Proposals for withdrawal period of sheep milk for some commonly used veterinary medicinal products: A review

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A B S T R A C T
This paper summarises the results of studies performed in Greece, in order to establish withdrawal time of sheep milk for some commonly used antimicrobial drugs. Several studies have been performed to establish milk withdrawal time for the following veterinary medicinal products, commonly used in the therapeutics of sheep: lincomycin, spectinomycin, procaine penicillin G, dihydrostreptomycin, kanamycin, oxytetracycline, trimethoprim and sulfadiazine, albendazole. For oxytetracycline, two pharmaceutical forms were tested, specifically long-acting injectable solution and spray. For albendazole, the pharmaceutical form of oral suspension was tested. For all the other antimicrobials, the pharmaceutical form of injectable solution was tested. The animal phase of all the trials was performed at the Veterinary Faculty of the University of Thessaly. Each product was administered at the highest recommended dose and the frequency currently licensed for administration to sheep. Subsequently, either liquid chromatography or liquid chromatography–mass spectrophotometry was used under GLP principles and as required each time, in order to establish residues of each antibiotic in milk. Most of the analytical work was carried out at GLP accredited laboratories. In each case, limit of detection and limit of quantification for each antibiotic tested were appropriately calculated. For calculation and proposal of a withdrawal period, the legally established minimum residue levels of each antibiotic and their concentrations detected in sheep milk were taken into account. The proposed withdrawal periods are as follows: lincomycin and spectinomycin inj. sol.: 4 milkings, procaine penicillin G and dihydrostreptomycin inj. sol.: 5 milkings, dihydrostreptomycin inj. sol.: 5 milkings, kanamycin inj. sol.: 4 milkings, oxytetracycline long-acting inj. sol.: 16 milkings, oxytetracycline spray: 0 milkings, trimethoprim and sulfadiazine inj. sol.: 7 milkings, albendazole or. susp.: 7 milkings after last administration of each test product. The above results provide standards and will help veterinarians to use the various veterinary pharmaceutical products in the therapeutics of dairy ewes.

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1. Introduction

In general, there is a paucity of official withdrawal periods for veterinary medicinal products in sheep milk. Nevertheless, there is a need for such information, as in Southern European countries raw sheep milk or sheep milk products are commonly used for human consumption. Currently, there are licensed withdrawal periods for various drugs available only for meat and offal of sheep. Lack of appropriate withdrawal period for ewes’ milk hinders their
use in dairy sheep and creates confusion among veterinarians and sheep farmers. The objective of this paper is to present in brief the results of studies performed in Greece, in order to establish withdrawal time of sheep milk for some commonly used antimicrobial drugs. Some of these have already been adopted by the Greek licensing authority ( Ethnikos Organismos Farmakon) as official withdrawal periods for sheep’s milk; others are currently under evaluation by the authority’s official reviewers. In any case, the withdrawal periods calculated herebelow for the various drugs may be used as guidelines for veterinarians, when using unlicensed products for dairy sheep.

2. Materials and methods

2.1. Experimental design—administration of test drugs

Several studies have been performed to establish milk withdrawal time for the following veterinary medicinal products, commonly used in the therapeutics of sheep: lincomycin (LINCOVET SE®, inj. sol.), spectino- mycin (LINCOVET SE®, inj. sol.), procaine penicillin G (NEO-AMPIVET®, inj. susp.), dihydrostreptomycin (NEO-AMPIVET SE®, inj. susp.), oxytetracycline (OXYVET®, inj. sol.), spectinomycin (LINCOVET SE®, inj. sol.), kanamycin (NIGER®, inj. sol.), oxytetracycline (OXYVET® spray), oxytetra- cycline long-acting (OXYVET® LA, inj. sol.), trimethoprim (OPTIPRIME®, inj. sol.), sulfadiazine (OPTIPRIME®, inj. sol.) and albendazole (ALBENDAZOLE ALAPIS, or. susp.). Each product was administered at the highest recommended dose and the frequency currently licensed for administration to sheep. Details are in Table 1. The whole work was supervised and audited by the Greek licensing authority (Ethnikos Organismos Farmakon).

The animal phase of all the trials was performed under Good Clinical Practice and Good Laboratory Practice principles at the Veterinary Faculty of the University of Thessaly, after obtaining appropriate licenses for animal experimentation by the Greek Ministry of Agriculture.

Animals used in the studies were of a Greek indigenous breed, Karagouniko. For each study, a group of 20–22 clinically healthy lactating ewes were used. Number of experimental animals varied among studies for each test drug. The animal experimentation by the Greek Ministry of Agriculture. Animals used in the studies were of a Greek indigenous breed, Karagouniko. For each study, a group of 20–22 clinically healthy lactating ewes were weighed and their milk yield was recorded. Administration of each test drug was performed on D0 of each trial, and was followed by subsequent administrations according to a licensed schedule, as detailed in Table 1. Dose administered to each ewe was calculated according to the weight of the animal; then, the dose was rounded up to the next available mark of the syringe (i.e., if the calculated dose was 4.3 ml, it was rounded up to 4.5 ml).

Milk samples were obtained as follows. Both mammary glands of each animal were emptied into the same bucket, in order to measure milk yield of the ewe. The milk was thoroughly mixed and a sample of 50 ml was collected. Milk samples were initially collected on D1 (i.e., before drug administration), to be used as negative controls; then, samples were again collected at the first milking immediately after the last administration of the test drug and thereafter at 12-h intervals until 20 d after last administration of the test drug. Milk samples were stored at −20 °C until transportation to the laboratory, during which samples were packaged in dry-ice. Animals were maintained for an extra “clearance” period of 7 days and then, released.

2.2. Laboratory studies

Negative control samples for each of the above drugs were tested on the next day after collection, in order to confirm the absence of any drug therein. Then, a known quantity of the drug (i.e., pure active ingredient) was added into three sets of sub-samples of that sample; 1 × MRL [minimum residue limit] was added into one set of sub-samples, then two multiples of MRL were added in the other two sub-sets of samples. These “spiked” samples were subsequently stored at −20 °C and tested alongside the milk samples after drug administration (CVMP-VICH, 1998a,b).

Milk samples collected after drug administration were tested according to the established stability of each active ingredient. Thus, samples collected after ampicillin administration (which has a known short-term stability) were tested the next day after collection, whilst samples collected after oxytetracycline administration (which has a longer stability) were tested within a maximum of 2 months after collection (Botsoglou and Fletouris, 2001).

Either liquid chromatography (LC) or liquid chromatography–mass spectrophotometry (LC–MS) was used under Good Laboratory Practice principles and as required each time, in order to establish concentration of each drug or its metabolites in milk samples. Liquid chromatography was used for the following drugs: lincomycin, spectinomycin, procaine penicillin G, dihydrostreptomycin, kanamycin, trimethoprim and sulfadiazine. Most of the analytical work, including method development and validation, was carried out at Alapis’ accredited laboratories (Athanasiou et al., 2008a,b). The analysis for albendazole was performed as described before (Botsoglou and Fletouris, 2001; Fletouris et al., 2003). In each case, limit of detection and limit of quantification for each antibiotic tested were appropriately calculated.

2.3. Calculation of withdrawal period

Initially, results of drug concentration into the “spiked” samples were used to provide reference values for stability of the test drug; if necessary, their results were employed to correct the results of the post-drug administration samples.

Table 1

<table>
<thead>
<tr>
<th>Veterinary pharmaceutical product (trade name and pharmaceutical form)</th>
<th>Dose administered</th>
<th>Route and frequency of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lincomycin (LINCOVET SE®, inj. sol.)</td>
<td>5 mg kg⁻¹ bw</td>
<td>i.m., s.i.d. for 5 days</td>
</tr>
<tr>
<td>Spectinomycin (LINCOVET SE®, inj. sol.)</td>
<td>10 mg kg⁻¹ bw</td>
<td>i.m., s.i.d. for 5 days</td>
</tr>
<tr>
<td>Procaine penicillin G (NEO-AMPIVET®, inj. susp.)</td>
<td>9100 IU kg⁻¹ bw</td>
<td>i.m., s.i.d. for 5 days</td>
</tr>
<tr>
<td>Dihydrostreptomycin (NEO-AMPIVET SE®, inj. susp.)</td>
<td>11.35 mg kg⁻¹ bw</td>
<td>i.m., s.i.d. for 5 days</td>
</tr>
<tr>
<td>Dihydrostreptomycin (DIHYDROSTREPTOMYCIN ALAPIS, inj. sol.)</td>
<td>10 mg kg⁻¹ bw</td>
<td>i.m., b.i.d. for 4 days</td>
</tr>
<tr>
<td>Kanamycin (NIGER®, inj. sol.)</td>
<td>12 mg kg⁻¹ bw</td>
<td>i.m., b.i.d. for 3 days</td>
</tr>
<tr>
<td>Oxytetracycline (OXYVET® spray)</td>
<td>1 spray from 15 cm for &gt;4 s on udder skin</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline long-acting (OXYVET® LA, inj. sol.)</td>
<td>20 mg kg⁻¹ bw</td>
<td>i.m., 2 administrations every 72 h</td>
</tr>
<tr>
<td>Trimethoprim (OPTIPRIME®, inj. sol.)</td>
<td>3.2 mg kg⁻¹ bw</td>
<td>i.m., b.i.d. for 5 days</td>
</tr>
<tr>
<td>Sulfadiazine (OPTIPRIME®, inj. sol.)</td>
<td>16 mg kg⁻¹ bw</td>
<td>i.m., b.i.d. for 5 days</td>
</tr>
<tr>
<td>Albendazole (ALBENDAZOLE ALAPIS, or. susp.)</td>
<td>15 mg kg⁻¹ bw</td>
<td>p.o., once</td>
</tr>
</tbody>
</table>

Explanation of abbreviations: bw: bodyweight, i.m.: intramuscular administration, i.v.: intravenous administration, p.o.: per os administration, s.i.d.: once per day, b.i.d.: twice per day.
Results

For lincomycin and spectinomycin, detection limit was 28.6 μg kg⁻¹ and 30.3 μg kg⁻¹, respectively, whilst quantification limit was 75.4 μg kg⁻¹ and 100.2 μg kg⁻¹, respectively. When drug concentration was below the quantification limits – and for calculation purposes – 50% of that limit was taken. Mean lincomycin concentration in milk was 4549.5 μg kg⁻¹ (detected in samples from 20/20 animals), 1188.5 μg kg⁻¹ (in 20/20 animals), 331.4 μg kg⁻¹ (in 18/20) and 60.7 μg kg⁻¹ (in 5/20), 12 h, 24 h, 36 h and 48 h after last injection; thereafter, it was not detected. Mean spectinomycin concentration in milk was 570.3 μg kg⁻¹ (detected in samples from 20/20 animals), 158.7 μg kg⁻¹ (in 15/20), 70.2 μg kg⁻¹ (in 4/20), 12 h, 24 h and 36 h after last injection; thereafter, it was not detected. Based on the above results and the legally defined MRLs of the drugs (lincomycin: 150 μg kg⁻¹, spectinomycin: 200 μg kg⁻¹), the following withdrawal periods (after last administration of the medicinal product) can be proposed: four milkings for lincomycin and two milkings for spectinomycin. Therefore, for the medicinal product containing both products (lincomycin and spectinomycin), a withdrawal period of four milkings is recommended for sheep milk (Athanasiou et al., 2008b).

Similar procedures were followed for the other test drugs. Again, based on their results and the legally defined MRLs of the drugs (procaine penicillin G: 4 μg kg⁻¹, dihydrostreptomycin: 200 μg kg⁻¹, kanamycin: 150 μg kg⁻¹, oxytetracycline: 100 μg kg⁻¹, trimethoprim: 50 μg kg⁻¹, sulfadiazine: 100 μg kg⁻¹, albendazole: 100 μg kg⁻¹), the following withdrawal periods (after last administration of the medicinal product) can be proposed; procaine penicillin G: 5 milkings, dihydrostreptomycin: 5 milkings, kanamycin: 4 milkings, oxytetracycline: 16 milkings for long-acting inj. sol. and 0 milkings for spray, trimethoprim: 7 milkings and sulfadiazine: 7 milkings (Athanasiou et al., 2008b), albendazole: 7 milkings (Fleitouris et al., 2003). Therefore, for the medicinal products containing two of the above products (procaine penicillin G and dihydrostreptomycin–trimethoprim and sulfadiazine), a withdrawal period of five or seven milkings, respectively, is recommended for sheep milk (Athanasiou et al., 2008b).

Discussion

The results of the present studies provide standards and can help veterinarians to use the various veterinary pharmaceutical medicinal products in the therapeutics of dairy sheep. For licensing purposes, sheep are considered a "minor" species, thus proposals for withdrawal period of drugs in ewes’ milk are not legally required. However, in many European countries, sheep are milked and thus, official withdrawal periods are useful for the prescription of the products for sheep.

There are some suggestions that in case of products already licensed for cows, one may observe the withdrawal period for cows’ milk even when the product is used in ewes. However, differences in metabolism between the two animal species, as well as in the composition of their milk, could lead to different pharmacokinetic properties of the drugs in the mammary gland and consequently to different excretion times in milk. This would lead to differences in withdrawal periods between the two animal species. Examples are illustrated in Table 2, where the proposed withdrawal periods for some drugs in ewes’ milk are compared to those licensed for cows’ milk. Differences in some of these drugs (procaine penicillin G–dihydrostreptomycin combination, oxytetracycline inj. sol. LA, trimethoprim–sulfadiazine combination) confirm that no extrapolations between the two animal species should be performed.

Drug residues in foods can have significant implications for the health of consumers. They are considered to contribute to the increased antimicrobial resistance observed in human intestinal bacterial populations. Also, they can affect enzymic systems of humans, consuming the animal products or may predispose humans (particularly babies) to drug-induced allergic reactions during food consumption.

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Withdrawal period for ewes’ milk¹</th>
<th>Withdrawal period for cows’ milk²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lincomycin–spectinomycin combination</td>
<td>4 milkings</td>
<td>4 milkings</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>4 milkings</td>
<td>Not available</td>
</tr>
<tr>
<td>Procaine penicillin G–dihydrostreptomycin combination</td>
<td>5 milkings</td>
<td>6 milkings</td>
</tr>
<tr>
<td>Dihydrostreptomycin</td>
<td>5 milkings</td>
<td>6 milkings</td>
</tr>
<tr>
<td>Oxytetracycline inj. sol. LA</td>
<td>16 milkings</td>
<td>14 milkings</td>
</tr>
<tr>
<td>Oxytetracycline spray</td>
<td>Zero</td>
<td>Zero</td>
</tr>
<tr>
<td>Trimethoprim–sulfadiazine combination</td>
<td>7 milkings</td>
<td>5 milkings</td>
</tr>
</tbody>
</table>

¹ Results of the presented studies.
² Licensed withdrawal periods.
However, apart from the direct human-safety implications, there are other problems associated with not meeting appropriate withdrawal periods. For example, it has long been known (Botsoglou and Fletouris, 2001) that presence of antimicrobial compounds (e.g., procaine penicillin G) in milk can alter the technological properties of milk, resulting to impaired quality of fermented dairy products (yogurt, cheese, buttermilk, sour cream, etc.).

Therefore, when no licensed withdrawal periods are available, good-quality studies performed under appropriate standards and published in the literature, can provide guidelines for the proper use of veterinary medicinal products and should be taken into account when prescribing drugs for sheep.

Acknowledgement

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References


