Induction of the Carrier State in Pigeons Infected with *Salmonella enterica* Subspecies *enterica* Serovar Typhimurium PT99 by Treatment with Florfenicol: a Matter of Pharmacokinetics

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Paratyphoid caused by *Salmonella enterica* subsp. *enterica* serovar Typhimurium is the main bacterial disease in pigeons. The ability of *Salmonella* serovar Typhimurium to persist intracellularly inside pigeon macrophages results in the development of chronic carriers, which maintain the infection in the flock. In this study, the effect of drinking-water medication with florfenicol on *Salmonella* infection in pigeons was examined. The pharmacokinetics of florfenicol in pigeons revealed a relatively high volume of distribution of 2.02 liters/kg of body weight and maximum concentrations in plasma higher than the MICs for the *Salmonella* strain used (4 μg/ml) but quick clearance of florfenicol due to a short half-life of 1.73 h. Together with highly variable bioavailability and erratic drinking-water uptake, these parameters resulted in the inability to reach a steady-state concentration through the continuous administration of florfenicol in the drinking water. Florfenicol was capable of reducing only moderately the number of intracellular salmonellae in infected pigeon macrophages in vitro. Only at high extracellular concentrations (>16 μg/ml) was a more-than-10-fold reduction of the number of intracellular bacteria noticed. Florfenicol treatment of pigeons via the drinking water from 2 days after experimental inoculation with *Salmonella* serovar Typhimurium until euthanasia at 16 days postinoculation resulted in a reduction of *Salmonella* shedding and an improvement in the fecal consistency. However, internal organs in florfenicol-treated pigeons were significantly more heavily colonized than those in untreated pigeons. In conclusion, the oral application of florfenicol for the treatment of pigeon paratyphoid contributes to the development of carrier animals through sub-MIC concentrations in plasma that do not inhibit intracellular persistency.

Paratyphoid in pigeons is typically associated with *Salmonella enterica* subsp. *enterica* serovar Typhimurium variant Copenhagen PT2 or PT99 strains. These strains cause a typhoid condition and are the main cause of bacterial disease in pigeons, resulting in gastroenteritis, arthritis, oophoritis or orchitis, granulomatous inflammation in all possible organs, and high mortality (7). Homing pigeons, as dealt with in this study, are kept in groups in pigeon lofts, facilitating long-term maintenance of a *Salmonella* infection. Although vaccination is a valuable part of a control program, it does not eliminate the possibility of a clinical infection in a pigeon aviary but mainly reduces clinical symptoms and mortality (12, 15). Antimicrobial treatment is often used as an aid to control salmonellosis in the aviary. However, *Salmonella* bacteria may persist inside pigeon macrophages, a niche in which these microorganisms are well protected from most antimicrobial agents (11). It has been suggested previously, but not clearly proven, that antimicrobial treatment may promote the carrier state (16).

Florfenicol distributes easily throughout the body (1) and possibly reaches sufficient intracellular concentrations to assist the macrophage in killing intracellular salmonellae. Florfenicol is a fluorinated derivative of thiamphenicol, and neither of these two compounds contains the nitro group, which is the cause of aplastic anemia that is rarely seen after the use of chloramphenicol. Florfenicol blocks the peptidyltransferase at the 50S ribosome subunit and acts against a wide variety of both gram-positive and gram-negative bacteria (3, 9, 13). The pharmacokinetic parameters of florfenicol in pigeons are not available.

It was the aim of the present study to determine the ability of florfenicol to eliminate *Salmonella* serovar Typhimurium from experimentally infected pigeons and isolated pigeon macrophages and to correlate this ability with the drug's pharmacokinetics in pigeons by comparing naive pooling with population analysis.

MATERIALS AND METHODS

Experimental animals. A total of 35 clinically healthy, 1- to 2-year-old homing pigeons (*Columba livia*) were used for the experimental infection and collection of macrophages. The animals were negative for the presence of *Salmonella* bacteria in the feces at sampling points at 2-week intervals and for the presence of agglutinating antibodies to *Salmonella* (14). For the pharmacokinetic experiment, 24 clinically healthy, 1- to 2-year-old homing pigeons were used. During the experiments, each animal was housed individually in a wire mesh cage. All
The two models were parameterized in terms of systemic clearance (CL), intercompartmental-distribution clearance (CLD), the volume of distribution in the central compartment (Vc), and the volume of distribution in the peripheral compartment (Vp). For oral administration, Vp/F, Vc/F, CL/F, and CLD/F ratios, in which F is the absolute bioavailability for the oral route, were estimated.

The pharmacokinetic parameters were assumed to be log-normally distributed, as indicated in the following equation:

$$\ln(\text{Par}) = \ln(\theta_{\text{Par}}) + \epsilon_{\text{Par}}$$

where Par is the parameter (CL/F, CLD/F, Vp/F, Vc/F, or ka) for the i-th pigeon and ϵPar is the population mean of the logarithm of the parameter. Also, ϵPar is a centered independent random variable assumed to be normally distributed, with a variance of var Par. These variances were estimated by the software, accounting for interindividual errors.

The concentration-time profile was described by the following equation:

$$C_{Pi}(t) = E(C_{Pi}) \cdot (1 + \epsilon)$$

where $C_{Pi}$ is the observed concentration in plasma of the i-th pigeon at time t, $E(C_{Pi})$ is the expected concentration in plasma of the i-th pigeon at time t, and $\epsilon$ is a normally distributed random variable with a mean of zero and a variance of var $\epsilon$. Accounting for the residual variability of the data resulting from intraindividual variability, assay errors, model misspecification, and any other sources of variability.

**Comparison of concurrent models.** The bicompartamental models described by equations 1 and 2 were compared to the corresponding monocompartmental models (for intravenous and oral administration routes). The comparison was based on Akaike's information criterion, conducing to the selection of the two bicompartamental models presented above (see Discussion).

**Salmonella serovar Typhimurium strains and growth conditions.** In all experiments, *Salmonella serovar Typhimurium* PT99 strain DAB66 was used. This strain was isolated from pigeons and has been proven to be highly pathogenic to pigeons (11). In all experiments, the strain was grown in Luria-Bertani broth at 37°C for 16 h without shaking. The MIC of florfenicol for this strain, as determined by both agar and broth dilution according to CLSI guideline M31-A2 (5), was 4 µg/ml. The minimal bactericidal concentration for the strain, determined accordingly, was >256 µg/ml. In vitro killing curves were prepared by plating 10-fold dilutions of Mueller-Hinton broth, inoculated with 5 × 10⁶ CFU of the *Salmonella* strain and exposed to 0, 2, 4, 32, or 128 µg of florfenicol/ml onto Mueller-Hinton agar at different time points postinoculation (see Fig. 5).

**Minimal concentration of florfenicol inhibiting proliferation of Salmonella serovar Typhimurium inside pigeon macrophages.** The minimal concentration of florfenicol in the extracellular environment needed to inhibit intracellular proliferation inside pigeon macrophages was determined as follows. Pigeon respiratory macrophages were collected according to a previously described method (10). Briefly, a pigeon was humanely killed, the trachea was aseptically exposed and cut in the midgular region, and a tracheotube was inserted. The respiratory tract was flushed through this tube with 150 ml of Hank's balanced salt solution without Ca²⁺ and Mg²⁺ at room temperature. The aspirate was centrifuged at 350 × g at 4°C, and the pellet was resuspended in RPMI medium with 1% glucose and 1% pyruvate at 0°C. The cells were counted and seeded into a 96-well plate at 10⁵ macrophages per well. After 2 h of incubation at 41°C and 5% CO₂, the wells were rinsed to remove nonadherent cells. Cell sample purity was determined using nonspecific esterase (Sigma Diagnostics, St. Louis, MO) and Haeacoclor (Merck, Darmstadt, Germany) staining. Then the cells were exposed to *Salmonella* bacteria at a concentration of 5 × 10⁵ CFU/ml (a multiplicity of infection of 10), centrifuged at 350 × g for 10 min at 41°C, and incubated for 1 h at 41°C in 5% CO₂. Cell culture medium containing gentamicin (final concentration of 50 µg/ml; Gibco) and different concentrations of florfenicol (0, 1, 2, 4, 8, 16, and 32 µg/ml) were added, and the wells were incubated for 16 h at 41°C in 5% CO₂. Finally, the wells were rinsed to remove the gentamicin and extracellular florfenicol, after which the macrophages were lysed by the addition of 1% Triton X-100 (Aeros, NJ). Bacteria were enumerated by plating six drops of 20 µl of 10-fold dilutions onto brilliant green agar (BGA; Oxoed Ltd., Hampshire, United Kingdom). The experiment was repeated with macrophages from five different pigeons.

**Oral florfenicol treatment of pigeons inoculated with Salmonella serovar Typhimurium.** Thirty pigeons were divided into the following three groups of 10 pigeons each: (i) pigeons inoculated and not treated; (ii) pigeons inoculated and treated with florfenicol; and (iii) pigeons not inoculated and not treated. Each pigeon was housed individually. The animals of the first two groups were inoculated in the crop with 10⁵ CFU of the *Salmonella* serovar Typhimurium strain DAB69 in 1 ml of phosphate-buffered saline. Treatment consisted of the addition
Counts of postinoculation and continued until euthanasia at 16 days postinoculation. Solubility of florfenicol in water is 0.68 mg/ml maximum) and was started 48 h of florfenicol to the sterilized drinking water at a concentration of 0.5 mg/ml (the of the curves are given in the text.

An intravenous (a) or oral (b) bolus of 30 mg of florfenicol/kg to pigeons as determined by the naïve pooling approach. The equations of the curves are given in the text.

of florfenicol to the sterilized drinking water at a concentration of 0.5 mg/ml (the solubility of florfenicol in water is 0.68 mg/ml maximum) and was started 48 h postinoculation and continued until euthanasia at 16 days postinoculation. Counts of Salmonella bacteria in the excreta were determined daily as numbers of CFU per gram by plating 10-fold dilutions of excreta onto BGA. If negative after direct plating, the samples were preenriched in buffered peptone water (Oxoid), enriched in tetrathionate brilliant green broth (Oxoid), and plated onto BGA. The fecal consistency was scored daily as a measure of the severity of gastrointestinal symptoms, as follows: 0, normal feces; 1, feces not well formed; 2, watery feces; 3, severe diarrhea; 4, presence of blood in the stools; 5, absence of fecal production combined with anorexia. Daily water intake was measured to the nearest milliliter. Polydypsia was defined as a level of water consumption higher than the sum of the average consumption by the negative control pigeons plus two times the standard deviation for these control animals, combined with the presence of polyuria. The pigeons were kept until 16 days postinoculation, after which they were humanely killed, weighed, and necropsied. The numbers of CFU per gram of tissue from the lungs, livers, spleens, kidneys, gonads, and intestines were determined as described above. Significant weight loss was defined as weight loss equal to the sum of the average weight loss of the negative controls plus two times the standard deviation for these controls.

Statistical analysis. A one-way analysis of variance of the cumulative daily fecal consistency scores, the cumulative measurements of daily fecal shedding of Salmonella, and the bacteriological counts per pigeon and per each separate organ was performed. Pairwise comparisons between the treatment groups were done using the least-significant-difference test with a confidence interval of 95%. A statistical software package (SPSS, version 12) was used for these calculations.

RESULTS

Pharmacokinetics of florfenicol in pigeons after oral and intravenous administration. Intravenous administration of the florfenicol solution induced a brief period of vomiting in 70% of the pigeons. No side effects after oral administration were noticed. Figure 1 presents the individual plasma florfenicol concentrations measured after intravenous and oral administration and the corresponding predicted concentration-versus-time curves obtained after the fitting of the pooled data. The pharmacokinetic parameters derived from this analysis are summarized in Table 1. Both absorption and elimination occurred rapidly. The CL for florfenicol was estimated to be 1.41 liters kg⁻¹ h⁻¹, and F for the oral route based on the average areas under the curve from time zero to infinity (AUCs) was about 59%.

Figure 2 presents the individual plasma florfenicol concentrations determined after intravenous and oral administration and the predicted population concentration-versus-time curve obtained after nonlinear mixed-effects modeling. The pharmacokinetic parameters derived from this analysis are summarized in Table 2. The median population estimates were a florfenicol CL of 1.32 liters kg⁻¹ h⁻¹ and F of 60% for the oral route. Interindividual variability of CL and F, expressed as percentages corresponding to coefficients of variation, were 29 and 49%, respectively. The residual errors of the models, expressed as percentages corresponding to coefficients of variation, were 55% for intravenous administration and 56% for oral administration by naïve pooling and 9.6% for intravenous administration and 8.8% for oral administration by nonlinear mixed-effects modeling.

The average concentrations of florfenicol in plasma after continuous administration in the drinking water are depicted in Fig. 3. Continuous administration did not result in a steady state. In 13 of 66 samples, no florfenicol was detected. In eight samples, a concentration in plasma higher than 1 mg/liter was detected. The highest concentration detected in plasma was 3.31 mg/liter.

The levels of protein binding of florfenicol in pigeon plasma were 88.8% at 0.5 μg/ml, 79.8% at 2.5 μg/ml, and 71.2% at 10 μg/ml.

Minimal concentration of florfenicol inhibiting proliferation of Salmonella serovar Typhimurium strain DAB69 inside pigeon macrophages. The addition of florfenicol to the extracellular medium reduced the number of viable intracellular bacteria compared to the numbers inside the untreated mac-

**TABLE 1. Pharmacokinetic parameters of florfenicol obtained by the naïve pooling approach after intravenous or oral administration of a single bolus of 30 mg/kg**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intravenous administration</th>
<th>Oral administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (liters kg⁻¹ h⁻¹)</td>
<td>1.41</td>
<td>1.25</td>
</tr>
<tr>
<td>Vₐ (liters kg⁻²)</td>
<td>2.02</td>
<td>1.55</td>
</tr>
<tr>
<td>Mean residence time (h)</td>
<td>1.43</td>
<td>1.25</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>1.73</td>
<td>1.25</td>
</tr>
<tr>
<td>AUC (mg · h liter⁻¹)</td>
<td>21.30</td>
<td>12.47</td>
</tr>
<tr>
<td>F (%)</td>
<td>58.54</td>
<td>58.54</td>
</tr>
<tr>
<td>kₑ (h⁻¹)</td>
<td>0.56</td>
<td>0.56</td>
</tr>
<tr>
<td>tₚ (h)</td>
<td>0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>Cₘₚₖ (mg liter⁻¹)</td>
<td>5.10</td>
<td>5.10</td>
</tr>
</tbody>
</table>

Abbreviations: Vₐ, volume of distribution at steady state; t₁/₂, half-life at β phase; t₁/₂ₑ, absorption half-life; Tₘₚₖ, time to reach the maximum concentration; Cₘₚₖ, maximum concentration in plasma.
Peroxides at 16 h after inoculation at all concentrations of florfenicol tested (Fig. 4). Only at 32 and 64 μg/ml was a more-than-10-fold reduction of the numbers of bacteria noticed. The inhibition of *Salmonella* growth in Mueller-Hinton medium after exposure to florfenicol was much more pronounced than the inhibition inside macrophages, with reductions of nearly 1 and 2 log10 CFU at 2 and 4 μg/ml, respectively, at 16 h postinoculation (Fig. 5).

**Oral florfenicol treatment of pigeons inoculated with *Salmonella* serovar Typhimurium strain DAB69.** The average daily level of florfenicol exposure for all birds the standard deviation, calculated from the drinking-water uptake, was 35 ± 5 mg/kg, and daily levels of exposure ranged from 26 to 45 mg/kg. The average minimum and maximum daily levels of florfenicol exposure were 17 ± 8 and 59 ± 19 mg/kg, respectively.

Significant weight loss was determined to be a loss of ≥17% of the initial body weight. One inoculated and florfenicol-treated pigeon and two inoculated untreated pigeons showed significant weight loss (23, 17, and 37%, respectively). The average minimum and maximum daily levels of florfenicol exposure were 17 ± 8 and 59 ± 19 mg/kg, respectively.

**TABLE 2. Pharmacokinetic parameters of florfenicol obtained from nonlinear mixed-effects modeling after intravenous or oral administration of a single bolus of 30 mg/kg**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median value (% variation) after:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intravenous administration</td>
</tr>
<tr>
<td>CL (liters kg⁻¹ h⁻¹)</td>
<td>1.32 (29)</td>
</tr>
<tr>
<td>CLD (liters kg⁻¹ h⁻¹)</td>
<td>2.97 (29)</td>
</tr>
<tr>
<td>V₁ (liters kg⁻¹)</td>
<td>0.88</td>
</tr>
<tr>
<td>V₂ (liters kg⁻¹)</td>
<td>0.56 (88)</td>
</tr>
<tr>
<td>AUC (mg · h liter⁻¹)</td>
<td>22.72 (29)</td>
</tr>
<tr>
<td>F (%)</td>
<td>60.0 (46)</td>
</tr>
<tr>
<td>kₑ (h⁻¹)</td>
<td>0.775 (33)</td>
</tr>
</tbody>
</table>

**FIG. 2.** Plasma florfenicol concentrations after administration of an intravenous (a) or oral (b) bolus of 30 mg of florfenicol/kg to pigeons as determined using nonlinear mixed-effects modeling. The equations of the curves are given in the text.

**FIG. 3.** Average concentrations (conc.) ± standard deviations of florfenicol in pigeon plasma after continuous administration in drinking water at 0.5 mg/ml.

5 mg/kg, and daily levels of exposure ranged from 26 to 45 mg/kg. The average minimum and maximum daily levels of florfenicol exposure were 17 ± 8 and 59 ± 19 mg/kg, respectively.

Significant weight loss was determined to be a loss of ≥17% of the initial body weight. One inoculated and florfenicol-treated pigeon and two inoculated untreated pigeons showed significant weight loss (23, 17, and 37%, respectively). The average daily water uptake of ≥63 ml per day and was noticed in only one inoculated and untreated pigeon (with an average daily water uptake of 89 ml). *Salmonella* was isolated from all inoculated pigeons and from none of the negative controls. Florfenicol-treated pigeons shed significantly fewer *Salmonella* bacteria than the untreated ones (P < 0.01) (Fig. 7). On average, levels of fecal shedding by the untreated animals between days 5 and 13 postinoculation were more than 100 times higher than those by the florfenicol-treated pigeons during this time period. At 16 days postinoculation, 9 of 10 untreated pigeons were still shedding *Salmonella* in the feces, as opposed to 4 of 10 treated animals. The sum of the bacteriological counts for all *Salmonella*-infected organs in the treated pigeons was higher than that for the untreated pigeons, but the difference was not significant (P = 0.166) (Fig. 8). The bacteriological counts of *Salmonella* CFU in the spleens and kidneys of treated pigeons were significantly higher (P < 0.05) than those in the spleens and kidneys of the untreated animals.

**DISCUSSION**

The naïve pooling approach is classically used to deal with sparse data in pharmacokinetic experiments. The main drawbacks of this approach are the risk of pharmacokinetic-model
misspecification and the absence of estimation of the interindividual variability in drug disposition. The application of nonlinear mixed-effects modeling is appropriate for the unbiased estimation of population means and the interindividual variability of pharmacokinetic parameters. In the present study, the naive pooling approach resulted in similar fitting performances with monocompartmental and bicompartamental models (based on Akaike’s information criterion) whereas nonlinear mixed-effects modeling indicated that the best fit was obtained with a bicompartamental model. This model was finally used with both the naive pooling approach and nonlinear mixed-effects modeling. The inability of naive pooling to distinguish both inter- and intraindividual variabilities was highlighted by the comparison of residual errors obtained with naive pooling and nonlinear mixed-effects modeling; these errors were close to the analytical error for florfenicol determination (about 10%) with nonlinear mixed-effects modeling, whereas they were much higher (more than 55%) with naive pooling.

In contrast, the comparison of pharmacokinetic parameter estimates indicated reasonable agreement between results from the naive pooling approach and nonlinear mixed-effect modeling. Only the second approach was able to estimate the interindividual variabilities of estimated parameters, expressed as percentages corresponding to coefficients of variation. For intravenous administration, the interindividual variabilities of

![FIG. 4. Numbers of viable intracellular Salmonella bacteria recovered 16 h after the inoculation of pigeon macrophages with Salmonella at a multiplicity of infection of 10 and the subsequent addition of different concentrations of florfenicol to the cell culture medium. Data are presented as the mean differences (Δ) between the log₁₀ numbers of CFU of intracellular bacteria per milliliter in cultures with the given concentrations of florfenicol and the log₁₀ numbers of CFU of intracellular bacteria per milliliter in cultures not exposed to florfenicol. Bars indicate standard errors of the means.](http://aac.asm.org/)

![FIG. 5. In vitro killing curves for Salmonella serovar Typhimurium strain DAB69.](http://aac.asm.org/)
the AUC and CL for florfenicol ranged between 24 and 29%, whereas AUC variability after oral administration was about 50%. The resulting $F$ of orally administered florfenicol was rather high (60%), but it could be deduced from the degree of variation (46%) that 95% of the pigeons exhibited $F$ values in the range from 25 to 100%. In other words, the same dose given orally can result in fourfold variations in levels of systemic exposure to florfenicol among pigeons. Such variability should be taken into account in evaluations of the ability of a given dose to provide appropriate exposure levels for a given percentage of animals in a group.

Florfenicol is a time-dependent antimicrobial agent that shows strong bactericidal activity at MICs for *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Mannheimia haemolytica*, and *Histophilus somni* and bacteriostatic activity at MICs for *Staphylococcus aureus* (2, 6). Therefore, it seems advisable to maintain concentrations in plasma above the MIC to successfully control a *Salmonella* infection in pigeons. The average peak concentration of florfenicol in the serum after oral bolus administration was slightly higher than the MIC for the *Salmonella* strain tested. Combined with the rather high volume of distribution, this pharmacokinetic parameter suggests that the oral florfenicol medication of pigeons with salmonellosis results in a bacteriostatic effect on *Salmonella* in vivo. However, the short half-life of the drug, together with the erratic drinking-water uptake patterns of the pigeons, resulted in the absence of a steady state and the absence of blood samples containing concentrations higher than 4 μg/ml in the plasma when florfenicol was given at a dose of 0.5 mg/ml in the drinking water. Oral resorption of florfenicol by pigeons occurred rapidly, but the oral $F$ was moderate (60%) and variable. This result may be due to incomplete intestinal resorption and/or a first-pass effect.

The volume of distribution of florfenicol in pigeons is rather...
high, indicating elevated tissue concentrations and possibly high intracellular concentrations. In the in vitro experiment with the pigeon macrophages, in which the macrophages were exposed to different florfenicol concentrations for 16 h, a marked reduction of the number of intracellular salmonellae was obtained only at high (>16 μg/ml) concentrations of florfenicol in the extracellular environment. The pronounced growth inhibition observed in broth combined with the limited inhibition in the pigeon macrophages at similar concentrations of florfenicol would even suggest that the Salmonella bacteria are protected from the florfenicol in Salmonella-containing vacuoles. The dose dependency of florfenicol for the reduction of intracellular salmonellae was not observed previously (4) for chloramphenicol by using murine macrophages. Chiu et al. noticed a significant reduction of intracellular viable bacteria at extracellular chloramphenicol concentrations equal to the MICs and 10 times the MICs, whereas in the present study, no significant decrease of bacterial numbers was noticed at an extracellular florfenicol concentration equal to the MIC. The reasons for these differences are not clear but may include the use of chloramphenicol instead of florfenicol, the use of murine instead of pigeon cells, and/or the use of a non-gentamicin-based intracellular proliferation assay. If the data concerning the reduction of intracellular bacteria are combined with the pharmacokinetic data and the erratic drinking behavior of the animals, these results indicate that it is highly improbable that the intracellular persistency of Salmonella inside pigeon macrophages could be effectively inhibited by the administration of florfenicol via the drinking water. Drinking-water medication of pigeons with florfenicol for the treatment of Salmonella infections would thus promote therapeutic failure.

The predicted therapeutic failure of the oral florfenicol treatment of pigeons after inoculation with Salmonella serovar Typhimurium was reflected in the creation of Salmonella carriers (6 of the 10 animals) that did not shed the bacteria in detectable numbers in the feces but in which high numbers of Salmonella bacteria persisted in the tissues. Actually, the internal organs of the florfenicol-treated pigeons exhibited

![Graph](http://aac.asm.org/DownloadedFrom.png)

FIG. 8. Results of the bacteriological examination of tissues from pigeons inoculated with Salmonella serovar Typhimurium. Panel a shows the fraction of Salmonella-positive tissues from untreated (black bars) and florfenicol-treated (white bars) pigeons. In panel b, the average Salmonella loads ± the standard deviations in the tissues of treated (white bars) and untreated (black bars) pigeons are presented.
higher *Salmonella* burdens than those of the untreated ones. This result is in agreement with the finding that the concentrations of florfenicol in plasma were not high enough to inhibit intracellular persistency inside macrophages. Such pigeons thus would pose a serious health threat to previously unexposed animals. This finding supports the hypothesis that geons thus would pose a serious health threat to previously unhibit intracellular persistency inside macrophages. Such pi-
trations of florfenicol in plasma were not high enough to in-

This result is in agreement with the finding that the concen-

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