

1 **Supplementary Tables (S1, S2 and S3)**

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3 **Table S1.** Bacteriological examination of lymph nodes and organs from sheep
 4 inoculated by conjunctival route with 10^9 viable cfu of *M. agalactiae* wild type strain
 5 (PG2) or Vpma phase locked mutants (PLMs) PLMU and PLMY and necropsied at
 6 Day 20 p.i.

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LN ^a	PLM Sheep					PG2 Sheep				
	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15
MAN L	- ^b	-	-	-	-	-	-	-	-	-
MAN R	+ 7x10 ^{3c}	+ 1.8x10 ⁴	-	+ NR ^d	+ 1.0 x10 ⁴	-	-	-	-	-
PAR L	-	-	-	-	-	-	-	-	-	-
PAR R	+ 7x10 ⁴	+ 0.9x10 ⁶	+ NR	+ 2.3x10 ⁵	+ 0.6 x10 ⁴	+ 4.2x10 ³	+ NR	+ 4.4x10 ⁴	+ 0.9x10 ⁵	+ 2.6x10 ⁵
RPHM L	-	-	-	+ 0.5x10 ⁵	-	-	-	-	-	+ 1.4x10 ³
RPHM R	-	+ 0.9x10 ⁴	-	-	-	-	-	-	+ NR	-
RPHL L	-	-	-	-	-	-	-	-	-	-
RPHL R	-	+ 0.6x10 ⁵	-	-	-	+ NR	-	+ 0.2x10 ²	+ 5.7x10 ³	-
CSUP L	-	-	-	+ 0.5x10 ²	-	-	-	-	-	-
CSUP R	-	-	-	+ NR	-	-	-	-	-	-
MED	-	-	-	-	-	-	-	-	-	-
JEJ	-	-	-	-	-	-	-	-	-	-
SCRO L	-	-	-	+ NR	-	-	-	-	-	-
SCRO R	-	-	-	-	-	-	-	-	-	-
Kidney	-	-	-	-	-	-	-	-	-	-
Spleen	-	-	-	-	-	-	-	-	-	-
Lungs	-	-	-	-	-	-	-	-	-	-
BALF ^e	-	-	-	-	-	-	-	-	-	-

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9 ^a LN (Lymph node) L (left) / R(right) –MAN: Mandibularis; PAR : Parotidialis; RPHM: Retropharyngeal
 10 medialis; RPHL: Retropharyngeal lateralis; CSUP: Cervical superficialis; MED: Medialis; JEJ: jejunal;
 11 SCRO: Scrotalis

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13 ^b -: negative for *M. agalactiae* isolation; +: positive for *M. agalactiae* isolation

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15 ^c Number of viable cfu/g of LN

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17 ^d NR: No reisolation when plated directly for quantitative mycoplasma analysis and thereby implying a
 18 very low initial bacterial count

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20 ^eBALF: Bronchoalveolar lavage fluids

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22 **Table S2:** Bacteriological examination of nasal and eye swabs from lambs inoculated
 23 with 10^9 viable cfu of *M. agalactiae*

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Day pi	Swabs	PLM Sheep					PG2 Sheep				
		# 6	#7	#8	# 9	#10	#11	#12	#13	#14	#15
1	N ^a	++ ^b	-	++	+	++	+	-	+	-	+
	E	+	+	-	+	+	-	-	+	-	-
2	N	++	+	++	++	+	+	-	-	-	+
	E	-	+	+	+	+	+	-	+	-	-
5	N	-	+	+	+	+	+	-	-	-	-
	E	+	+	+	+	+	+	-	+	+	-
7	N	-	+	+	-	+	+	-	-	+	+
	E	-	+	+	+	+	-	-	+	+	-
9	N	-		+	+	++	+	-	-	-	+
	E	-	+	+	+	+	+	+	+	+	-
12	N	-	-	+	-	-	-	-	-	-	-
	E	-	+	-	+	+	+	+	-	-	-
14	N	-	+	-	-	-	-	-	-	-	-
	E	-	+	-	-	+	-	-	-	-	-
16	N	-	+	-	-	+	-	-	-	-	-
	E	-	+	-	+	+	+	-	-	-	-
19	N	-	-	+	-	-	+	-	-	-	-
	E	-	+	-	-	+	+	-	+	+	-

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^a N: nasal Swab; E: eye swab

^b +: positive for right side; ++: positive for both left and right side; -: negative culture

31 **Table S3.** Quantitative bacteriological examination of lymph nodes and organs from
 32 sheep inoculated by intramammary route with 10^9 viable cfu of *M. agalactiae* wild
 33 type strain (PG2) or Vpma phase locked mutants (PLMs) PLMU and PLMY and
 34 necropsied at Day 28 p.i.

LN ^a	PLM Sheep					PG2 Sheep				
	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15
SUPM L	- ^b	+ 1.9x10 ^{2c}	-	-	-	-	-	-	-	-
SUPM R	-	+ 1.5x10 ³	-	-	-	+ 1.0x10 ²	-	+ 3.9x10 ⁴	-	+ 6.1x10
ILI L	+ 9.1x10	-	+ 2.7x10 ²	-	-	+ 2.2x10 ³	-	+ 1.4x10 ²	+ 6.2x10	-
ILI R	+ 3.5x10 ²	-	+ 0.7x10 ²	-	+ NR ^d	+ NR	+ NR	+ 1.3x10 ²	+ 2.5x10	+ 5.8x10 ²
MES	+ 3.2x10 ⁵	-	+ 6.0x10 ²	-	-	+ 3.0x10 ²	-	+ 1.6x10	-	-
MED	-	-	+ 1.6x10 ²	-	-	-	-	-	-	-
CSUP L	-	+ NR	-	-	-	-	-	-	-	-
CSUP R	-	-	-	-	-	-	-	-	-	-
PAR L	-	+ NR	-	-	-	-	-	-	-	-
PAR R	-	+ NR	-	-	-	-	-	-	-	-
Udder L	+ NR	+ 5.3 x10 ⁴	+ 2.5x10 ⁴	-	+ NR	+ 1.1x10 ⁵	+ 1.1x10 ³	+ 2.8x10 ⁴	+ 3.3x10 ²	+ 2.6x10 ³
Udder R	+ 2.2x10 ⁷	+ 1.9 x10 ⁷	+ 1.1x10 ⁷	+ 2.3x10 ²	-	+ 8.9x10 ⁵	+ 1.2x10 ⁷	+ 2.2x10 ⁸	+ 3.3x10 ⁶	+ 3.3x10 ⁶

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 36 ^a LN (Lymph node) L (left) / R(right) – SUPM: Supramammary; ILI: Iliacis; MES: Mesenterialis; MED:
 37 Medialis; CSUP: Cervicalis superficialis; PAR : Parotidialis

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 39 ^b -: negative for *M. agalactiae* isolation; +: positive for *M. agalactiae* isolation

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 41 ^c Number of viable cfu/g of LN

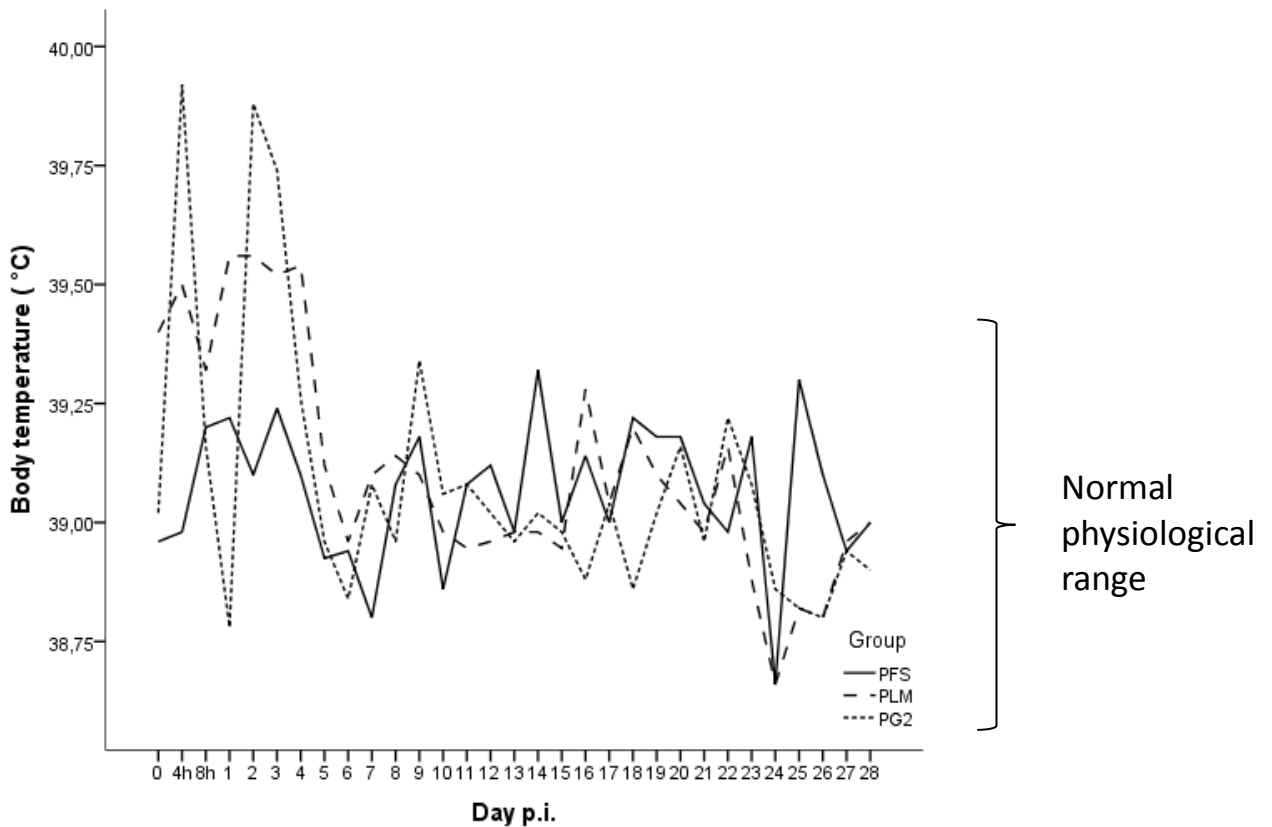
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43 ^d NR: No reisolation when plated directly for quantitative mycoplasma analysis and thereby implying very
44 low initial mycoplasmal counts

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49 **Supplementary figures (S1, S2, S3 & S4)**

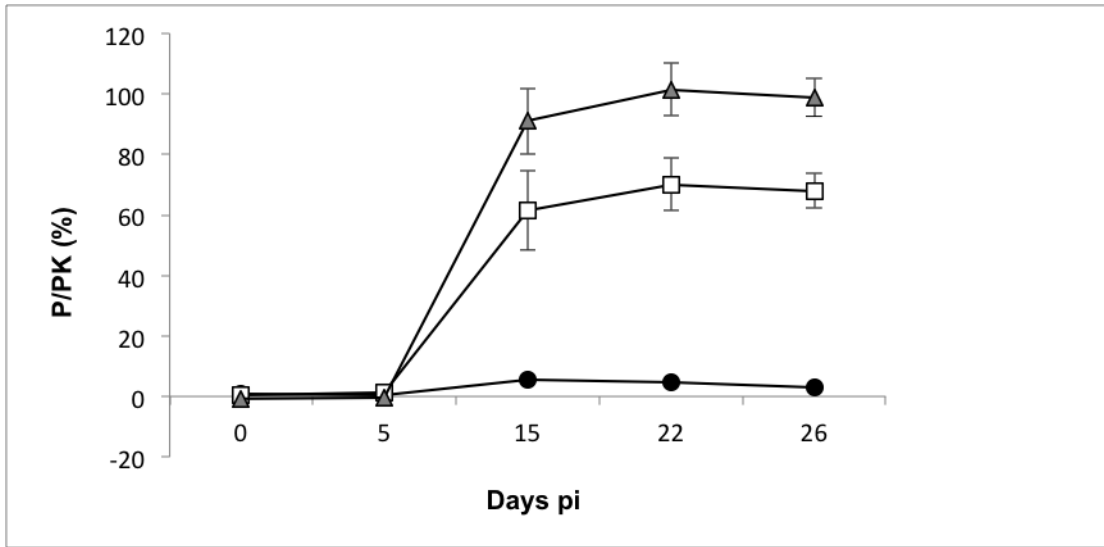
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72 **Fig S1.** Mean body temperatures (°C) monitored via the rectal route shown as a function
73 of time in the three groups of five ewes each, infected intramammarily with *M. agalactiae*
74 wild type strain PG2 (...), Vpma phase invariable PLMs (---) and PFS (—), respectively. A
75 biphasic increase in the mean body temperature of the PG2 infected sheep is observed
76 at 4 h and Day 2 pi.

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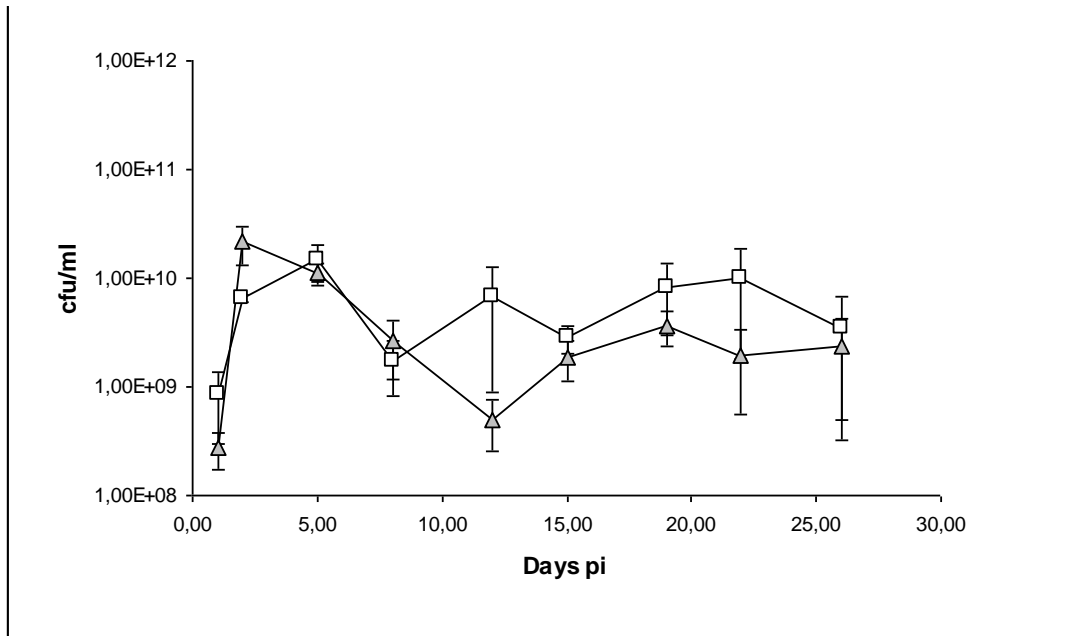


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Fig. S2 Time-course of *M. agalactiae* specific serological antibody response (measured by the IDEXX *M. agalactiae* Ab test kit) in sheep infected with PG2 (grey Δ), PLMs (white □) and PFS (black O) via the intramammary route. P/PK (%) values greater than 60 are considered positive as per manufacturer's instructions.

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93 **Fig. S3.** Time-course of *M. agalactiae* excretion in milk obtained from the inoculated right
94 udder halves of sheep experimentally infected with 10⁹ viable cfu of type strain PG2 (grey
95 Δ) and PLMs (white □). Mean log₁₀ cfu values of mycoplasmas per millilitre of milk are
96 depicted and bars represent standard error of the mean.

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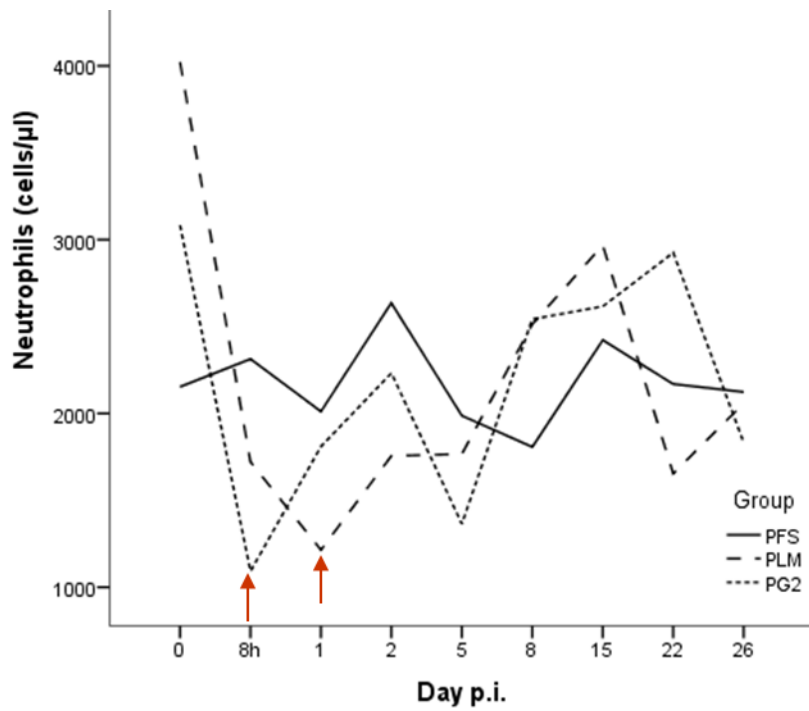
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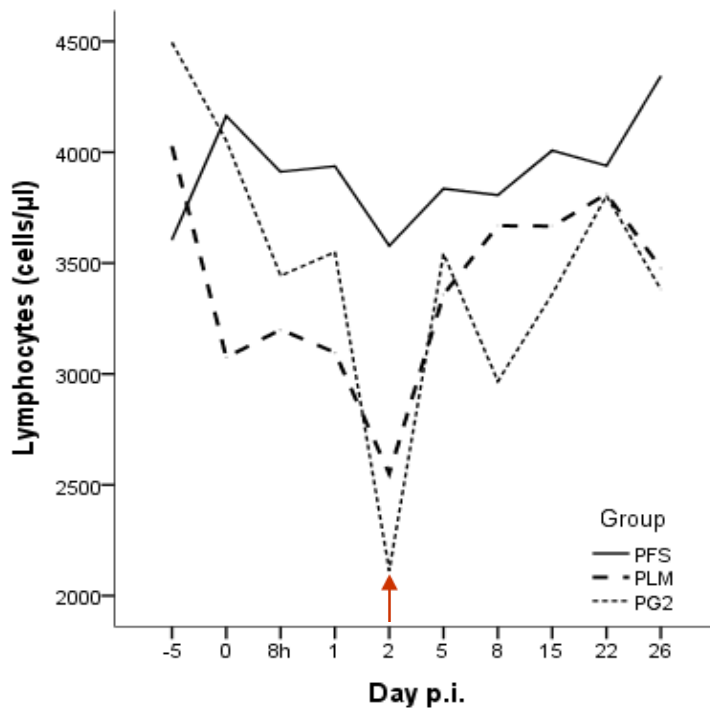
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Fig. S4. Systemic responses to intramammary *M. agalactiae* infection. Blood samples were collected at regular intervals after inoculation and analyzed for differential neutrophil granulocyte (A) and lymphocyte counts (B). Mean cell counts

122 are indicated for five animals of each group at a given time point. Arrows indicate
123 lymphopenia and neutropenia, respectively, with significant ($P < 0.05$) differences
124 between the control (PFS) and *M. agalactiae* infected animals (PLMs and PG2), and
125 also between the PG2 and PLM groups in terms of time and/or extent of
126 lymphopenia/neutropenia.

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