Abortion and serological reaction of ewes after conjunctival instillation of Salmonella enterica subsp enterica ser abortusovis
R Sanchis, P Pardon, G Abadie

To cite this version:
Abortion and serological reaction of ewes after conjunctival instillation of *Salmonella enterica* subsp *enterica* ser *abortusovis*

R Sanchis¹*, P Pardon², G Abadie¹

¹ Ministère de l’Agriculture et de la Forêt, CNEVA, Laboratoire de Pathologie des Petits Ruminants et des Abeilles, Route des Colles, Sophia Antipolis 06410 Biot;
² Institut National de la Recherche Agronomique, Station de Pathologie de la Reproduction, Unité de Pathologie Infectieuse Ovine, 37380 Nouzilly, France

(Received 27 April 1990; accepted 18 September 1990)

Summary – The aim of the experiment was to evaluate in pregnant ewes the clinical, bacteriological and serological effects of conjunctival instillation of *S abortusovis*. An individual dose of $1 \times 10^{10}$ viable *S abortusovis* were administered to 20 ewes 92 d after mating. Among 18 pregnant ewes, 8 aborted 110 ± 13 d after mating, 4 had stillborn lambs and 6 a clinically normal lambing (114 ± 1.3 d). *S abortusovis* was isolated from placentas, swabs and foetuses of the 12 lambings. Evolution of "H" agglutination titers measured by means of a microtechnique using a coloured antigen was found to be independent of the clinical results. Instillation by conjunctival route could constitute an experimental model closer to a natural mode of infection than the subcutaneous route.

ovine salmonellosis / experimental infection / conjunctival route

Résumé – Avortement et réaction sérologique après instillation conjonctivale de *Salmonella enterica* subsp *enterica* ser *abortusovis*. Une dose unique de $1 \times 10^{10}$ UFC de *S abortusovis* a été administrée par instillation conjonctivale à 20 brebis, 92 j après la lutte. Sur 18 brebis gestantes, 8 avortements à 110 ± 13 j de gestation, 4 agnelages à terme d’agneaux morts nés et 6 agnelages normaux 144 ± 1,3 j après la lutte ont été observés. *S abortusovis* a été isolée à partir de tous les écouvillons, placentas et foetus prélevés après les 12 mises bas anormales et la cinétique des anticorps agglutinants est indépendante de l’évolution clinique des animaux. Comparée à l’inoculation sous-cutanée, l’instillation conjonctivale pourrait fournir un modèle expérimental plus proche des modalités naturelles de contamination.

salmonellose ovine / infection expérimentale / voie conjonctivale

* Correspondence and reprints
INTRODUCTION

Salmonella enterica subsp enterica ser abortusovis (thereafter called Abortusovis), a sheep adapted serotype, causes a contagious infectious disease with abortion as the main symptom and the main source of contamination. Abortion was regularly reproduced by Abortusovis subcutaneous injection from the third month of gestation (Pardon et al, 1983; Sanchis and Pardon, 1984). This model simulating bacteriemia and foetoplacental colonization was used to evaluate means of diagnosis and prevention of Abortusovis infection (Pardon et al, 1980, 1983; Sanchis and Pardon, 1981).

The subcutaneous challenge does not simulate early events of the natural infection, which probably takes place at the mucosal level. However, experimental contamination by oral (Jack, 1968), intragastric (Pardon et al, 1983), vaginal (Tadjebakhche et al, 1974) or conjunctival (Jack, 1968) route was shown not to regularly reproduce infection leading to abortion, even with high challenge doses. As the conjunctival route is currently used in other experimental ovine infections such as brucellosis (Fensterbank et al, 1982; Plommet and Fensterbank, 1984), we first reinvestigated conjunctival contamination with Abortusovis in rams; a serological response was regularly reproduced, but genital colonisation was not observed (Sanchis and Pardon, 1986). The aim of the present experiment was to evaluate the clinical, bacteriological and serological effects of an Abortusovis conjunctival instillation in pregnant ewes.

MATERIALS AND METHODS

Animals and protocol

Twenty-three-month-old Préalpes – Péone ewes were purchased from a salmonellosis-free flock. They were fed and allowed to remain outdoors in an enclosed yard. In 3 successive examinations between purchase and mating, they were shown to be negative to routine tests for brucellosis, chlamydiosis, Q fever, toxoplasmosis and Abortusovis infection. When 12 months old, they were synchronised in oestrus and mated with rams negative to the same serological tests. All ewes were housed in the same pen in an isolated sheep barn and challenged at d 92 after mating. The experiment ended 3 months after the normal term of gestation, without necropsy of ewes and surviving lambs.

Bacteria and challenge

The challenge strain Abortusovis 15/5 and the bacterial cultures have been described previously (Pardon and Marly, 1979). An individual dose of $1 \times 10^{10}$ viable Abortusovis 15/5 in 0.2 ml of sterile saline was administered by conjunctival route. The bacterial suspension was tested for purity and titrated by plating, as previously described (Pardon and Marly, 1979).

Sample collection

Ewes were bled every second day from challenge until end of gestation, and then every week. Uterine discharge was sampled with vaginal swabs on the day after each parturition. Placentae were recovered. Aborted foetuses were necropsied; spleen, brain, lung and stomach contents were sampled.

Examination procedures

Ewes were observed daily. Agglutinating antibodies against "H" Abortusovis antigens were measured in serum by means of a microtitration technique using whole
Abortusovis was coloured with tetrazolium red as previously described (Pardon et al., 1983, Sanchis et al., 1985). Swabs, organs or tissue samples were subjected to differential research for bacteria causing infectious abortion (Sanchis, 1982). Abortusovis was cultivated and identified as previously described (Pardon and Marly, 1979).

Terminology and expression of results

Parturitions were considered as abortions before 140 d of gestation, and as being infected when any sample led to isolation of Abortusovis. Antibody titers were expressed as means and standard errors of inverse of serum dilution.

RESULTS

Serological responses (Fig 1)

Individual serologic responses to challenge were similar whatever the clinical and bacteriological outcome of the gestation. Titers were maximal at = 15 d after instillation and then decreased to reach a plateau at = 60 d lasting until the end of the experiment.

Clinical results (table I)

After challenge, no visible reaction in the eye to the instillation was noticed, and the comportment of ewes remained normal. Two ewes did not become pregnant. Eight abortions were observed between d 100 and 137 after mating (mean ± SE: 110 ± 13), ie between 8 and 45 d after inoculation. Ten ewes lambed at term, between d 143 and 147 after mating (mean ± SE: 144 ± 1.3); in 4 of them, lambs were stillborn. Lambs of the 6 remaining ewes were clinically normal.

Bacteriological results (table I)

Among the 18 lambings, 12 were infected. Abortusovis was isolated from vaginal swabs, placentas and all recovered foetal organs taken from the 8 lambing before term, and from the 4 at term lambings with dead lambs. From the 6 at term parturitions with living lambs, the same samples were found to be negative.

DISCUSSION

Experimental conjunctival contamination about mid-gestation with $1 \times 10^{10}$ viable Abortusovis strain 15/5 induced a serological response in the 20 ewes. In 12 of the 18 pregnant ewes, this contamination led to foeto-placental colonisation and abortion or stillbirth. This high chal-
lenge dose was already shown necessary to obtain a reproducible serologic response in sheep (Pardon and Marly, unpublished results). In a preliminary experiment, this dose administered to 3 pregnant ewes induced 2 infected abortions and one infected at term lambing (Sanchis, unpublished results).

If effects of differences between the environmental conditions or the genetic background of the rams are considered as relatively low, these results could be compared to those of earlier experiments using the subcutaneous route with a similar challenge dose administered at a similar stage of gestation (Sanchis and Pardon, 1981; Pardon et al, 1983; Sanchis and Pardon, 1984). In these previous works, the subcutaneous route seemed to be more severe, with hyperthermia and prostration immediately after challenge. A greater proportion of abortions occurred earlier after inoculation. Maximal serologic response was also reached earlier, but with similar maximal "H" agglutination. So comparing the interval between challenge and effects after subcutaneous or conjunctival challenge, a direct relation between serological response and clinical effects could be contemplated. Taken as a whole, conjunctival contamination induces a longer course of infection and a greater variability in clinical and bacteriological effects than subcutaneous injection. Such a conclusion seems biologically plausible but would need a more comprehensive experiment to be carried out before it could be verified.

In this experiment, the relatively long period of time between the first abortions and the last infected

Table 1. Clinical and bacteriological results after instillation of 1 × 10¹⁰ S abortusovis 92 d after mating.

<table>
<thead>
<tr>
<th>Time of pregnancy (days)</th>
<th>Lambs</th>
<th>S abortusovis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Clinic</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td>101</td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>104</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td>105</td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>107</td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>110</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td>125</td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>137</td>
<td>2</td>
<td>A</td>
</tr>
<tr>
<td>143</td>
<td>2</td>
<td>CN</td>
</tr>
<tr>
<td>144</td>
<td>1</td>
<td>SB</td>
</tr>
<tr>
<td>144</td>
<td>2</td>
<td>SB</td>
</tr>
<tr>
<td>144</td>
<td>1</td>
<td>CN</td>
</tr>
<tr>
<td>145</td>
<td>1</td>
<td>CN</td>
</tr>
<tr>
<td>146</td>
<td>1</td>
<td>SB</td>
</tr>
<tr>
<td>146</td>
<td>2</td>
<td>CN</td>
</tr>
<tr>
<td>147</td>
<td>2</td>
<td>SB</td>
</tr>
<tr>
<td>147</td>
<td>1</td>
<td>CN</td>
</tr>
</tbody>
</table>

a Two ewes were not pregnant with negative bacteriological results on swab examination. b A : abortion; SB : stillborn; CN : clinically normal.
lambings could be attributed to an overall variability in the status of the ewes on the day of injection. However, the housing in the same experimental barn suggests another hypothesis: the last infections could result not from the contaminating instillation, but from the massive contamination due to the first abortions. However, the serological response of all animals which underwent instillation was similar, whatever the clinical or bacteriological issue of gestation. Thus those possible reinfections do not induce a detectable rise in the production of circulating antibodies, whereas an effect on the serologic response is regularly observed after vaccine boosters or after a challenge following vaccination with dead or living vaccines (Sanchis and Pardon, 1981). So, depending on the number of bacteria translocating through mucosal epithelia or on prolonged restriction to lymphatic dissemination, a transient, low-level and delayed bacteriemic phase could lead to foeto-placental colonisation without any detectable serological consequences.

The present protocol, using a dose similar to that used with the subcutaneous route (Pardon et al, 1983) partially meets the need to regularly reproduce an infection leading to foeto-placental colonisation so that a rather restricted number of animals can be used in experimental groups. In this experiment, the birth of infected lambs which were apparently healthy but which died during the weeks following lambing was not observed. But the clinical and bacteriological effects correspond to the more frequent issues of gestation in natural conditions: infected abortions and some infected at term par-turitions with stillborn lambs. This route probably simulates the course of the natural infection better than the subcutaneous one. The dose – effect relationship is currently under study.

REFERENCES

Jack EJ (1968) Salmonella abortus ovis, an atypical Salmonella. Vet Rec 82, 558-551