

## Supplementary material

**Table S1:** Details of statistical tests performed to compare bacterial copy numbers in biopsies and swabs. Associations with  $P < 0.05$  (also indicated by \*) were considered as significant. BFR: Bovine foot rot; BDD: Bovine digital dermatitis; Inc: Inconclusive; M: M-stage classification system of BDD; Fnec: *Fusobacterium necrophorum*; Fmort: *Fusobacterium mortiferum*; Bpyo: *Bacteroides pyogenes*; Plev: *Porphyromonas levii*; Tmed: *Treponema medium*; Tped: *Treponema pedis*; Tphg: *Treponema phagedenis*; SE: standard error; OR: odds ratio; CI: confidence interval; SC: subcutaneous.

Comparisons	Count regression group (punch biopsy samples as reference and swab as test variable)					Zero-inflated part		
	Estimate	SE	P-value	OR	95% CI for OR	Intercept	SE	P-value
<b>BFR</b>								
Fnec	8.8237	0.4471	*	6793.5	2828.4, 16317.2	-15.40	653.50	0.982
Fmort	7.4158	0.7390	*	1662.0	390.5, 7073.9	-2.5978	0.7072	0.0002*
Bpyo	9.0700	0.8116	*	8690.6	1770.9, 42648.1	-2.3706	0.8596	0.006*
Plev	6.5153	0.6778	*	675.4	178.9, 2549.8	-12.07	115.85	0.917
Tmed	2.4203	0.7142	*	11.2	2.8, 45.6	0.9781	0.2906	0.00008*
Tphg	5.7885	0.9293	*	326.5	52.8, 2018.2	-1.0517	0.3856	0.00638*
Tped	9.5061	1.1539	*	13441.4	1400.4, 129017.5	-0.4938	0.7111	0.487
<b>BDD-M2</b>								
Fnec	7.1630	0.7685	*	1290.8	286.2, 5821.9	-2.820	1.068	0.00829*
Fmort	6.6552	0.5108	*	776.8	285.5, 2114	-3.654	1.411	0.0096*
Bpyo	6.1580	1.285	*	472.3	38.1, 5857.1	-18.99	5016.93	0.997
Plev	7.3762	0.5860	*	1597.5	506.6, 5038.2	-16.1	572	0.978
Tmed	4.1790	0.1754	*	65.3	46.3, 92.1	0.3738	0.3762	0.32
Tphg	2.6622	0.6357	*	14.3	4.1, 49.8	-20.69	5685.04	0.997
Tped	6.887	1.184	*	979.7	96.1, 9981.9	-10.62	36.99	6.06e-09*
<b>BDD-M4</b>								
Fnec	6.6855	0.7664	*	800.7	178.3, 3596	-17.57	1847	0.992
Fmort	60.94	0.6673	*	407.2	110.1, 1506.2	-2.1096	0.7866	0.00732*
Bpyo	7.588	2.009	*	1973.9	38.5, 101278.7	-1.2174	0.8319	0.143
Plev	3.7269	0.9273	*	41.5	6.7, 255.8	-2.824	1.227	0.0214*
Tmed	2.3605	0.3231	*	10.6	5.6, 20	-2.368	1.046	0.0236*

Tphg	4.929	1.061	*	138.2	17.3, 1105.8	-18.29	2245.6	0.994
Tped	8.3064	1.2101	*	4049.7	377.9, 43400.6	-10.02	76.31	0.895
Inconclusive								
Fnec	7.7216	1.2040	*	2256.5	213.1, 23893.8	-16.44	2197.86	0.994
Fmort	7.560	1.070	*	1920.7	235.8, 15642.4	-1.2958	0.6681	0.0524
Bpyo	7.1119	1.3625	*	1226.5	84.9, 17717.9	-14.58	655.7	0.982
Plev	6.1958	0.9429	*	490.7	77.3, 3114.7	-3.432	1.428	0.0162*
Tmed	1.3090	0.7979	*	3.7	0.8, 17.7	-0.4831	0.4875	0.322
Tphg	8.1146	1.1753	*	3343.1	334, 33463.4	-17.4	4854.7	0.997
Tped	8.846	1.692	*	6949.6	252.2, 191535.8	-1.98	3.575	0.58
Healthy								
Fnec	5.2653	0.5167	*	193.5	70.3, 532.7	-1.432	0.502	0.00434*
Fmort	2.6371	0.6662	*	14	3.8, 51.6	0.003147	0.303857	0.992
Bpyo	4.4324	0.6283	*	84.1	24.6, 288.3	0.1001	0.3015	0.74
Plev	3.4673	0.8052	*	32	6.6, 155.3	-2.4591	0.8138	0.00251*
Tmed	4.2874	0.7202	*	72.8	17.7, 298.5	1.0413	0.3532	0.0032*
Tphg	1.7646	0.6476	*	5.8	1.6, 20.8	-1.0378	0.3857	0.00713*
Tped	4.3883	0.7323	*	80.5	19.2, 338.2	0.2186	0.3127	0.485

**Table S2:** Details of statistical tests performed to compare bacterial copy numbers in biopsies and subcutaneous samples. Associations with  $P < 0.05$  (also indicated by \*) were considered as significant. BFR: Bovine foot rot; BDD: Bovine digital dermatitis; Inc: Inconclusive; M: M-stage classification system of BDD; Fnec: *Fusobacterium necrophorum*; Fmort: *Fusobacterium mortiferum*; Bpyo: *Bacteroides pyogenes*; Plev: *Porphyromonas levii*; Tmed: *Treponema medium*; Tped: *Treponema pedis*; Tphg: *Treponema phagedenis*; SE: standard error; OR: odds ratio; CI: confidence interval.; AIC: Akaike's information criterion; SC: subcutaneous; ULoA: upper limit of agreement; LLoA: lower limit of agreement; CCC: concordance correlation coefficient.

Comparisons	Count regression group (punch biopsy samples as reference and SC as test variable)	Zero-inflated part

	Estimate	SE	P-value	OR	95% CI for OR	Intercept	SE	P-value
<b>BFR</b>								
Fnec	-1.241	0.8031	0.122	0.3	0.1, 1.4	-16.83	7469.93	0.998
Fmort	-2.2189	0.811	0.00622*	0.1	0.02, 0.5	-1.932	1.479	0.191
Bpyo	-1.1765	0.8598	0.171	0.3	0.06, 1.7	-0.229	0.3912	0.558
Plev	-0.6784	0.6779	0.317	0.5	0.1, 1.9	-2.7475	0.7613	0.00031*
Tmed	-0.1318	0.9875	0.894	0.9	0.1, 6.1	0.93	0.4975	0.0616
Tphg	-0.1453	1.0457	0.889	0.9	0.1, 6.7	-18.36	9887.69	0.999
Tped	1.4554	0.5942	0.0143*	4.3	1.3, 13.7	0.5032	0.3664	0.17
<b>BDD-M2</b>								
Fnec	-1.833	1.378	0.183	0.2	0.01, 2.4	-19.49	8950.82	0.183
Fmort	-1.2065	0.9905	0.223	0.3	0.04, 2.1	-3.552	2.42	0.142
Bpyo	-2.6107	0.7025	0.0002*	0.1	0.01, 0.3	-1.479	0.717	0.0391*
Plev	-1.7249	0.8472	0.0417*	0.2	0.03, 0.9	-20.09	5429.7	0.997
Tmed	0.3438	0.3603	0.34	1.4	0.77, 2.9	0.6931	0.5	0.166
Tphg	-3.1204	0.8923	0.0005*	0.04	0.008, 0.3	-20.75	7566.85	0.998
Tped	-1.247	1.555	0.422	0.3	0.01, 6	-19.18	3453.79	0.996
<b>BDD-M4</b>								
Fnec	0.8266	1.2785	0.5179	2.3	0.2, 28	-19.35	12821	0.999
Fmort	0.4439	1.7721	0.802	1.6	0.04, 50.3	0.6931	0.6124	0.258
Bpyo	-1.968	1.1922	0.0988	0.1	0.01, 1.4	-1.463	1.203	0.224
Plev	-3.877	3.99	0.331	0.02	8.3e-06, 51.6	-0.7949	0.6908	0.25
Tmed	-5.351	0.0093	*	0.005	0.005, 0.005	-1.062	1.160	0.36
Tphg	-0.036	1.2326	0.97645	0.96	0.09, 10.8	-20.9	9974.1	0.998
Tped	-1.355	1.268	0.2851	0.3	0.02, 3.1	-19.65	9359.92	0.998

**Table S3:** Details of statistical tests performed to compare bacterial copy numbers in biopsies and subcutaneous samples. Associations with  $P < 0.05$  (also indicated by \*) were considered as significant. BFR: Bovine foot rot; BDD: Bovine digital dermatitis; Inc: Inconclusive; M: M-stage classification system of BDD; Fnec: *Fusobacterium necrophorum*; Fmort: *Fusobacterium mortiferum*; Bpyo: *Bacteroides pyogenes*; Plev: *Porphyromonas levii*; Tmed: *Treponema medium*; Tped: *Treponema pedis*; Tphg: *Treponema phagedenis*; SE: standard error; OR: odds



	Bland-Altman's statistics between swabs and punch biopsy samples (bias (ULoA, LLoA))					Bland-Altman's statistics between SC and punch biopsy samples (bias (ULoA, LLoA))		
Fnec	-9561616 (- 24512368, - 43635599) *	-2490597 (6693817, - 11675010)	-5571282 (30902194, -42044758)	-8159781 (25740246, -42059808)	- 3756.724 (14205.1 9, - 21718.64 )	1097.116 (7134.98 7, - 4940.755 )	28340.42 (188621. 6, - 131940.8 )	310.24 (1943.05 9, - 1322.579 )
Fmort	-543581.5 (1728795, - 2815958) *	-1668377 (2307916, - 5644671) *	-1670794 (5312315, - 8653903)	-507238.3 (2100278, - 3114755)	- 187.2264 (2104.13 9, - 2478.592 )	494.0981 (4146.56 7, - 3158.371 )	- 127.7556 (12390.6 3, - 12646.14 )	132.1667 (841.509 1, - 577.1758 )
Bpyo	-13485421 (56262033, -83232875) *	-1040019 (4268784, - 6348821)	-30771664 (160017933, - 221561261)	-50369744 (277607882 , - 378347369)	- 60214.44 (521732. 7, - 642161.6 )	454.3262 (5921.29 1, - 5012.638 )	488.3544 (1940.70 4, - 963.9954 )	149.2 (692.484 6, - 394.0846 )
Plev	-715060507 (303280228 4, - 446292329 8) *	-701238945 (109398233 1, - 249646022 0) *	-203951893 (857976128, - 1265879915 )	-324863162 (138730670 5, - 203703302 8)	-805683 (272165 2, - 4333018 ) *	529439.1 (360715 1, - 2548273 )	386606.7 (2232415 , - 1459202)	1179562 (683679 5, - 4477672 )
Tmed	-203.3925 (1450.954, - 1857.739)	-309.4933 (674.2829, - 1293.27) *	-4075037 (17107567, -25257640)	-293.7142 (956.2004, - 1543.629)	- 220.2021 (804.238 6, - 1244.643 ) *	1.435625 (80.0336 3, - 77.16238 )	2.555556 (36.7671 2, - 31.65601 )	384826.2 (223241 8, - 1462765 )
Tphg	-2154069 (18575068, -22883206)	-8898197 (11720394, -29516788) *	-4839551 (9177873, - 18856975) *	-1513738 (7878360, - 10905836)	- 179.1972 (1034.41 4, - 1392.809 )	- 144.0006 (1265.16 4, - 1553.166 )	181586.6 (791989. 3, - 428816.2 )	212764.5 (123309 3, - 807563.8 )
Tped	-50012108 (396280138 , - 496304354)	- 182297521 1 (312171960 8, - 676767003 1) *	- 1859116151 (660743909 6, - 1032567139 8)	-38020304 (219940706 , - 295981314)	- 12229.59 (52667.7 4, - 77126.92 )	-199.2 (1275.02 8, - 1673.428 )	2693500 (1468283 7, - 9295838)	97487.78 (489737. 3, - 294761.7 )
	Lin's CCC between swabs and punch biopsy samples CCC (95% CI)					Lin's CCC between SC and punch biopsy samples CCC (95% CI)		
Fnec	0 (0, 0)	0.01 (0, 0.03)	0 (0, 0.01)	0 (0, 0)	0 (0, 0)	-0.01 (- 0.08, 0.07)	0.01 (0, 0.02)	0.11 (0.03, 0.2)

Fmort	0 (0, 0.01)	0 (0, 0)	0 (0, 0.01)	0 (0, 0)	-0.08 (-0.44, 0.31)	0 (-0.01, 0.01)	-0.16 (-0.68, 0.46)	-0.05 (-0.27, 0.17)
Bpyo	0 (0, 0)	-0.05 (-0.25, 0.14)	0 (0, 0)	0 (0, 0)	0.01 (0, 0.01)	-0.04 (-0.38, 0.3)	-0.01 (-0.06, 0.05)	0.13 (-0.17, 0.41)
Plev	0 (0, 0)	0 (0, 0)	0 (0, 0.02)	0 (0, 0)	-0.03 (-0.13, 0.08)	-0.01 (-0.04, 0.02)	-0.12 (-0.51, 0.32)	0.01 (0, 0.01)
Tmed	0.21 (0.16, 0.25)	-0.01 (-0.03, 0.02)	0.1 (0.06, 0.14)	-0.14 (-0.54, 0.3)	0 (-0.01, 0.01)	0.39 (-0.01, 0.68)	0.08 (-0.55, 0.65)	0.01 (0, 0.01)
Tphg	0 (0, 0)	0.05 (0, 0.11)	0 (0, 0)	0 (0, 0)	0.02 (-0.26, 0.3)	0.02 (-0.11, 0.15)	-0.05 (-0.2, 0.1)	0.01 (0, 0.01)
Tped	0 (0, 0)	0 (0, 0)	0.03 (-0.02, 0.07)	0 (0, 0)	0 (-0.01, 0.01)	-0.03 (-0.13, 0.07)	-0.05 (-0.23, 0.13)	0.37 (0.15, 0.55)

**Table S4:** A. Values associated with multivariable modeling of various risk factors in various cattle groups with healthy group as a reference. B. Values associated with multivariable modeling of various risk factors with lameness scores as the outcome. In each model, the lower lameness score was considered as the reference group. BFR: Bovine foot rot; BDD: Bovine digital dermatitis; Inc: Inconclusive; M: M-stage classification system of BDD; AW: arrival weight; SW: sampling weight; LS: lameness scores compared to lameness score of 0 as a reference; AIC: Akaike's information criterion.

<b>A. Multivariable model with animal groups as outcomes</b>				
Group	Risk factor	Odds ratio	95% CI	P-value
<b>Model 1</b>				
BFR	AW	0.995	0.992, 1.001	0.03
	Score (score 0 as baseline)			
	LS1	0.551	0.13, 8.73	0.41
	LS2	1.29e-08	0 - ∞	1
	LS3	4.56e-09	0 - ∞	0.99
	Intercept	38.83	1.85, 811.41	0.02
<b>Model 2</b>				
BDD-M2	SW	0.997	0.993, 1.003	0.03
	Intercept	117.21	1.91, 3.7e+05	0.02
<b>Model 3</b>				
BDD-M4	SW	0.996	0.99, 1.01	0.13
	LS1	0.3	0.04, 14.8	0.24
	Intercept	1082.5	0.79, 1.5e+09	0.06
<b>Model 4</b>				
Inc	SW	1.004	1, 1.01	0.08
	Intercept	0.007	3.8e-05, 161.4	0.06
<b>B. Multivariable model with lameness scores as outcomes</b>				
Group	Risk factor	Odds ratio	95% CI	P-value
<b>Model 1</b>				
LS1	ADG	1.44	0.88 – 3.79	0.15

	Group (healthy as baseline)			
	BFR	2.66	0.71 – 35.33	0.15
	BDD-M2	1.9	0.46 – 30.65	0.37
	BDD-M4	4.09	0.61 – 171.33	0.15
	Inc	4.86	0.75 – 191.43	0.1
	Intercept	0.19	0.03, 7.730	0.09
<b>Model 2</b>				
LS2	None			
<b>Model 3</b>				
LS3	Wentry	1	0.99, 1.01	0.21
	Intercept	2.35	0.1, 1260.25	0.6
<b>Model 4</b>				
LS1 vs LS2	None			
<b>Model 5</b>				
LS1 vs LS3	AW	1	0.99, 1.004	0.11
	Intercept	2.26	0.14, 504.28	0.11
<b>Model 6</b>				
LS2 vs LS3	AW	0.99	0.98, 1.01	0.15
	Intercept	2077.66	0.15, 2.63e+11	0.12

**Table S5:** General histopathological observations in cattle samples taken from punch biopsies. BFR: Bovine foot rot; BDD: Bovine digital dermatitis; Inc: Inconclusive; M: M-stage classification system of BDD; SC: stratum corneum; SG: stratum granulosum; SS: stratum spinosum; SB: stratum basale; O: overall thickness indicating the thickness from SC to the end of rete pigs (derived by averaging 3 measurements from the center and 2 edges for each sample) PMSD: perivascular mononuclear superficial dermatitis; WF: Warthin-Faulkner's; HE: hematoxylin-eosin.

Cattle group	General histopathological observations from punch biopsy samples		Notes
	Epidermis (morphological changes)	Dermis	
Healthy (n=13)	SC: Intact, uniformly thick, SG: clearly visible granules; SS and SB: occasional and sparse vacuolated keratinocytes (likely artifacts); O: rete ridges present, with uniformly sized projections that are not extended to deep dermis.	No inflammatory infiltration, occasionally scattered (1-5/section) neutrophils on dermis-epidermis junction. Microscopic structures intact.	Hyperkeratosis observed in 2 samples with SC approximately 180 $\mu\text{m}$ (>15 cells) and rete

	<p><i>Estimated range of measurements (4X)</i></p> <p>SC: 35-70 µm thick, approximately 100 µm thick in a few cases, 5-10 cell layers; SG: 1-6 cell layers: O: 200-300 µm (upper range: 520 µm)</p>		<p>ridges 730 µm thick. One healthy specimen had SC layer 2600 um thick.</p>
<p>BFR (n=20)</p>	<p>SC: Erosion to ulceration in samples obtained from within lesion borders, hyperkeratotic in samples taken from area adjacent to the lesions. SG: 3-8 layers. SS and SB: Spongiosis (8/20), serum lakes (6/20), and ballooning degeneration of keratinocytes (6/20) occasionally observed. All sections were acanthotic.</p> <p><i>Estimated range of measurements (4X)</i></p>	<p>Generalized increase in inflammatory cell infiltration composed of neutrophils and lymphocytes, mostly localized in papillary tips at dermis-epidermis junction. Papillary inflammation-associated necrosis (7/20), hemorrhage (9/20), and edema common (6/20) present. PMSD common (9+new/20)</p>	<p>Three sections had either none or minimal dermis included.</p>

	<p>SC: 200-700 <math>\mu\text{m}</math> thick, beyond 1000 <math>\mu\text{m}</math> thick in a few cases, &gt;20 cell layers. SG: 5-10 layers. O: 2000-4000 <math>\mu\text{m}</math> thick.</p>	<p>frequently observed. Vasculitis infrequent (4/20) and limited to few superficial blood vessels.</p>	
<p>BDD-M2 (n=7)</p>	<p>SC: Ulceration in samples obtained from within lesion borders, hyperkeratotic in samples taken from area adjacent to the lesions. SG: Indiscernible in samples with erosion; hypergranulosis common; SS and SB: Acanthosis, occasional ballooning degeneration of keratinocytes and serum lake epidermis observed in samples with ulceration or erosion. Rete pegs extended deep into dermis. All sections were acanthotic.</p> <p><i>Estimated range of measurements (4X)</i></p> <p>SC: 500-1200 <math>\mu\text{m}</math>, &gt;15 cell layers; SG: 3-15 cell layers; O: 600-1300 <math>\mu\text{m}</math>.</p>	<p>Increased inflammatory cell infiltration composed of neutrophils, plasma cells, variable-sized lymphocytes, and macrophages. Neutrophils and lymphocytes often formed columns at the dermis-epidermis junction and papillary tips. Severe PMSD (5/7) present but localized and no vasculitis. Papillary inflammation-associated necrosis, hemorrhage, and edema infrequent (2/7).</p>	<p>No spirochetes detected in any of the samples tested with WF stain.</p>

<p>BDD-M4 (n=4)</p>	<p>SC: Erosion with some samples having partially intact hyperkeratotic SC layer: SG: Indiscernible due to erosion. SS and SB: spongiosis (2/4), serum lakes (3/4), ballooning degeneration of keratinocytes (2/4) commonly observed.</p> <p><i>Estimated range of measurements (4X)</i></p> <p>SC: 700-1200 <math>\mu\text{m}</math>, &gt;15 cell layers, when present; Rete pegs: 700-2200 <math>\mu\text{m}</math>.</p>	<p>Increased inflammatory cell infiltration with neutrophils primarily located in papillary tips, superficial dermis, and occasionally in deep dermal plexus. Papillary inflammation-associated necrosis, hemorrhage, and edema common (3/4). PMSD was observed in 2 samples with dermis. Dense nuclear debris and inflammatory infiltrates in papillary tips.</p>	<p>HE staining indicated bacteria in skin surface, in general. WF staining in one sample showed extensive damage to structures with dermal necrosis and hemorrhage with aggregate of spirochetes in epidermis.</p>
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			Two sections did not have dermis included.
Inconclusive (n=5)	<p>SC: Erosion or partial erosion in samples obtained from within lesion borders; hyperkeratotic in samples taken from area adjacent to the lesions. SG: Hypergranulosis uncommon (1/5). SS and SB: spongiosis (1/4), serum lakes (1/4), ballooning degeneration of keratinocytes (1/4) uncommon. All sections were acanthotic.</p> <p><i>Estimated range of measurements (4X)</i></p>	<p>Neutrophils increased in sections (4/5) and located primarily in papillary tips, superficial dermis, and occasionally in deep dermal plexus. Papillary inflammation-associated necrosis (4/5), hemorrhage (3/5), and edema (5/5) either diagnosed or suspected. Mild PMSD observed (5/5). Vasculitis commonly suspected (4/5).</p>	One section did not have dermis included.

	<p>SC: A few samples had 60-80 <math>\mu\text{m}</math> thick SC (5-10 cells) and most had 120-280 <math>\mu\text{m}</math> thick SC (&gt;20 cells).</p> <p>SG: 2-5 cell layers. O: 500-2600 <math>\mu\text{m}</math>.</p>		
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**Figure S1:** Perceived ranking of various lameness-associated conditions as ranked by 5 feedlot producers. The x-axis represents the rank in a chronological order from 1-6, with ‘others’ being a category excluding the diseases that are explicitly mentioned. BFR: Bovine foot rot; BDD: Bovine digital dermatitis

