 0.001) and 2 (P < 0.05). During the period of darkness (7:30 PM to 6:30 AM) on both days, significantly (P < 0.001) less water was consumed, compared with the other periods, regardless of group. During the medication period (9:30 AM to 1:30 PM) on both days, mean water consumption and thus the mean doses of amoxicillin did not differ significantly between groups.

Mean dose-adjusted plasma concentrations of amoxicillin for pigs with diarrhea and control pigs were calculated (Figure 2). Pigs with diarrhea had significantly lower plasma concentrations of amoxicillin at 2, 4, 6, and 8 hours (P < 0.01) and 10 hours (P < 0.05) on day 1, compared with corresponding concentrations for control pigs, whereas plasma concentrations for pigs with diarrhea at the various time points on day 2 did not differ significantly from the corresponding concentrations for control pigs on day 1.

Pharmacokinetic variables were calculated from plasma concentration curves generated for each pig (Table 1). Dose-adjusted AUC and dose-adjusted Cmax were significantly (P < 0.01) reduced (56% and 62%,
respectively) for pigs with diarrhea on day 1, compared with values for the control pigs on day 1, whereas no significant difference was observed for the dose-adjusted AUC and dose-adjusted C\text{max} on day 2 for pigs with diarrhea, compared with values for the control pigs on day 1. For the control group, dose-adjusted AUC and dose-adjusted C\text{max} were significantly (dose-adjusted AUC, 40% \[P < 0.05]\]; dose-adjusted C\text{max}, 45% \[P = 0.01]\) lower on day 2, compared with values on day 1. The T\text{max} was 2 hours longer in pigs with diarrhea, compared with the T\text{max} for the control pigs on day 1; these T\text{max} values differed significantly \(P < 0.001\) between groups. The t\text{1/2} was significantly increased in pigs with diarrhea on day 1 and control pigs on day 2, compared with the t\text{1/2} for control pigs on day 1. The amount of time that the dose-adjusted plasma concentration was >0.025 µg/mL was 12 hours on day 1 in pigs with diarrhea, which was significantly less than the amount of time (20 hours) for the control pigs.

Mean ± SEE rectal temperatures for control pigs were 38.6 ± 0.11°C, 38.7 ± 0.11 °C, and 38.7 ± 0.11°C for the morning of day 1, morning of day 2, and afternoon of day 2, respectively. Corresponding values differed significantly \(P < 0.01\) for pigs with diarrhea (38.8 ± 0.14°C, 39.0 ± 0.13°C, and 39.4 ± 0.21°C, respectively). No signs of severe systemic sepsis (which included behavioral disturbances or cyanosis) were observed. Mean ± SEE percentage of fecal dry matter was significantly \(P < 0.001\) lower for pigs with diarrhea on days 1 (10 ± 2% \([n = 10\) pigs\]) and 2 (14 ± 3% \([4\) pigs\]), compared with corresponding values for control pigs (38 ± 4% \([14\) and 40 ± 3% \([13\) for days 1 and 2, respectively). Only 13 fecal samples were available on day 2 on the basis that feces from 1 pig was only visually assessed because the sample was not sufficient to enable determination of the dry-matter content.

Rotavirus was detected in all fecal samples obtained on the day of arrival from both groups, whereas coccidia were not detected. We consistently isolated \textit{E coli} O149:F4 subtype indistinguishable from the inoculum strain from feces of pigs with diarrhea.

\textbf{Discussion}

In the study reported here, diarrhea attributable to \textit{E coli} O149:F4 subtype ac did not change mean daily water consumption of pigs. This is in agreement with the observation of another study, although all pigs in the study reported here were not systemically affected by diarrhea. The reduced water consumption we observed during the period of darkness has also been reported in another study, whereas peaks in water consumption associated with feeding were not observed in our study. Collection of blood samples from pigs of both groups every 2 hours from 9 AM to 7:30 PM may have disturbed drinking behavior.

\begin{table}[h]
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\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Variable} & \textbf{Day 1} & \textbf{Day 2} \\
\hline
\text{Dose (mg/kg)} & 26 (9–41) & 32 (11–39) & 32 (19–40) & 31 (11–36) \\
\text{AUC/dose (hL/kg)} & 2.5 (1.3–5.9) & 1.1 (0.5–3.6)* & 1.5 (0.9–2.9)† & 2.0 (1.0–8.9) \\
\text{C\text{max}/dose (kg/L)} & 0.29 (0.14–1.03) & 0.11 (0.04–0.38)* & 0.16 (0.06–0.29)* & 0.21 (0.10–0.69) \\
\text{T\text{max} (h)} & 2 (2–6) & 4 (2–6)* & 2 (2–6) & 4 (2–6)† \\
\text{K\text{e}} & 0.14 (0.09–0.16) & 0.10 (0.08–0.25)† & 0.11 (0.07–0.15)† & 0.15 (0.13–0.18) \\
\text{t\text{1/2} (h)} & 4.9 (4.4–7.8) & 7.0 (2.8–8.7)† & 6.4 (4.7–9.3)† & 4.7 (3.9–5.3) \\
\hline
\end{tabular}
\caption{Median (range) values of pharmacokinetic variables for recently weaned 24- to 28-day-old pigs with diarrhea induced by inoculation with \textit{Escherichia coli} O149:F4 subtype ac or healthy control pigs administered amoxicillin in the drinking water (0.75 mg/mL) for a 4-hour period on 2 consecutive days.}
\end{table}
Although mean doses of amoxicillin did not differ significantly between groups, doses differed among pigs, which required adjustment prior to pharmacokinetic evaluation.

Diarrhea attributable to *E. coli* O149:F4 subtype ac significantly reduced the dose-adjusted AUC of amoxicillin by approximately 50% on the first day of medication. This result corresponds well with, and is similar to, the AUC of amoxicillin reported in another study conducted by our laboratory group in which pigs with diarrhea experimentally induced by *E. coli* O149:F4 subtype ac were administered a single dose of amoxicillin by use of a stomach tube.

*Giardia lamblia* can infect the proximal portion of the small intestine and cause secretory diarrhea. Plasma concentrations of amoxicillin are reduced after oral administration in rats and humans with *G. lamblia*-induced diarrhea.48

The dose-adjusted AUC and dose-adjusted Cmax of pigs with diarrhea on day 1 differed significantly from values of control pigs on day 1; however, the dose-adjusted AUC and dose-adjusted Cmax of pigs with diarrhea on day 2 did not differ significantly from values of control pigs on day 1. This result was unexpected and may have been influenced by the low number of pigs with diarrhea (n = 4) on day 2. However, the low number of pigs with diarrhea on day 2 would be anticipated because a large number of pigs receiving therapeutic doses of amoxicillin cannot generally be expected to have diarrhea for 2 full days. However, fecal scores and dry-matter percentages for day 2 did not suggest a full recovery of diarrhea pigs and therefore did not offer an explanation of our results. In another study, an increase in the AUC of amoxicillin by 50% to 170% was found after IV administration of *E. coli* endotoxin in dogs and was explained by a decrease in renal clearance. Endotoxins are possibly released during amoxicillin administration; however, involvement of endotoxins in results for the diarrhea group on day 2 is not likely because the T1/2 of amoxicillin was not prolonged in the diarrheic pigs.

The dose-adjusted AUC and dose-adjusted Cmax on day 2 for pigs in the control group were significantly reduced, compared with values on day 1 for the control pigs. Analysis of fecal scores and dry-matter percentages did not indicate that a possible diarrheic response was developing; thus, results for those variables cannot explain the unanticipated results. However, it has been reported that intestinal absorption of amoxicillin is reduced by mutual competitive inhibition of the saturable carrier mechanism. The time to reestablish typical absorption capacity was not described in that study. In the study reported here, absorption capacity of amoxicillin could have been partially saturated on day 2 as a result of the relatively large dose of amoxicillin administered on day 1 (32 mg/kg).

Microbiological and fecal findings are consistent with diarrhea attributable to *E. coli* O149:F4. Fecal dry-matter percentage was an effective method of differentiating between pigs with diarrhea and healthy control pigs.

In the study reported here, we detected a complex set of changes in pharmacokinetics resulting from diarrhea attributable to *E. coli* O149:F4 and treatment of diarrheic pigs by use of amoxicillin administered in the drinking water. Thus, diarrhea attributable to *E. coli* O149:F4 subtype ac did not alter mean daily water consumption or the mean doses of amoxicillin administered. However, in agreement with findings of another study conducted by our laboratory group, diarrhea attributable to *E. coli* O149:F4 subtype ac significantly reduced plasma concentrations of amoxicillin, Cmax, and AUC for amoxicillin on day 1, possibly because of a reduction in intestinal absorption. Unexpectedly, we observed an increase for these variables on day 2 in diarrheic pigs, compared with values for control pigs. Renal dysfunction caused by dehydration could explain these findings, but no signs of dehydration were observed during the study. Additionally, a typical degree of hydration was observed in diarrheic pigs in another study because the pigs in that study drank more and urinated less to compensate for the dehydrating effects of diarrhea. A disease-induced increase in hepatic metabolism is also unlikely because amoxicillin is eliminated primarily via renal or biliary secretion and liver metabolism is extremely low or totally lacking. Thus, the mechanism or mechanisms responsible for the effect observed on day 2 in the study reported here remain unclear.

We detected a significant reduction in the amount of time for which the dose-adjusted plasma concentration was > 0.025 µg/mL in pigs with diarrhea on day 1, compared with the amount of time for control pigs. Efficacy of amoxicillin is determined by the amount of time for which concentrations are greater than the minimum inhibitory concentration of pathogenic organisms. On the basis of our results, the therapeutic efficiency of amoxicillin would be expected to be reduced in diarrheic pigs for pathogens with minimum inhibitory concentration values greater than an adjusted plasma concentration of 0.025 µg/mL. Thus, although the results reported here may be ambiguous, we believe that this study may suggest the use of a high initiating dose of amoxicillin on the first day for the treatment of recently weaned pigs with diarrhea attributable to infection with *E. coli* O149:F4 when the amoxicillin is administered via medicated water to which the diarrheic pigs have access for 4 hours. However, additional studies are needed to confirm these findings.

a. Provegaaarden, Denmark.
   b. Nag Startpiller (138-10-31), Nordsjællands Andels Grovfareforening, Helsinge, Denmark.
   c. Identification No. 9910049-1, Danish Institute for Food and Veterinary Research, Copenhagen, Denmark.
   d. Columbia agar (Oxoid) supplemented with 5% calf blood, Danish Institute for Food and Veterinary Research, Copenhagen, Denmark.
   e. Infusion broth, Difco, Detroit, Mich.
   g. Fluorescence detector, Waters, Milford, Mass.
   h. Sperisorb S500D2, C18 column, Mikrolab, Aarhus, Denmark.
   i. Security Guard Phenomenex, Torrance, Calif.
References


