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The use of an experimental metritis model to study antibiotic distribution in genital tract secretions in the ewe

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Summary — The influence of experimentally-induced metritis on spiramycin disposition in genital secretions was investigated in six ovariectomized ewes. A crossover study design was selected to compare control with metritis pharmacokinetics. A clinically-relevant metritis was obtained under progestagen priming by inoculation in the uterine lumen of a bacterial suspension of Actinomyces pyogenes and Fusobacterium necrophorum. Ewes were given a single iv administration of spiramycin at a dose of 20 mg·kg⁻¹. Plasma and genital secretions were regularly sampled up to 96 h post-injection and spiramycin activity was measured using a microbiological method. Experimental metritis did not affect plasma spiramycin disposition and the antibiotic was more concentrated and lasted longer in genital secretions than in plasma regardless of the animal’s state of health. The area under the concentration–time curve of spiramycin in genital secretions was twofold higher (p < 0.05) in infected than in healthy ewes (3361 ± 112 µg·h·g⁻¹ and 175 ± 41 µg·h·g⁻¹ respectively). The mean residence time of spiramycin in genital secretions was significantly longer in diseased ewes (32 ± 4 h) than in control ewes (23 ± 4 h). The maximum concentration of spiramycin in genital secretions was equal for both studies but occurred later in infected ewes (2.7 ± 1.0 h versus 8.6 ± 4.5 h). It was concluded that a uterine infection had a marked influence on the disposition of spiramycin in genital tract secretions and that this uterine infection model in the ewe merits consideration for the study of drug treatments of genital tract infection.

experimental metritis / genital secretion / pharmacokinetic / spiramycin

* Correspondence and reprints
Résumé — Utilisation d’un modèle de métrite expérimentale pour l’étude de la distribution des antibiotiques dans les sécrétions génitales chez la brebis. L’influence d’une métrite expérimentale sur le passage de la spiramycine dans les sécrétions génitales a été étudiée chez six brebis ovariectomisées. Un plan expérimental croisé a permis de comparer les cinétiques réalisées chez les animaux témoins à celles réalisées chez les mêmes animaux après induction d’une métrite. La métrite expérimentale a été obtenue chez des brebis sous imprégnation progestérénique en introduisant dans la lumière utérine une suspension bactérienne d’Actinomyces pyogenes et de Fusobacterium necrophorum. Les brebis ont reçu une administration intraveineuse de spiramycine (20 mg kg⁻¹) ; le plasma et les sécrétions génitales ont été régulièrement recueillis pendant les 96 heures qui ont suivi l’administration. L’activité de la spiramycine dans les échantillons a été mesurée selon une méthode microbiologique. La métrite expérimentale n’a pas modifié la cinétique plasmatique de la spiramycine ; les concentrations d’antibiotique ont été élevées et se sont maintenues plus longtemps dans les sécrétions génitales que dans le plasma, chez la brebis saine et infectée. Les aires sous la courbe des concentrations de spiramycine dans les sécrétions génitales ont été deux fois plus importantes chez les brebis infectées (361 ± 112 µg.h.g⁻¹) que chez les témoins (175 ± 41 µg.h.g⁻¹). Le temps moyen de résidence de la spiramycine dans les sécrétions génitales a été plus long chez les animaux infectés (32 ± 4 heures) que chez les sains (23 ± 4 heures). Les concentrations maximales de spiramycine dans les sécrétions génitales ont été similaires mais sont apparues plus tard chez les brebis infectées (2,7 ± 1,0 versus 8,6 ± 4,5 heures). Cette étude montre que la métrite expérimentale modifie le passage de la spiramycine dans les sécrétions génitales et que ce modèle d’infection utérine mérite d’être retenu pour l’étude des médicaments utilisés dans le traitement des métrites.

métrite expérimentale / sécrétion génitale / pharmacocinétique / spiramycine

INTRODUCTION

To obtain effective treatment of a genital tract infection, an antibiotic should be administered at the most appropriate dose, dosing interval and route of administration (local versus general) to provide drug concentrations at the uterine site of infection which maximize bacterial killing. The intrauterine route is usually selected in veterinary medicine, because it is generally considered to be the most convenient and because it is assumed that the antibiotic concentration will then be higher at the infection site. In fact, when bacteria spread out of the endometrial lumen and invade the myometrium and oviducts, a general route of administration is in order (Gustafsson, 1984; Bretzlafl, 1986; Gilbert and Schwark, 1992). The selection of an appropriate dosage for a systemic route of absorption requires kinetic information on antibiotic disposition in plasma and its passage through the uterine barrier. Most experiments published to date have studied uterine antibiotic disposition in healthy animals although it is recognised that metritis can modify the ability of a drug to cross the uterine barrier. Genital infections may increase blood flow to the inflamed uterus or damage the endometrium (Ayliffe and Noakes, 1982). The purpose of the present experiment was to develop an experimental animal model of metritis to document the passage of an antibiotic through the uterine barrier when a systemic route of administration was selected.

MATERIALS AND METHODS

Animals

Six cyclic, multiparous Romanov-cross ewes (weighing 43 ± 6 kg) were used. At least one month before the experiments, the ewes were ovariectomized under aseptic surgical conditions and a silicone catheter (Rhodorsil; od, 3 mm) was inserted into the upper third portion of each uterine horn via a small puncture hole in the uterine wall. The catheters were maintained inside the
uterus with ligatures and the free ends of the catheters were exteriorized to the flank of the animals and clamped. After surgery, the animals were placed in individual cages and were provided oats, hay and water ad libitum.

The body temperature and the pH of the genital secretions were measured with an electronic thermometer and electronic pH-meter respectively, in three control and three metritis ewes once a day before spiramycin administration and for the four days following antibiotic administration.

**Experimental design**

Ewes were randomly assigned to groups A (n = 3) or B (n = 3) in a crossover study with respect to uterine pathologic status. In group A the control disposition of spiramycin was investigated before the disposition during metritis, and vice versa in group B. A washout period of five weeks was observed between the two studies. After experiments performed during metritis, ewes were given a daily intramuscular administration of penicillin-dihydrostreptomycin combination (Mixtencilline, Rhône-Mérieux, Toulouse, France) at a dose of $10^6$ international units (IU) in toto for five days.

**Control pharmacokinetics**

Control kinetics of spiramycin were examined in uninfected ewes under oestrogen priming. The ewes were treated with progestagen by means of a 40-mg fluorogestone acetate (FGA) sponge (Chronogest, Intervet, Angers, France) placed in the vagina for 14 days. Twenty four hours after sponge withdrawal, 30 µg of 17β oestradiol (oestradiol Benzoate, Intervet, Angers, France) was injected intramuscularly. The administration of 17β oestradiol benzoate was continued daily for five days.

**Induction of metritis**

The procedure used for metritis induction was adapted from a method described by Farin et al (1989) who were studying the role of *Actinomyces pyogenes* and Gram-negative anaerobic bacteria in producing a pyometra. The day of the first inoculation with the bacterial suspension was designated as Day 0. On Day-4, a polyurethane sponge impregnated with 40 mg fluorogestone acetate was inserted into the vagina. One day before the first inoculation (Day-1), the uterus was infused with 5 mL per uterine horn of an irritating iodine solution via the uterine catheters. Both ovine uterine horns were inoculated on three consecutive days (Day 0, Day 1 and Day 2) with 5 mL per horn of bacterial suspension (*Actinomyces pyogenes*: $5 \times 10^7$ colony-forming units·mL$^{-1}$, *Fusobacterium necrophorum*: $5 \times 10^7$ cfu·mL$^{-1}$).

A *pyogenes* used in the inocula was obtained using swabs from the uterus of a cow with metritis and *F necrophorum* was isolated from a sheep with foot rot. The bacteria were stored at −80°C until used to prepare the bacterial suspension which was then infused into the uterus. After thawing, 0.1 mL of each bacterial strain was streaked on to Columbia Blood Agar (Bio-Mérieux, Marcy-l'Étoile, France) and incubated under anaerobic conditions for 72 h at 37°C. After purity control and identification by standard methods, the bacteria were transferred into a tryptase-soy broth (Bio-Mérieux, Marcy-l'Étoile, France) and diluted to a final concentration of $10^8$ cfu·mL$^{-1}$. The final suspension was prepared with an equal volume of each bacterial suspension, homogenized and dispensed into 30-mL sterile flasks. These suspensions were stored at −80°C until they were infused into the ovine uterus. Before inoculation, the bacterial suspension was thawed for 3 h at 37°C.

**Drug administration and samples collection**

One day before the experiments, a catheter filled with heparinized 0.9% saline solution was inserted into each jugular vein. Spiramycin (Suanovil 20, Rhône-Mérieux, Toulouse, France) was injected intravenously as a single dose of 20 mg·kg$^{-1}$ via one of the catheters. The control kinetic study began 15–18 h after the first administration of benzoate oestradiol while the metritis kinetic study started 48 h after the last bacterial uterine infusion and 3 h after withdrawal of the FGA vaginal sponge.

Blood samples were collected from the other catheter into heparinized tubes before dosing.
(baseline sample, time 0) and then 1, 2, 4, 8, 15, 30, 45, 60 and 90 min and 2, 4, 6, 8, 9, 10, 24, 30, 48, 54, 72 and 96 h after antibiotic injection. Plasma was separated from blood by centrifugation and frozen until assayed.

Genital tract secretions were withdrawn using a method adapted from that described by Lindsay and Francis (1968). In brief, polyurethane sponges (Chrono-Gest placebo, Intervet, Angers, France) were inserted into the vagina of the ewes for one hour. Genital secretions samples were obtained before injection and 1, 3, 6, 9, 24, 30, 48, 54, 72 and 96 h after antibiotic injection. The secretions were squeezed out of the sponge and stored at \(-20\,^\circ\text{C}\) until being assayed for spiramycin.

**Analytical assay**

The antibiotic activity of spiramycin in plasma and genital secretions was measured by agar gel diffusion using *Sarcina lutea* ATCC 9341 as the test organism according to the procedure described by the European Pharmacopoeia (1980). Just before the analytical assay, the genital secretions samples were centrifuged at 6000 g for 10 min and the supernatant removed. Spiramycin concentrations were measured in the supernatant obtained after centrifugation of genital tract secretions. Plasma and genital secretions were assayed simultaneously with known spiramycin standard solutions prepared in plasma and genital secretions respectively, obtained from untreated ewes. The level of quantitation was 0.0625 mg·L\(^{-1}\) for both plasma and uterine secretions and the coefficient of variation of repeatability and reproducibility was less than 18%.

**Pharmacokinetic analysis**

The plasma concentrations of spiramycin were analysed with a program for non-linear regression analysis adapted from Multi (Yamaoka et al, 1981). Each data point was weighted with the reciprocal of the fitted value:

\[
W_i = \frac{1}{\hat{Y}_i^2}
\]

where \(W_i\) was the weight, and \(\hat{Y}_i\) the fitted value of the \(i\)th datum. Initial estimates of the parameters of the equation describing the data were obtained by linear regression methods (Gibaldi and Perrier, 1982). The following exponential equation was fitted to the data:

\[
C_t = \sum_{i=1}^{n} Y_i \exp(-\lambda_i t)
\]

where \(C_t\) (mg·L\(^{-1}\)) represented spiramycin activity at time \(t\), \(Y_i\) (mg·L\(^{-1}\)) were the coefficients and \(\lambda_i\) (h\(^{-1}\)) the exponents. The selection of the number of exponents of equation [2] was determined according to the Akaike information criterion (Yamaoka et al, 1978). The pharmacokinetic parameters (elimination half-time, body clearance, volume of distribution) were calculated using the classic equations associated with compartmental analysis (Gibaldi and Perrier, 1982). Plasma clearance (\(Cl\)) was calculated using the equation:

\[
Cl = D / \text{AUC}_{0,\infty}
\]

where \(D\) was the dose, and \(\text{AUC}_{0,\infty}\) the area under the plasma concentration–time curve calculated using the trapezoidal rule from time 0 to infinity. The extrapolated part of the curve was calculated between the last experimental point and infinity using the equation:

\[
\text{AUC}_{0,\text{last}} = C_{\text{last}} / \lambda_z
\]

where \(C_{\text{last}}\) was the concentration of the last sample and \(\lambda_z\), the slope of the terminal phase. The mean residence time (MRT) was calculated by the linear trapezoidal rule with and without extrapolation to infinity.

Equations describing the fate of drugs administered by a non-vascular route, ie, including an invasion phase and a lag-time (\(t_{\text{lag}}\)) before the onset of invasion, were fitted to the spiramycin concentrations in genital secretions:

\[
C_t = -(Y_1 + Y_2) \exp[-\lambda_A(t - t_{\text{lag}})] + \ Y_1 \exp[-\lambda_1(t - t_{\text{lag}})] + Y_2 \exp[-\lambda_2(t - t_{\text{lag}})]
\]

In equation [5], \(C_t\) (µg·g\(^{-1}\)) represents spiramycin activity in genital secretions at time \(t\); \(Y_1\) and \(Y_2\) (µg·g\(^{-1}\)) are coefficients; \(\lambda_1\) and \(\lambda_2\) (h\(^{-1}\)) are exponents; \(\lambda_A\) (h\(^{-1}\)) is the invasion rate constant. No weighting factor was used to fit the equation to genital secretions data.

The spiramycin concentrations were also analysed using a non-compartmental approach. The
AUC and MRT for plasma and genital secretions were selected to evaluate the extent of the diffusion from blood to genital secretions and to quantify the influence of metritis on spiramycin disposition in genital tract secretions. The $\text{AUC}_{0-\text{last}}$ and $\text{MRT}_{0-\text{last}}$ were calculated using the trapezoidal rule from time 0 to the last concentration measured in plasma or in secretions. The secretions-to-plasma concentration ratio was calculated using the $\text{AUC}_{0-\text{last}}$ for both plasma and genital secretions for both kinetic studies.

Statistical analysis

The pharmacokinetic parameters derived from all plasma and genital tract secretion data were expressed as mean ± standard deviation (SD) for the six ewes. Harmonic means were calculated for the half-times of invasion and elimination; standard deviations were computed using a jackknife technique (Lam et al, 1985).

Statistical analysis was performed using Statgraphics (Statistical Graphics Corporation, 1988). The pharmacokinetic parameters of the control and metritis kinetic studies were compared using a four-factors ANOVA (pathological status, period, sequence and animals nested in sequence). The level of significance was 0.05. The plasma and genital secretions-derived parameters were compared using a two-factor ANOVA (animal, biological matrix).

RESULTS

Animals

Clinically-relevant metritis was confirmed in all ewes by the presence of purulent vaginal discharge while control ewes showed typical clear oestral mucus. Body temperature was not modified in metritis ewes and behaviour looked normal throughout the experiment. The pH values of genital tract secretions were $8.4 \pm 0.1$ and $8.3 \pm 0.1$ in control and metritis ewes respectively.

![Fig 1. Semi-logarithmic plots of spiramycin concentrations in plasma and in genital secretions (GS) versus time, after a single iv administration of spiramycin (Suanovil 20) at a dose of 20 mg kg$^{-1}$ in six ewes. (Plasma: $\bullet$ $\bullet$ $\bullet$ $\bullet$ $\bullet$ $\bullet$ and $\bigcirc$ $\bigcirc$ in control and inoculated ewes respectively; genital secretions: $\square$ $\square$ $\square$ $\square$ and $\blacksquare$ $\blacksquare$ in control and inoculated ewes respectively).]
Plasma kinetics

Figure 1 shows the mean semi-logarithmic plots of spiramycin activity in plasma and genital secretions in six ewes with and without uterine infection.

According to the Akaike information criterion, a triexponential equation best fitted the plasma experimental data for each ewe (equation [2]). Consequently, a three-compartmental model was used to analyse the plasma disposition of spiramycin. Mean pharmacokinetic values for the six ewes are given in table I. Comparison of plasma pharmacokinetic parameters did not show any significant difference between the two groups ($p > 0.05$, ANOVA). The half-times of elimination were ($t_{1/2\lambda_2}$) $17.0 \pm 3.3$ and $18.0 \pm 3.9$ h and the mean residence times (MRT) were $10.5 \pm 3.8$ and $12.7 \pm 3.2$ h for control and metritis kinetics respectively. These parameters indicated a slow disappearance of spiramycin from plasma. The steady state volume of distribution ($V_{ss}$) was high ($6.0 \pm 1.7$ and $7.9 \pm 1.9$ L·kg$^{-1}$), suggesting considerable distribution of spiramycin in the tissues.

Genital tract secretion parameters

The area under the time–concentration curves and the mean residence time were significantly higher in genital secretions than in plasma ($p < 0.001$, ANOVA) whatever the health condition of the ewes (tables II

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Animal health status</th>
<th>Healthy ewes</th>
<th>Metritis ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Healthy ewes</td>
<td>Metritis ewes</td>
</tr>
<tr>
<td>$Y_1$</td>
<td>mg·L$^{-1}$</td>
<td>117.01 ± 42.51</td>
<td>111.89 ± 22.38</td>
<td></td>
</tr>
<tr>
<td>$Y_2$</td>
<td>mg·L$^{-1}$</td>
<td>8.02 ± 2.77</td>
<td>6.14 ± 0.89</td>
<td></td>
</tr>
<tr>
<td>$Y_3$</td>
<td>mg·L$^{-1}$</td>
<td>0.533 ± 0.090</td>
<td>0.570 ± 0.136</td>
<td></td>
</tr>
<tr>
<td>$\lambda_1$</td>
<td>h$^{-1}$</td>
<td>11.04 ± 2.29</td>
<td>12.44 ± 1.60</td>
<td></td>
</tr>
<tr>
<td>$\lambda_2$</td>
<td>h$^{-1}$</td>
<td>0.741 ± 0.252</td>
<td>0.740 ± 0.105</td>
<td></td>
</tr>
<tr>
<td>$\lambda_3$</td>
<td>h$^{-1}$</td>
<td>0.041 ± 0.008</td>
<td>0.039 ± 0.008</td>
<td></td>
</tr>
<tr>
<td>$V_c$</td>
<td>L·kg$^{-1}$</td>
<td>0.181 ± 0.072</td>
<td>0.175 ± 0.042</td>
<td></td>
</tr>
<tr>
<td>$V_{ss}$</td>
<td>L·kg$^{-1}$</td>
<td>5.99 ± 1.74</td>
<td>7.92 ± 1.91</td>
<td></td>
</tr>
<tr>
<td>$V_{area}$</td>
<td>L·kg$^{-1}$</td>
<td>14.56 ± 1.45</td>
<td>16.81 ± 3.82</td>
<td></td>
</tr>
<tr>
<td>$C_{0\text{-Inf}}$</td>
<td>L·kg$^{-1}$·h$^{-1}$</td>
<td>0.591 ± 0.100</td>
<td>0.634 ± 0.132</td>
<td></td>
</tr>
<tr>
<td>$t_{1/2\lambda_2}$</td>
<td>h</td>
<td>16.96 ± 3.25$^a$</td>
<td>17.96 ± 3.88$^a$</td>
<td></td>
</tr>
<tr>
<td>$\text{MRT}_{0\text{-Inf}}$</td>
<td>h</td>
<td>10.48 ± 3.79</td>
<td>12.72 ± 3.24</td>
<td></td>
</tr>
<tr>
<td>$\text{AUC}_{0\text{-Inf}}$</td>
<td>mg·h·L$^{-1}$</td>
<td>34.59 ± 5.46</td>
<td>32.59 ± 6.19</td>
<td></td>
</tr>
</tbody>
</table>

$a$ Harmonic mean. $Y_1$, $Y_2$ and $Y_3$: extrapolated Time 0 plasma drug concentrations of the first, second and third phases of spiramycin disposition respectively; $\lambda_1$, $\lambda_2$ and $\lambda_3$: slopes of the first, second and third phases of spiramycin disposition; $V_c$: volume of the central compartment; $V_{ss}$: steady state volume of distribution; $V_{area}$: volume of distribution calculated from Time 0 to infinity; $C_{0\text{-Inf}}$: plasma clearance calculated using the trapezoidal rule from Time 0 to infinity; $t_{1/2\lambda_2}$: elimination half-time; $\text{MRT}_{0\text{-Inf}}$: mean residence time calculated using the trapezoidal rule from Time 0 to infinity; $\text{AUC}_{0\text{-Inf}}$: area under the concentration–time curve calculated from Time 0 to infinity.
The area under the time-concentration curves calculated from genital secretion data was twofold higher for infected ewes ($p < 0.05$, ANOVA): ie, $175 \pm 41$ and $361 \pm 112 \text{ pg} \cdot \text{h} \cdot \text{g}^{-1}$ respectively (table II). Similarly, the mean residence time of spiramycin in genital secretions was significantly longer in ewes with metritis than in control ewes ($32.5 \pm 3.8$ versus $22.8 \pm 4.3$ h, $p < 0.001$ ANOVA) (table III).

The compartmental pharmacokinetic analysis showed that a biexponential equation best fitted the genital secretions data with an invasion phase and with a lag-time for some data sets ($n = 2$ for healthy ewes and $n = 4$ for metritis ewes) (equation [5]). The mean values for genital secretion parameters are given in table IV. The passage of spiramycin from blood to genital tract secretions (half-time of invasion, $t_{1/2,A}$) was markedly slower in ewes with metritis than in control conditions ($p < 0.05$, ANOVA). This was confirmed by a maximal concentration (equal for both groups) that occurred later in metritis ewes ($8.6 \pm 4.5$ h) than in healthy ewes ($2.7 \pm 1.0$ h). The difference between these values was significant for $p = 0.053$ (ANOVA). The elimination rate constants for uterus ($\lambda_2$) were significantly lower in infected than in control ewes ($p < 0.001$, ANOVA), leading to a twofold longer half-time of elimination ($t_{1/2,2}$) of spiramycin from the uterus ($28.2 \pm 9.2$ versus $50.2 \pm 10.7$ h in control and metritis ewes respectively).

**DISCUSSION**

In the present experiment, genital tract secretions were chosen to assess diffusion from blood to the uterine lumen of an antibiotic administered by a systemic route. Female genital pathogens invade the deep layers of the uterine wall from the uterine lumen and the presence of antibiotic in uterine and cervical secretions after an iv administration guarantees the passage of drug.

### Table II. Area under the spiramycin concentration-time curve (AUC) in plasma and in genital tract secretions in six ewes with and without metritis.

<table>
<thead>
<tr>
<th>Ewe</th>
<th>Healthy ewes</th>
<th>Metritis ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{pl}$</td>
<td>$T_{sec}$</td>
</tr>
<tr>
<td>1</td>
<td>72 h</td>
<td>96 h</td>
</tr>
<tr>
<td>2</td>
<td>72 h</td>
<td>96 h</td>
</tr>
<tr>
<td>3</td>
<td>54 h</td>
<td>96 h</td>
</tr>
<tr>
<td>4</td>
<td>72 h</td>
<td>96 h</td>
</tr>
<tr>
<td>5</td>
<td>72 h</td>
<td>96 h</td>
</tr>
<tr>
<td>6</td>
<td>72 h</td>
<td>96 h</td>
</tr>
<tr>
<td>Mean</td>
<td>33.52</td>
<td>175.02</td>
</tr>
<tr>
<td>SD</td>
<td>5.04</td>
<td>41.04</td>
</tr>
</tbody>
</table>

$T_{pl}$ (h) and $T_{sec}$ (h): times corresponding to the last concentration measured in plasma and genital tract secretions respectively; AUC$_{pl}$ (mg·h·L$^{-1}$): area under the plasma concentration-time curve calculated using the trapezoidal rule from Time 0 to $T_{pl}$; AUC$_{sec}$ (mg·h·g$^{-1}$): area under the secretions concentration-time curve calculated using the trapezoidal rule from Time 0 to $T_{sec}$; ratio: genital secretion-to-plasma concentrations ratio calculated from the AUC.
through the uterine barrier and its presence throughout the biophase.

The procedure of intrauterine infusion of bacteria described in this experiment successfully induced uterine infection in all ewes. The choice of *A. pyogenes* and *F. necrophorum* in our experimental model was guided by the fact that these bacteria have often been isolated from the uterus of diseased cows and ewes (Bane, 1980; Roberts, 1967b; Olson et al, 1984) and *A. pyogenes* is considered as the most harmful pathogen in metritis. Moreover, several investigations have demonstrated a synergistic relationship between *A. pyogenes* and *F. necrophorum* in producing pathologic changes (Sokkar et al, 1980; Roberts, 1967a; Farin et al, 1989). Progestagen priming and iodine treatment were used respectively to reduce defense mechanisms and damage the endometrium, thereby promoting bacterial invasion of the genital tract. The presence of metritis was ascertained by vaginal speculum examination: infected ewes had cloudy, malodorous genital secretions with small clumps of pus whereas the oestral mucus of healthy ewes was clear. Infected ewes showed no modification in behaviour and general health status and rectal temperature remained within the normal range.

The iv administration of spiramycin produced higher and longer concentrations in genital secretions than in plasma regardless of animal health status. These results are in agreement with those reported for spiramycin in previous works (Cester et al, 1990, 1992). The area under the time–concentration curve for genital secretions and the secretion-to-plasma concentration ratio were higher in metritis than in control ewes. Similarly, experimental metritis increased spiramycin persistence in genital secretions. The statistical analysis did not reveal any significant difference in the plasma pharmacokinetic parameters between control and metritis ewes. The increased amounts and longer persistence of spiramycin in genital secretions of metritis ewes cannot be attributed to modification of the spiramycin plasma disposition. This is consistent with the fact that

### Table III. Mean residence time (MRT) of spiramycin in plasma and in genital tract secretions in six ewes with and without metritis.

<table>
<thead>
<tr>
<th>Ewe</th>
<th>Healthy ewes</th>
<th>Metritis ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T$<em>{pl}$ T$</em>{sec}$</td>
<td>MRT$<em>{pl}$ MRT$</em>{sec}$</td>
</tr>
<tr>
<td>1</td>
<td>72 h 96 h</td>
<td>6.13 21.78</td>
</tr>
<tr>
<td>2</td>
<td>72 h 96 h</td>
<td>6.02 20.61</td>
</tr>
<tr>
<td>3</td>
<td>54 h 96 h</td>
<td>5.24 16.45</td>
</tr>
<tr>
<td>4</td>
<td>72 h 96 h</td>
<td>10.03 27.70</td>
</tr>
<tr>
<td>5</td>
<td>72 h 96 h</td>
<td>9.67 27.28</td>
</tr>
<tr>
<td>6</td>
<td>72 h 96 h</td>
<td>10.02 23.11</td>
</tr>
<tr>
<td>Mean</td>
<td>7.85</td>
<td>22.82</td>
</tr>
<tr>
<td>SD</td>
<td>2.28</td>
<td>4.25</td>
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</tbody>
</table>

*T$_{pl}$* (h) and *T$_{sec}$*: times corresponding to the last concentration measured in plasma and genital tract secretions, respectively; MRT$_{pl}$ (h): mean residence time of spiramycin in plasma calculated using trapezoidal rule from Time 0 to *T$_{pl}$*; MRT$_{sec}$ (h): mean residence time of spiramycin in genital secretions calculated using trapezoidal rule from Time 0 to *T$_{sec}$*.
Experimental metritis did not induce fever syndrome or impair general health status. It may be assumed, therefore, that the modification of spiramycin disposition in the genital secretions was due solely to a new local condition induced by the metritis. This cannot be attributed to a modification in pH partitioning with respect to genital tract infections. The pH values of genital secretions were in fact similar (8.4 ± 0.1 versus 8.3 ± 0.1) in both diseased and healthy oestrogen priming ewes and cannot be advocated as a factor influencing the passage of antibiotic from blood to genital secretions.

The compartmental analysis suggested that the experimental metritis was associated with a decrease in spiramycin transfer through the uterine barrier. The passage of spiramycin from blood to genital secretions was slower in diseased ewes as indicated by a longer invasion half-time ($t_{1/2\lambda A}$) and a later time of occurrence of the maximal concentration ($T_{\text{max}}$) of spiramycin in the secretions. The faster passage of drug from blood to the uterine lumen in control ewes was certainly due to an increased genital blood flow. It is currently recognized that oestrogen increases the passage of antibiotics through the uterine barrier by increasing uterine blood supply or endometrial capillary (Bretzlaff, 1986). Our control animals were under oestrogen priming because the direct collection of genital secretions is impossible in the ewe under progestagen. In a previous work (Cester et al, 1992), the passage of spiramycin into uterine secretions was investigated in uninfected oestrogens and progestagen ewes: the amounts of spiramycin that penetrated into uterine secretions were higher under oestrogen priming than under progestagen priming. In the present experiment, the amounts of spiramycin in genital secretions were higher in infected progestagen ewes than in uninfected oestro-

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**Table IV.** Pharmacokinetic parameters describing the disposition of spiramycin (Suanovil 20) in genital secretions, after a single intravenous administration of spiramycin in six ewes with and without metritis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Healthy ewes</th>
<th>Metritis ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_1$</td>
<td>μg*g⁻¹</td>
<td>26.62 ± 11.89</td>
<td>32.82 ± 24.13</td>
</tr>
<tr>
<td>$Y_2$</td>
<td>μg*g⁻¹</td>
<td>3.094 ± 1.137</td>
<td>5.088 ± 1.449</td>
</tr>
<tr>
<td>$\lambda_1$</td>
<td>h⁻¹</td>
<td>0.323 ± 0.197</td>
<td>0.131 ± 0.084</td>
</tr>
<tr>
<td>$\lambda_0$</td>
<td>h⁻¹</td>
<td>0.025 ± 0.008</td>
<td>0.014 ± 0.003</td>
</tr>
<tr>
<td>$\lambda_A$</td>
<td>h⁻¹</td>
<td>0.890 ± 0.288</td>
<td>0.276 ± 0.169</td>
</tr>
<tr>
<td>$t_{\text{lag}}$</td>
<td>h</td>
<td>0.54 ± 0.42</td>
<td>0.22 ± 0.38</td>
</tr>
<tr>
<td>$t_{\text{max}}$</td>
<td>h</td>
<td>2.74 ± 1.00</td>
<td>8.61 ± 4.53</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>μg*g⁻¹</td>
<td>11.25 ± 1.83</td>
<td>9.32 ± 2.69</td>
</tr>
<tr>
<td>$t_{1/2\lambda_0}$</td>
<td>h</td>
<td>28.17 ± 9.18 *</td>
<td>50.19 ± 10.65 *</td>
</tr>
<tr>
<td>$t_{1/2\lambda_A}$</td>
<td>h</td>
<td>0.78 ± 0.26 *</td>
<td>2.51 ± 1.62 *</td>
</tr>
</tbody>
</table>

* Harmonic mean. $Y_1$ and $Y_2$: extrapolated Time 0 drug concentrations in genital secretions of the first and second phases of spiramycin disposition respectively; $\lambda_1$ and $\lambda_0$: slopes of the first and second phases of spiramycin disposition; $\lambda_A$: slope of the invasion phase; $t_{\text{lag}}$: lag-time; $C_{\text{max}}$: maximal concentration of spiramycin in genital tract secretions; $t_{\text{max}}$: occurrence of time of $C_{\text{max}}$; $t_{1/2\lambda_0}$: half-time of elimination of spiramycin from genital secretions; $t_{1/2\lambda_A}$: half-time of diffusion of spiramycin from blood to genital secretions.
gen ewes, this steroid environment being considered as the most favourable steroid conditions of a good uterine penetration of spiramycin. Then the increased spiramycin amounts in genital secretions may be attributable to infection and not to steroid hormones. Besides, it is known that oestrogens increase the uterine defense mechanisms and experimental metritis should not be induced under oestrogen priming. In a previous work we have shown that spiramycin uterine availability, but not its residence time in genital secretions, was increased in ewes under oestrogen priming as compared in ewes under progestagen priming (Cester et al., 1992) and it was concluded that this increase was certainly due to an increased blood flow and/or uterine barrier permeability attributable to oestrogens. In this experiment, it can therefore be assumed that the uterine blood flow was probably increased less by inflammation following bacteria inoculation in our metritis conditions than by the oestrogen priming in our controls. The greater amounts of spiramycin in genital tract secretions of metritis ewes cannot be explained, therefore, by a greater passage of spiramycin from the blood to the uterus but more likely by less spiramycin passing from the uterus to the blood. This is consistent with the half-time of elimination (t1/2) and MRT for genital secretions which are longer in diseased ewes. This might be attributable to an increased binding of spiramycin to uterine contents (eg, secretions, pus, subendometrial tissues) or to the penetration of spiramycin into the polynuclear neutrophil population which dramatically increases during infections. It should be noted that considerable leucocyte passage into the uterine lumen occurs in the presence of A pyogenes (Constantin, 1987), and that spiramycin penetrates easily into macrophages and polynuclear cells as has been demonstrated for the respiratory tract (Zenebergh and Trouet, 1982; Pocidalo et al., 1985; Harf et al., 1988).

The efficacy of a treatment using a bacteriostatic antibiotic requires the persistence of sufficient concentrations in the genital tract for a sufficient time to allow the elimination of pathogens by uterine defence mechanisms. The minimal inhibitory concentration (MIC) of spiramycin for A pyogenes, considered to be the most harmful pathogen in metritis, is approximately 3 mg·L⁻¹ (Atkinson, 1986). In the present study, spiramycin concentrations in genital tract secretions remained close to 3 µg·g⁻¹ for approximately 48 h. These results support the use of the intravenous route for spiramycin administration and it can be assumed that the systemic route is likely to provide a good distribution of the drug in genital tract tissues and fluids, even of diseased animals.

The present experiment has clearly demonstrated that an experimentally-induced metritis can easily be obtained in the ewe and that the uterine disposition of a systemic administration of antibiotic was largely influenced by genital tract infection. It can be concluded, therefore, that a relevant antibacterial treatment for metritis cannot be based only on kinetic results involving healthy cycling animals. It is likely that the same considerations hold for genital tract infections of women.

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