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EFFICACY OF A LONG-ACTING OXYTETRACYCLINE* AGAINST CHLAMYDIAL OVINE ABORTION

Annie RODOLAKIS¹, A. SOURIAU¹, J.-P. RAYNAUD² and G. BRUNAUT²

with the technical assistance of Liliane GESTIN¹, J. TAILLANT² and M. FOUASSE²

¹Institut National de la Recherche Agronomique, Station de Pathologie de la Reproduction,
Centre de Tours-Nouzilly, 37380 Nouzilly, France.

²Station de Recherche et Développement Vétérinaire et Nutrition Animale, Pfizer International,
37400 Amboise, France.

Résumé

EFFICACITÉ D'UNE OXYTÉTACYCLINE À LONGUE ACTION CONTRE LA CHLAMYDIOSE ABORTIVE OVINE. — Le traitement de la chlamydiose abortive ovine par la Terramycine/L A 200 a été tenté dans trois expérimentations successives, dans lesquelles des brebis ont été inoculées avec 6×10^6 PFU chlamydia par voie intradermique à 80 jours de gestation. L'efficacité du traitement a été estimée par comparaison entre groupes témoin et traité par : le nombre d'agneaux vivants, le nombre de mises-bas normales, la durée de gestation, le poids moyen des agneaux à la naissance, l'excrétion génitale des chlamydia à la mise-bas.

Soixante à 80 % des brebis témoins ont avorté. Dans ces conditions, une seule injection de 20 mg/kg de Terramycine/L A au 105^e jour de gestation, soit quatre semaines après l'inoculation, est inefficace. En revanche, deux injections faites respectivement trois et cinq semaines après l'inoculation augmentent significativement la durée moyenne des gestations, diminuent le nombre d'avortements, augmentent le nombre d'agneaux vivants. Le traitement ne modifie pas cependant, ni le poids des agneaux vivants à la naissance, ni l'excrétion vaginale des chlamydia à la mise-bas. La transposition d'un tel traitement à la pratique et son intérêt sont discutés.

Chlamydial abortion is one of the main causes of ovine abortion in the South East of France and in the Roquefort basin (Fontaine, 1975). Losses due to this disease are difficult to calculate accurately, but they are probably the most severe encountered by producers. Whether sheep breeding is oriented to the production of meat, of milk or of replacement stock, in all cases the birth of lambs is requi-

red. During the acute phase of the disease, 30 % of the pregnant ewes may abort. The only means of eliminating this first wave of abortions is an appropriate antibiotic treatment. Tetracyclins are reputed to be active against chlamydia both *in vitro* and *in vivo* (Storz, 1971 ; Mitscherlich and Leiss, 1957 ; Frank *et al.*, 1962). However, with classic presentations of antibiotics an adequate concentration is maintained in the blood only for a short time. Consequently, to control the infection, it is necessary to prescribe a series of

* : Terramycin LA, trademark of Pfizer Inc.

Table 1. — Experimental design.

	Experiments		
	1	2	3
<i>Total number of animals</i>	42	20	50
Treated	20	10	24
Controls	22	10	26
Age (in years)	1 to 2	1	1
Breed	Rouge de l'Ouest and Prealpe × Lacaune	Charmoise × Suffolk	Limousin
<i>Inoculation by intradermal route of Chlamydia psittaci strain AB7</i>			
Dose (PFU/ewe)	4×10^6	8×10^6	6×10^6
No. of days of pregnancy	77	86	77
<i>No. of days of pregnancy at time of Terramycin/LA treatment</i>			
first treatment	105	110	98
second treatment	133 ^a	124	108
<i>Was contact possible between controls and treated group ?</i>	±	Yes	No

a : treatment not planned but made necessary by the occurrence of abortions in the treated group.

three injections on three consecutive days, to be repeated if necessary two weeks later. To reduce the number of injections a long-acting oxytetracyclin has been developed by Pfizer Laboratories (Terramycin/LA injectable solution 200 mg/ml) so that a single injection gives the same amount of antibiotic as three classic injections. It ensues that animals are handled less often, and in the specific case of abortion diseases, this is a particular advantage with pregnant ewes.

In this project we studied the efficacy of Terramycin/LA in the prevention of chlamydial abortion. Naturally infected flocks would have been more appropriate for a study such as this, but the cyclical evolution of the abortions, the fact that it is impossible to define the individual level of infection for ewes in a flock, the need for a control group, all of these make observations difficult to record, and to interpret. To start with we preferred to test the efficacy of the product in an experimental infection. We demonstrated that in these conditions a single treatment is not sufficient to eliminate abortions. Two treatments at an interval of 10 or 15 days make it possible to significantly increase the average duration of pregnancy, and to reduce the number of abortions and stillbirths.

Materials and Methods

Animals (table 1)

The ewes came from four different flocks with no previous record of abortions. However, before the start of the experiment serology tests (titre $\leq 1/40$) were slightly positive for all ewes vis-a-vis the chlamydia antigen (Rakeia, Roger Bellon). This antibody titer is due to the intestinal chlamydia which sheep usually carry.

Inoculation

In the three experiments, the inoculation was carried out by intradermal route with a suspension of *Chlamydia psittaci*, strain AB 7 (Faye *et al.*, 1972) cultivated on egg, and passaged twice on embryonated eggs after having been reisolated from the foetus of an aborted ewe.

Ewes were inoculated during the third month of gestation (at 77, 86 and 77 days of pregnancy in the three different experiments) with approximately the same dose of chlamydia (4×10^6 , 8×10^6 and 6×10^6 plaque-forming units (PFU) per ewe) determined by plaque lysis on monolayer McCoy cells (Banks *et al.*, 1970).

Table 2. — Effect of the treatment on lambings of ewes inoculated during the third month of pregnancy with *Chlamydia psittaci*.

	Experiments					
	1		2		3	
	Treated 105 + 133 ^a	Controls	Treated 110 + 124 ^a	Controls	Treated 98 + 108 ^a	Controls
Lambings ^b						
Normal (%)	13 (65)	9 (41)	9 (90)	2 (20)	16 (67)	8 (31)
Abnormal (%)	7 (35)	13 (59)	1 (10)	8 (80)	8 (33)	18 (69)
premature births	0	1	1	4	1	0
abortions	5	8	0	4	2	14

a : number of days of pregnancy at time of first or second treatment.

b : Normal : Lambs that live for three days or more, born after at least 140 days of pregnancy.

Abnormal : Premature births : lambing taking place before 140 days of pregnancy.

Abortions : Births, premature or not, of stillborn lambs.

Other abnormal lambings : « normal lambings » but with very weak lambs that die within 3 days or multiple lambings where at least one of the lambs is very weak and dies within 3 days.

Treatment

Injections of Terramycin/L.A. were made by deep intramuscular route in the neck at the rate of 20 mg/kg. A different batch of Terramycin/LA was used for each experiment.

Experimental design (table 1)

In each experiment lots were drawn to assign the ewes into two groups after inoculation : one group infected and treated (treated group) and the other infected and non-treated (controls). In the first experiment animals of both groups were raised in two paddocks separated by an open wooden fence allowing some contact between the ewes ; in the second experiment both groups were in the same paddock during the whole experiment, whereas in the third experiment the two groups were kept in two separate buildings and were looked after by two different shepherds.

The 20 ewes of the treated group in the first experiment were treated 28 days after the inoculation, i.e., at 105 days of pregnancy. As a single treatment proved to be insufficient, the 17 ewes still pregnant 28 days later, at 133 days of gestation, were treated a second time.

In the second experiment, the 10 ewes of the treated group were first treated 24 days

after the inoculation, and again 14 days later, i.e., at 110 and 124 days of pregnancy, respectively.

In the third experiment, the first treatment was made 21 days after inoculation, and the second 10 days later, at 98 and 108 days of pregnancy, respectively.

Serological examinations

Blood samples were taken each week during the four weeks after the inoculation and then each month until one month after lambing.

The complement-fixing antibodies were detected by Kolmer type micromethod (Rodolakis *et al.*, 1977) with *Rakeia* antigen (Roger Bellon). The highest serum dilution showing less than 50 % hemolysis was taken as the midpoint. A serum was considered positive when its endpoint was 1/80 or higher, negative when the endpoint was 1/20 or lower, and doubtful if 1/40.

Bacteriological examination

Vaginal mucus was sampled by swabs on the day of lambing and on the two following days. Swabs were kept at -80°C in a phosphate buffer with sucrose. Chlamydia were isolated by plaque assay on McCoy cells (Rodolakis and Chancerelle, 1977).

Table 3. — Effect of treatments on excretion of *Chlamydia psittaci* at lambing

	Experiments					
	1		2		3	
	Treated 105 + 133 ^a	Controls	Treated 110 + 124 ^a	Controls	Treated 98 + 108 ^a	Controls
Number of ewes tested ^b	12	8	9	8	20	22
excreting chlamydia at lambing	8	8	9	8	20	22

a : number of days pregnancy at time of first or second treatment.

b : examinations of vaginal swabs for chlamydia were carried out on all ewes in experiments 2 and 3 and only on ewes having normal lambings in experiment 1. We eliminated ewes whose swabs were too contaminated for correct interpretation of results.

Results

Experimental infection

All ewes became febrile in the 24 hours following the inoculation of *Chlamydia psittaci*, strain AB7, and remained so for five to six days. During this period they were slightly depressed, lost appetite, and some had dyspnoea. However, all animals recovered quickly and remained clinically normal until abortions started to occur without any prior symptoms. In the control groups, the inoculation induced abnormal lambings in at least 60 % of the animals (59, 69 and 80 % in the

three different experiments, table 2). In addition, all ewes excreted chlamydia at lambing (table 3).

Duration of pregnancy (table 4)

In the three experiments, pregnancies lasted significantly longer in the treated group than in the controls. The first abortions occurred much later in the treated group than in the controls. In addition, in experiments 2 and 3 (two treatments at 10 and 14 day intervals), a significantly lower number of abnormally short pregnancies was observed in the treated group.

Table 4. — Effect of treatment on pregnancy of ewes inoculated during the third month of pregnancy with *Chlamydia psittaci*.

	Experiments					
	1		2		3	
	Treated 105 + 133 ^a	Controls	Treated 110 + 124 ^a	Controls	Treated 98 + 108 ^a	Controls
Duration of pregnancy (in days)						
Average	142.5	137.2	141.4	134.3	143.9	141.4
Minimum	132	113	135	114	137	130
Maximum	147	147	143	142	148	148
Pregnancy of abnormal duration (< 140 days)						
Total number	4	8	1	8	8	9
Percentage	20	36	10	80	8	35

a : number of days of pregnancy at time of first or second treatment.

Table 5. — Effect of treatment on the condition of lambs.

	Experiments					
	1		2		3	
	Treated 105 + 133 ^a	Controls	Treated 110 + 124 ^a	Controls	Treated 98 + 108 ^a	Controls
Number of lambs						
liveborn	20	17	12	6	28	9
stillborn	11	14	0	5	5	29
very weak at birth (die within 3 days)	1	2	0	0	5	3
Average weight (kg)						
live lambs	3.00	2.81	2.68	2.71	2.77	2.40
number of lambs per ewe ^b	4.44	4.00	3.21	2.71	4.15	2.60

a : number of days of pregnancy at time of first or second treatment.

b : ewes having aborted are not included.

Condition of lambs

A significantly higher number of live lambs and of normal lambings was obtained in the groups having had two treatments close together (experiments 2 and 3, tables 2 and 5). However, in all the experiments, the average weight of the liveborn lambs in the treated group was not significantly different from that

of the control group. The only significant difference occurs in the weight of lamb per ewe not having aborted in the third experiment.

Excretion of chlamydia at lambing (table 3)

At lambing all ewes excreted chlamydia except those in the treated group of experiment 1, where 4 of the 13 ewes with normal

Table 6. — Influence of a second treatment at 133 days of pregnancy (Experiment 1)

	Ewes	
	Treated ^a 105 + 133 ^b	Control
Number of pregnancies of abnormal duration (%)	1 (...)	3 (36)
<i>Lambings</i>		
Normal	13	9
Abnormal (abortions)	4 (2)	13 (8)
<i>Lambs</i>		
Liveborn	20	17
Stillborn	6	16

a : only the ewes having received two treatments are included.

We have eliminated the three ewes that aborted before the second treatment (one at 132 days of pregnancy, two at 133 days).

b : number of days of pregnancy at time of first or second treatment.

lambings excreted no chlamydia and two others only excreted very few (less than 100 PFU/ml vaginal mucuous suspension).

Number of treatments required

In our experimental conditions a single treatment at 105 days of pregnancy is insufficient to prevent abortions. In the first experiment this single treatment prolonged pregnancies as the first abortion occurred in the treated group 19 days after the first abortion in the control group, i.e., at 132 days of gestation. On day 133, three ewes of the treated group had aborted (one at day 132 and two at day 133), and five in the non-treated controls (on days 113, 117, 121, 123 and 128). As a single injection of Terramycin/L.A. was insufficient, we decided to give a second injection on day 133 of gestation to the 17 ewes still pregnant. After this injection there were four further abnormal lambings: two abortions (on days 138 and 140), the birth of twin lambs, one live and the other stillborn, on day 140, and on day 146 the birth of a very weak lamb which died within 72 hours. The 13 other ewes had normal lambings.

When the results of the lambings of the 17 ewes having received two treatments are compared to the results of the control group (table 6), there is a significantly higher number of normal lambings and of live lambs in the treated group than in the controls.

Evolution of antibody titers

All ewes responded to the inoculation by an increase of the complement-fixing antibodies.

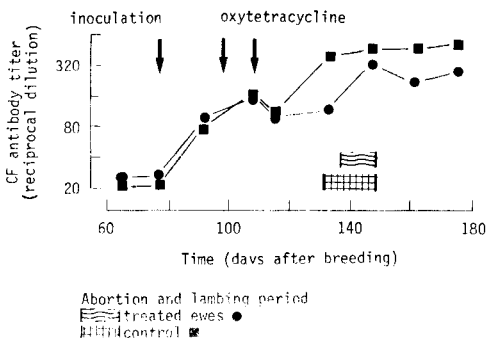


Fig. 1. — Complement-fixing antibodies response of ewes following intradermic inoculation with 6×10^6 Chlamydia. Experiment 3.

After treatment, the averages of the complement-fixing antibodies are slightly lower in the treated group than in the controls (figure 1), but this difference is not significant.

Discussion

In our experimental conditions, a single treatment with 20 mg/kg of Terramycin/LA at day 105 of pregnancy is not sufficient to limit the number of abortions. However, two treatments at 10 or 15 days interval during the fourth month of pregnancy result in a higher number of live lambs and more pregnancies of normal duration.

It should be noted that our experimental conditions were quite different to those occurring naturally in a flock:

- Here, all the animals received parenterally a high dose of chlamydia and this resulted in a large number of abortions (60 to 80 % of the ewes) and a high contamination of all the flock. All ewes of the control group excreted chlamydia at lambing.

- The time of the first treatment was chosen arbitrarily. In practice a flock is generally treated after the third or fourth abortion, without knowing either the individual level of infection or how long each animal has been infected. In these experiments we treated the animals before the first abortion but we waited for at least three weeks after the inoculation, by which time the chlamydia would have reached the placenta and started to multiply (Novilla and Jensen, 1970).

In these conditions, which are much more severe than those encountered in practice, the failure of the single treatment in the first experiment can be explained by two hypotheses:

- In this experiment the ewes were not completely separated, and it is possible that the treated ewes became contaminated again when the first abortions occurred in the control group at about day 113 of pregnancy. At this time the first injection was no longer effective, and yet it was early enough for ewes which became infected to abort.

- The injection of 20 mg/kg of Terramycin/LA is not sufficient to allow the ewes to eliminate the chlamydia. When the concentration of Terramycin/LA diminishes in the blood and in the tissues, the chlamydia start to multiply again. Abortions may then

occur if the treatment has been applied too long before the end of pregnancy. This second hypothesis is the more probable as ewes having had two treatments 20 and 30 days before lambing (experiments 2 and 3) excrete large numbers of chlamydia despite the fact that they give birth to live and apparently healthy lambs. When the second treatment is made later, at day 133 of pregnancy, it reduces the quantity of chlamydia excreted at lambing (experiment 1).

Several repeated treatments should eliminate the infection. It is possible to suppress completely the excretion of *Chlamydia trachomatis* in human venereal chlamydiosis if the treatment is continued for a sufficiently long time (Schachter and Dawson, 1979). However, for economical and practical reasons, it is not reasonable to consider giving several

treatments to control ovine chlamydial abortion.

These experiments seem to suggest that two treatments during the fourth month of pregnancy are sufficient to reduce the incidence of the disease. When abortions due to *Chlamydia psittaci* var *ovis* occur in a flock where abortions are grouped, two injections of Terramycin/LA at an interval of two weeks should reduce the number of abortions. However, it should be remembered that the infection remains present in the flock, and that the danger of abortion remains. The vaccination of ewes one month before mating should limit this risk (Rodolakis and Souriau, 1979).

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Summary

The treatment of ovine abortifacient chlamydiosis with Terramycin/LA 200 was examined in three successive experiments in which ewes were inoculated intradermally with 6×10^6 PFU chlamydia at 80 days of pregnancy. The efficacy of the treatment was estimated by comparing a control group with a treated group for number of live lambs, number of abnormal lambings, length of pregnancy, average weight of lambs at birth and genital excretion of chlamydia at lambing. Sixty to eighty per cent of the ewes in the control group aborted. Under these conditions a single treatment of 20 mg/kg of Terramycin/LA at day 105 of pregnancy, i.e. four weeks after the inoculation, is not effective. On the other hand, a combination of injections, one three weeks and one five weeks after the inoculation, result in longer pregnancies on average, fewer abortions and more liveborn lambs. However, the treatment does not modify either the weight of liveborn lambs or the vaginal excretion of chlamydia at lambing. The adaptation of this treatment to practical conditions and its interest are discussed.

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