Supplementary material

Article

Detection of cathelicidin-1 in the milk as an early indicator of mastitis in ewes

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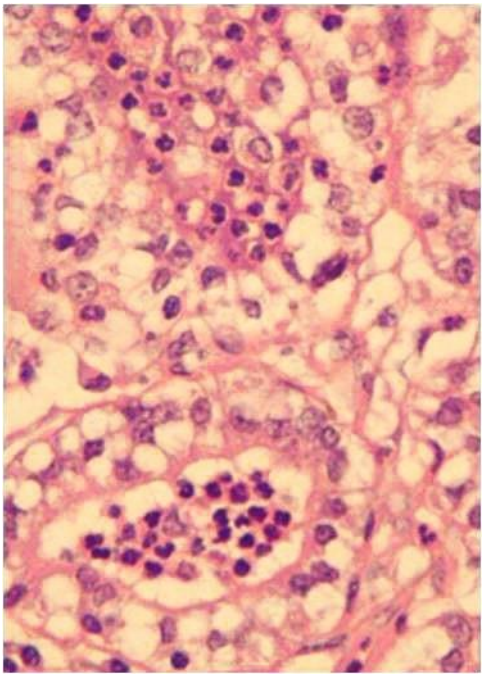

Figure S1. Histological section of mammary parenchyma, from inoculated side of the udder, with marked intra-alveolar neutrophilic infiltration and destruction of mammary alveoli (experiment 1).

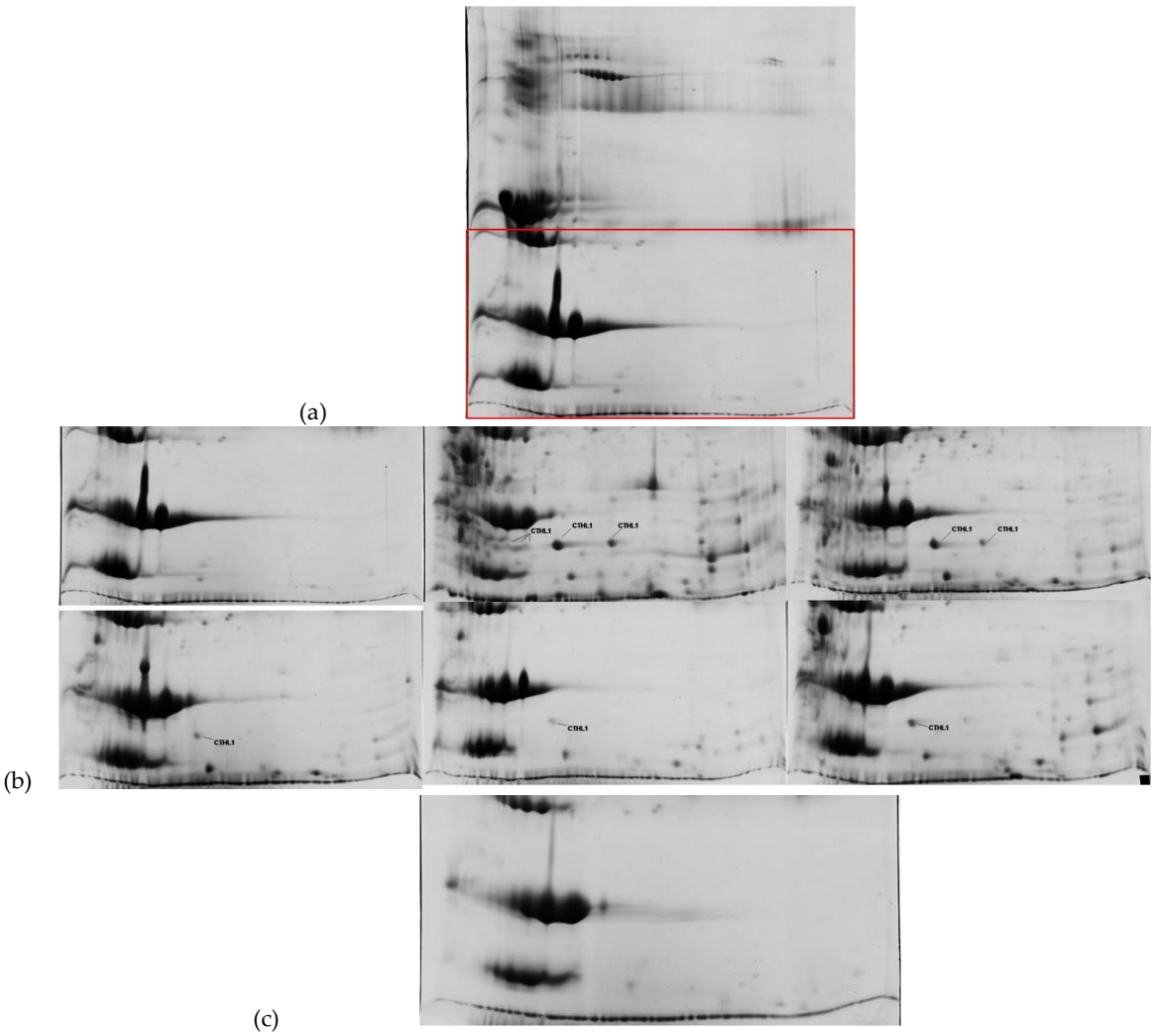

Figure S2. 2-DE gels with annotation of cathelicidin-1, obtained from milk samples (whey) collected from the inoculated side of the udder of a ewe before or after inoculation of the ipsilateral teat with *M. haemolytica* (a, b) or from the uninoculated side of the udder of the same ewe (c) (protein identification by MALDI-TOF MS) (experiment 1).

(a) 2-DE gel obtained from a whey sample before challenge, from the inoculated side of the udder of a ewe; the area in red indicates the region of the gels shown in detail in (b) and (c). (b) Region of 2-DE gels obtained from whey samples before or sequentially after challenge, from the inoculated side of the udder of a ewe; from top left to the right and from bottom right to the right: before inoculation (D0), 12 h after inoculation (D0+12 h), 1 d after inoculation (D1), D2, D3, D4. (c) Region of 2-DE gel obtained from whey sample 12 h after inoculation of the contralateral side of the udder.

Horizontal axis: isoelectric point 3 to 10 (non-linear) from left to right; vertical axis: molecular weight 10 to 100 kDa (non-linear) from bottom to top.

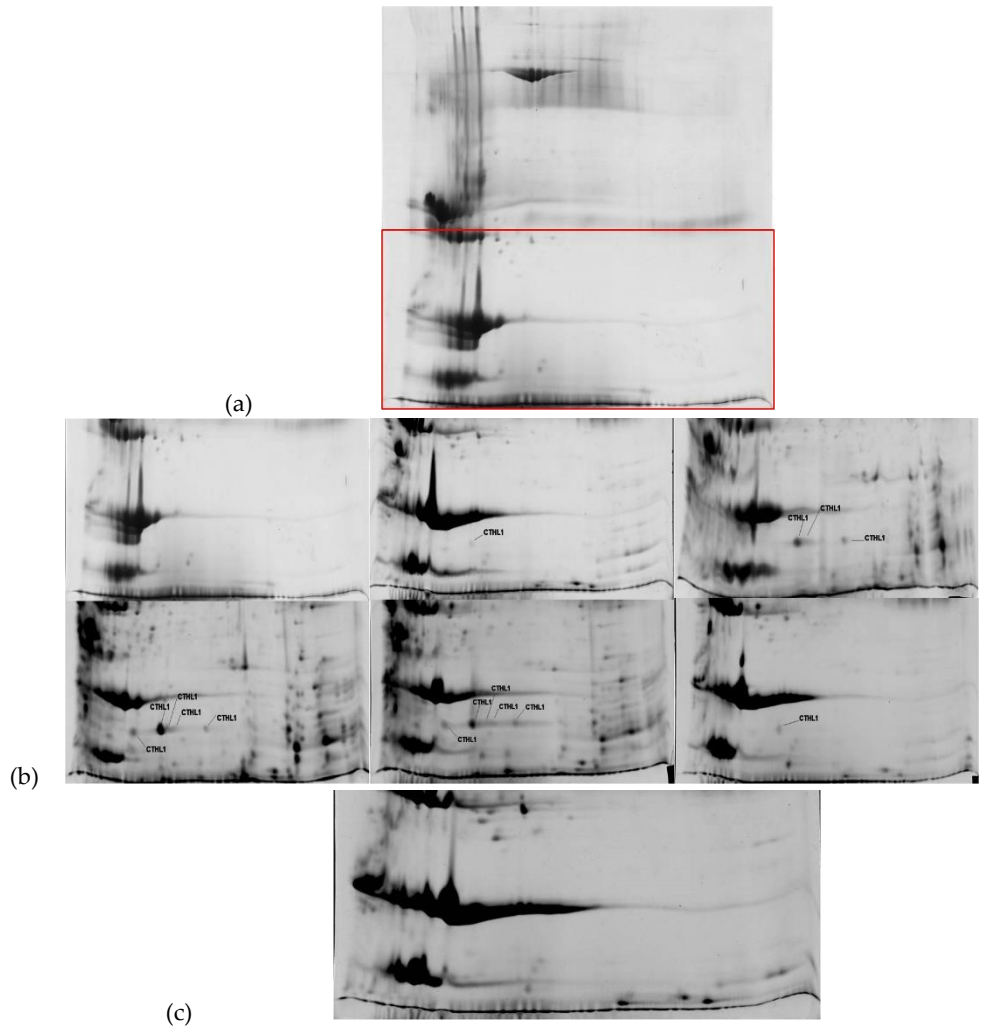

Figure S3. 2-DE gels with annotation of cathelicidin-1, obtained from milk samples (whey) collected from the inoculated side of the udder of a ewe before or after inoculation of the ipsilateral gland with *S. chromogenes* (a, b) or from the uninoculated side of the udder (c) (protein identification by MALDI-TOF MS) (experiment 2).

(a) 2-DE gel obtained from whey sample before challenge, from the inoculated side of the udder of a ewe; the area in red indicates the region of the gels shown in detail in (b) and (c). (b) Region of 2-DE gels obtained from whey samples before or sequentially after challenge, from the inoculated gland; from top left to the right and from bottom right to the right: before inoculation (D0), 3 h after inoculation, 6 h after inoculation, 9 h after inoculation, 12 h after inoculation, 24 h after inoculation. (c) Region of 2-DE gel obtained from pooled whey samples, from the contralateral to inoculated gland. Horizontal axis: isoelectric point 3 to 10 (non-linear) from left to right; vertical axis: molecular weight 10 to 100 kDa (non-linear) from bottom to top.

Table S1. Spot densities (mean ± standard error of the mean) of cathelicidin-1 in 2-DE gels from sequential milk samples (whey) from inoculated or uninoculated side of the udder, subsequently to bacterial challenge into one side of the udder (protein identification by MALDI-TOF MS).

Udder side	**Total spots (n)**	**Before challenge**	**After challenge**				
			Experiment 1				
		D0	D + 12 h	D1	D2	D3	D4
Inoculated	2.6 ± 0.7[a]	0.0 ± 0.0[x]	3357.9 ± 2687.6[a,k,l,x]	2408.3 ± 2142.0[a,k,m,x]	431.7 ± 278.9[l,m]	529.0 ± 334.8[a,x]	1573.1 ± 1577.8
Un-inoculated	0.8 ± 0.4[a]	0.0 ± 0.0	0.0 ± 0.0[a]	68.2 ± 60.3[a]	14.8 ± 14.7	5.7 ± 5.7[a]	0.0 ± 0.0
			Experiment 2				
		D0	3 h	6 h	9 h	12 h	24 h
Inoculated with any organism	2.3 ± 0.4	0.0 ± 0.0[x]	89.1 ± 25.8	1499.8 ± 721.3[a]	3679.8 ± 1726.0[a,x]	3732.0 ± 1293.2[a,x]	1515.7 ± 1057.2[a,x]
Inoculated with *M. haemolytica*	3.0 ± 0.6	0.0 ± 0.0[x]	101.1 ± 55.5	2283.5 ± 1408.1	4673.1 ± 3661.0[x]	4951.1 ± 2387.6	2465.5 ± 2156.8
Inoculated with *S. chromogenes*	1.7 ± 0.3	0.0 ± 0.0[x]	77.2 ± 10.1	716.2 ± 76.8	2686.5 ± 716.4	2512.9 ± 1085.5	565.9 ± 192.5
Un-inoculated	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0

Within each experiment: a = $p \leq 0.05$ between inoculated and non-inoculated glands, k-m = $p \leq 0.05$ between inoculated glands and x = $p \leq 0.05$ for inoculated glands compared to D0.